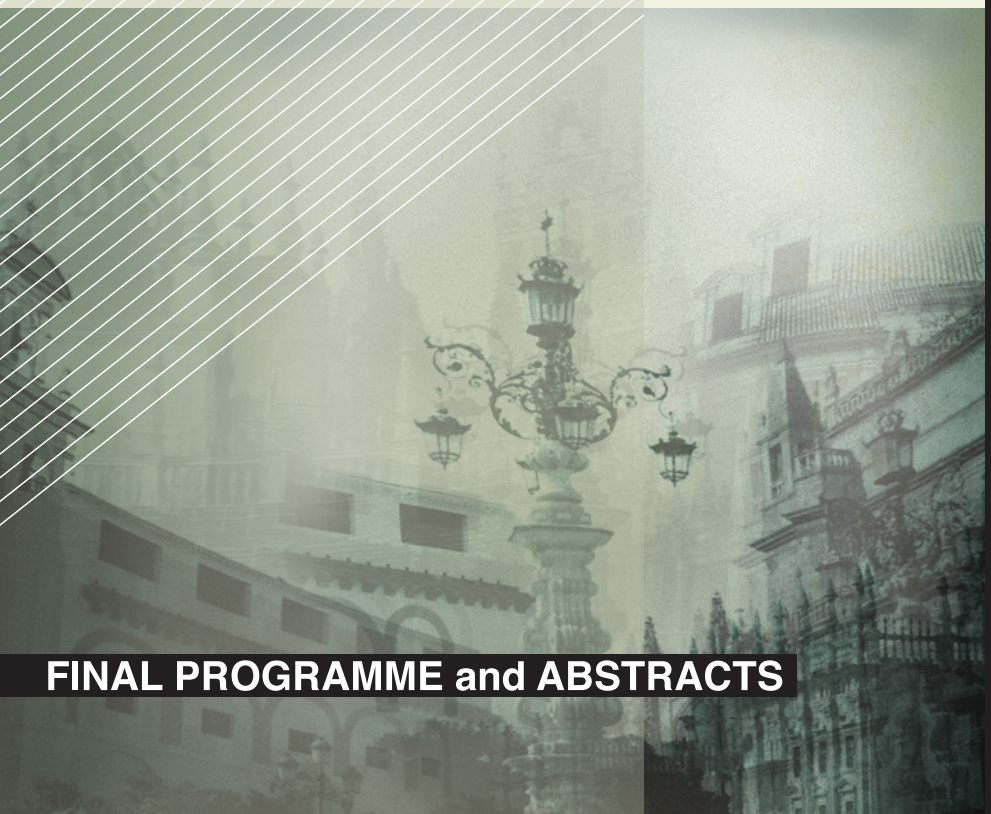
VII EUROPEAN CONGRESS OF PROTISTOLOGY

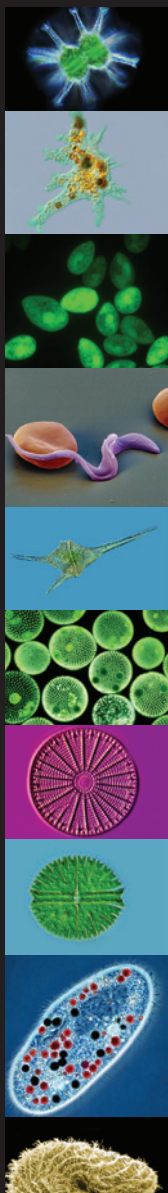
in partnership with

THE INTERNATIONAL SOCIETY OF PROTISTOLOGISTS

5-10 September 2015

SEVILLE - SPAIN





FINAL PROGRAMME and ABSTRACTS

Content

VII ECOP - ISOP Joint Meeting

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Wellcome Address

VII ECOP – ISOP Joint Meeting

Dear participants,

On behalf of the Organizing Committee, it is our great pleasure to welcome you to Seville and to the VII European Congress of Protistology (VII ECOP), hosted by the Specialized Group of Protistology (Grupo Especializado de Protistología, GEP) of the Spanish Society of Microbiology. The European Congresses of Protistology (ECOP) are the most visible activities of the Federation of European Protistological Societies (FEPS), a nonprofit association of 12 national and cross-national protistological societies in Europe.

The VII ECOP congress will be organized for the first time as a joint meeting in partnership with the International Society of Protistologists (ISOP), a global society which has members across the world with interests in all areas of Protistology, and some of its affiliated societies are also constituent societies of FEPS. This will be a very special occasion, so we thank you for your active participation in this outstanding meeting point for protistologists worldwide.

The Congress has been designed to provide a comprehensive overview of the latest research developments in different protistological fields, from the molecular to the ecological ones. Contributions will be presented in the form of 8 Plenary Lectures, 11 Symposia, 10 Workshops, 9 General Oral Sessions, 3 Special Sessions for PhD Students and Young Postdocts and 3 Poster Sessions. These activities assure that the meeting will be a major scientific event.

We are especially grateful to ISOP which organized and generously supported two symposia and two plenary lectures, one of them being the 2015 Hutner Award lecture. Therefore, the 2015 ISOP Meeting will take place in close connection with VII ECOP, one could say in a symbiotic-like way. Hence the name VII ECOP – ISOP Joint Meeting.

The meeting will be held in two venues located near the centre of the city and at a walking distance from each other, the Hotel-Conference Center Sylken Al-Andalus Palace and the Reina Mercedes Scientific Campus of the University of Seville.

We would like to deeply express our thanks to the University of Seville for its generous support, to I3 Congresos y Eventos for the excellent arrangement of logistical and technical aspects of the Congress, and to our dedicated staff, colleagues, friends and families for their untiring help, support and advice in planning and arranging this meeting.

We hope that you will enjoy the Congress and that your interaction with your colleagues from different countries will stimulate a creative exchange of ideas and will be personally rewarding. We also hope and trust that you will enjoy your visit to the charming and beautiful Seville, an ancient historical city that for almost three centuries was the gateway to the Americas and a unique space for cultural and commercial exchange between Europe and the New World.

We very much look forward to your participation and to welcoming you in Seville.

Yours sincerely,

Aurelio Serrano *President of the Organizing Committee of the VII ECOP – ISOP Joint Meeting*

Ana Martín *President of GEP – SEM*

Eduardo Villalobo *Secretary of the Organizing Committee of the VII ECOP – ISOP Joint Meeting*

VENUES

The VII ECOP – ISOP Joint Meeting will be held in two venues located near the centre of the city and at a walking distance from each other (ca. 800 meters), the **Silken Al Andalus Palace Hotel Conference Center** (Saturday 5th and Sunday 6th) and the **Reina Mercedes Scientific Campus** of the University of Seville (Monday 7th - Thursday 10th).

SILKEN AL ANDALUS PALACE HOTEL

CATEGORY:
Four Star Hotel

ADDRESS:
Avenida de la Palmera, 41012 Seville.

The **Silken Al Andalus Palace** is located at a 7 minute drive (3.4 km) from the historic city centre of Seville.

This modern design hotel offers spacious, air conditioned rooms with free Wi-Fi. The Silken Al Andalus' restaurants specialize in gourmet and Italian cuisine. There is also a cafe, poolside bar and a piano bar with live music.

Set in Seville's Heliopolis district, **Silken Al Andalus Palace** is a 5 minute drive from Plaza España. Regular public bus services connect the hotel with the historic city center in about 20 min. **The Silken Al Andalus Palace** is 1 km from Pineda Golf Club and 2 km from Maria Luisa Park.

The **Silken Al Andalus Palace Hotel** has 623 rooms where decoration and facilities give to customers a comfortable atmosphere. Victorio & Lucchino floor, decorated by two of the best Spanish fashion designer, has nine exclusive suites full of Andalusian flavour.

How to reach the Hotel Silken Al Andalus

Airport to the hotel

Taxis: There is a flat rate from the airport to Seville. The basic rate is 22.20 euros, but will increase if you arrive late in the night or during weekends. Each piece of luggage over 10 kg will increase the rate by 0.49 euros. Detailed information about taxi fares can be obtained here: http://www.aena-aeropuertos.es/csee/Satellite/Aeropuerto-Sevilla/en/Page/1237554620989//Taxi.html

Bus: There is a bus line between the airport to the bus station *"Plaza de Armas"*, in the city centre, with stops at convenient points, including the Santa Justa rail and AVE station. You can see the bus stop as you exit the airport and walk to the left. The journey takes about 35 minutes. One-way ticket is 4 euros. The bus driver sells the bus tickets. You should stop at the last stop, the *"Plaza de Armas"* stop, and then take a bus of line 3 in the *"Bellavista"* direction. Ask the driver for a stop near the hotel Silken. The stop is the next one after the *"Benito Villamarín"* football stadium. The bus stop is only a few meters from the hotel. The price of the line 3 bus ticket is 1.4 euros.

Information about the Airport of Seville:
http://www.aena-aeropuertos.es/csee/Satellite/Aeropuerto Sevilla/en/Page/1048243388846/

Train Station to the hotel

Taxi: Use the standard rate as shown in the link above. The cost of a taxi from the Santa Justa train station to the hotel should be about 10 euros, but it may increase depending on traffic conditions, number of pieces of luggage, and time of the day.

Bus: You can take the airport bus line (EA) to the *"Plaza de Armas"* and follow the instructions as if you were coming from the airport as described above. There are other alternatives combining line C1 from *"Santa Justa"*, stop at *"Prado de San Sebastián"*, and taking line 34 direction *"Los Bermejales"*. The stop is the same for line 3 described above. Please, see information and maps about the bus system here (in Spanish only, sorry): http://www.tussam.es

Moving around Seville:

Bus: There one bus stop a few meters from the hotel. It is located in Avenue Holanda, as you exit the hotel to the left, and take the second street to the right. The bus lines are 3 (direction *"Pino Montano"*) and 34 (direction *"Prado de San Sebastián"*) and can take you to the city center in about 15-30 min. Bus tickets are 1.4 euros. A bus card for 10 tickets can be bought in press stands by 1.5 euros and can be charged with a minimum of 7 euros. The cost of the bus ticket with the pre-paid card is 0.69 euros.

Taxi: there is a taxi stop at the hotel entrance. A taxi can take you to the city center in about 5-10 min and should cost about 8 euros, but the rate may differ depending on traffic conditionsand time of the day.

UNIVERSITY OF SEVILLE – *REINA MERCEDES SCIENTIFIC CAMPUS*

The ***Reina Mercedes Scientific Campus*** (Reina Mercedes Avenue s/n, 41012 Seville) is one of the main four campuses of the University of Seville, with the Faculties of Mathematics, Chemistry, Physics, and Biology. It also includes the Faculties of Pharmaceutical Sciences, Computer Engineering and the High School of Advanced Architecture. It is a quite compact Campus, with gardens and pedestrian areas.
Three different buildings in *Reina Mercedes Scientific Campus* will hold Congress activities:

Escuela Técnica Superior de Ingeniería Informática or ETSII (**Faculty of Computer Engineering**). All Plenary Lectures (PL) will take place at the *Salón de Actos* (**Auditorium Maximum**) of ETSII. Several Symposia and Workshops will take place also at the **Auditorium Maximum** of ETSII. Posters of several topics of the three Poster Sessions will be displayed at the entrance hall of the Auditorium Maximum of ETSII.

Facultad de Biología - Edificio Rojo (**Faculty of Biology – Red Building**). Several Symposia and Workshops will take place at the *Salón de Grados* (Conference Classroom) of **Faculty of Biology – Red Building.** Posters of several topics of the three Poster Sessions will be displayed at the lobby of **Faculty of Biology – Red Building.**

Edificio Celestino Mutis (CITIUSII) (**Celestino Mutis Building or CITIUSII**). Several Symposia and Workshops will take place at the Conference Classroom of **Celestino Mutis Building or CITIUSII.**

How to reach the *Reina Mercedes Scientific Campus* at *Avenida Reina Mercedes* (Reina Mercedes Avenue)

The ***Reina Mercedes Scientific Campus*** of the University of Seville has entrances both from *Avenida de la Raza* and from *Avenida Reina Mercedes*. The campus has a parking lot available only to university staff and students.

Lines 3, 6 and 34 of the Seville urban bus service (TUSSAM) stop at *Avenida Reina Mercedes,* and lines 1 and 33 have stops at walking distance from the campus as well. The campus can also be reached by local trains (*"Cercanías RENFE"):* walking from the *Virgen del Rocío* train station takes less than 15 minutes.

To and from the Airport

The Seville airport code is SVQ, and it is located about 10 Kms from the campus. Taxis to and from the airport have a fixed price (that depends on the day and the hour you take them), around 25 Euros in average. There is a bus service connecting the airport and Puerta de Jerez,in the city center. The line is called *"EA: Especial Aeropuerto Prado"* and you can get more information at the TUSSAM site.

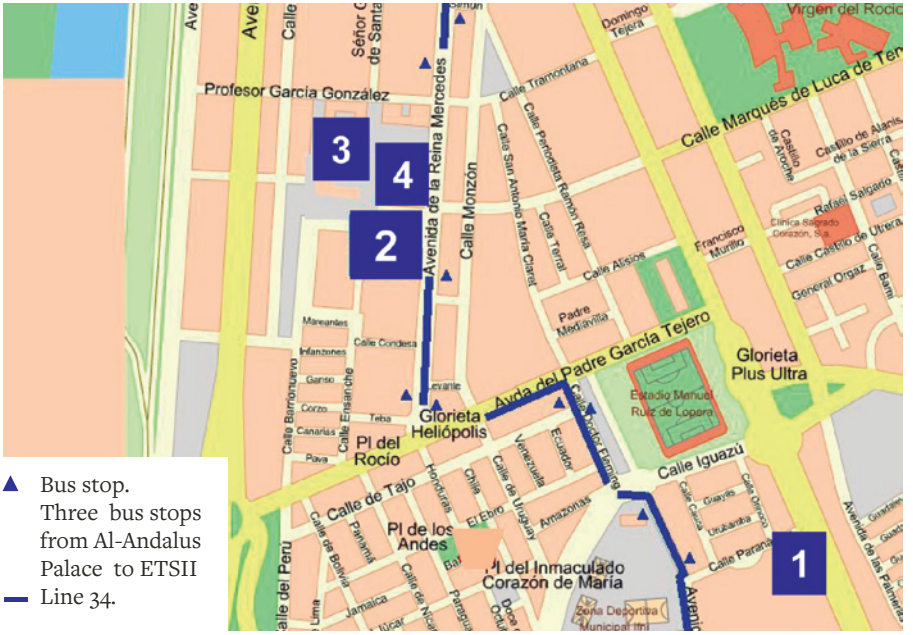
To and from *Santa Justa* Train Station

Santa Justa Station is the stop of the high-speed trains (AVE) connecting Seville from Madrid, Barcelona or Malaga. It is located in the Nervión district. Taxis are the easiest way to get to and from *Reina Mercedes Campus,* and the average cost is around 10 euros. You can also use the local train from *Santa Justa* to *Virgen del Rocío* or the bus, taking the line C1 and changing at *Prado de San Sebastián* to get line 34.

Using Your Own Car

The *Reina Mercedes Campus* is easy to reach by car. Once you get to the SE-30 (Seville's ring highway) drive in direction to Cádiz and take exit 9 or 9A. From the exit drive to *Avenida de la Raza* and follow the signs to *Avenida Reina Mercedes.*

1. *Silken Al-Andalus Palace* Hotel-Conference Center.
2. Faculty of Computer Engineering. (*Escuela Técnica Superior de Ingeniería Informática*)
3. Faculty of Biology – Red Building (*Facultad de Biología – Edficio Rojo*).
4. Celestino Mutis building, CITIUS II. (*Edificio Celestino Mutis, CITIUS II*). Behind this building is located the University canteen.



▲ Bus stop. Three bus stops from Al-Andalus Palace to ETSII
— Line 34.

Escuela Técnica Superior de Ingeniería Informática or ETSII (**Faculty of Computer Engineering**), ***Reina Mercedes Campus.***

Facultad de Biología – Edificio Rojo (**Faculty of Biology – Red Building**), ***Reina Mercedes Campus.***

Congress Registration Counter

All congress material is available at the Congress Counter. The Congress Counter will be located in the lobby of the **Al-Andalus Palace Hotel** (Sunday 6) and the entrance hall of the **Auditorium Maximum** of ESTII, ***Reina Mercedes Campus*** (Monday 7 to Thursday 10).

Name Badges

Participants are kindly requested to wear their name badge at all times during the congress including the Welcome Reception, Opening Ceremony and Social Programme activities. Name badges of Organizing Committee members and Collaborators will be marked with red and green spots, respectively.

Congress Language

The official language of the **VII ECOP – ISOP Joint Meeting** is English.

Certificate of Attendance

A certificate of attendance will be handed out upon demand at the registration counter.

Programme Changes

The organizers cannot assume liability for any changes in the conference programme due to external or unforeseen circumstances.

Internet Access

Free WiFi access is available at the **Al-Andalus Palace Hotel** (Sunday 6) and the ***Reina Mercedes Campus*** (Monday 7 to Thursday 10) during Congress activities. Specific instructions on how to access the Wireless networks will be provided at the Congress Counter.

Speakers' Centre

Speakers are kindly asked to submit their contributions to the Congress Counter on the day before their presentation or during lunch break. Staff will be present to assist you. Please bring your presentation either on a memory stick or a CD. It is not possible to use your own laptop in the conference rooms.

Mobile Phones

Participants are kindly requested to keep their mobile-phones turned off while attending the scientific sessions in the conference rooms.

Poster Exhibition

Three evening Poster Sessions of 1 h will take place:

- **Poster Session I, Monday 7th (18.00-19.00 h).**
- **Poster Session II, Tuesday 8th (18.00-19.00 h).**
- **Poster Session III, Wednesday 9th (15.00-16.00 h).**

Each poster is assigned to a specific session of 1 h. Posters will be displayed in the lobby of the **Faculty of Biology – Red Building** and the entrance hall of the ***Auditorium Maximum*** of the **Faculty of Computer Engineering** (*ETSII*) throughout the duration of all congress activities of the day.

It is the responsibility of the presenting author to ensure that at least one of the authors is present during that time, and that the poster is removed at the end of the session. Material to put up the poster will be available in the poster areas.

Comedor Universitario (University Canteen), *Reina Mercedes Campus.*

Coffee Breaks

Coffee, tea and refreshments will be served to all registered participants during the coffee breaks from Sunday to Thursday. Coffee bar stations are located at the lobby of the **Faculty of Biology – Red Building** and the entrance hall of the ***Auditorium Maximum*** (**Faculty of Computer Engineering**).

Lunch Breaks

From Sunday through Thursday, lunch is included in the registration fee for registered participants and accompanying persons. On Sunday 6, lunch will be served during the lunch break at the **Al-Andalus Palace Hotel.** On Monday and Thursday lunchs will be served between 13.00 and 15.00 h at the University Canteen of the ***Reina Mercedes Campus*** (*Comedor Universitario*) which is located just behind the **Celestino Mutis Building.** On Wednesday, picnic lunch boxes will be provided in stations located at the lobby of the **Faculty of Biology – Red Building** and the entrance hall of the ***Auditorium Maximum*** (**Faculty of Computer Engineering**).

Welcome Reception

Saturday, September 5th from 19.00 to 21.00 h.
The **VII ECOP – ISOP Joint Meeting** will begin with a Welcome Reception in the gardens and lobby of the **Al-Andalus Palace Hotel,** *Avenida de la Palmera,* 41012 Seville. The Welcome Reception is free of charge for registered participants and accompanying persons.

Guided Visit to Reales Alcázares of Seville (Royal Palace and Gardens)

ADDRESS: *Patio de Banderas s/n,* 41004 Seville

Wednesday, September 9th from 19.30 to 20.30 h (Special buses will depart from hotels and ***Reina Mercedes Campus*** at 18.30 h)

All registered participants and accompanying persons are invited to a guided visit to the ***Reales Alcázares,*** the most impressive monument of Seville and the oldest Royal Palace still in use in Europe, and probably in the World. Many movies and he 5th season of famous TV series Game of Thrones were filmed in that unique historical scenario.

The Real Alcazar (*or Reales Alcázares*) of Seville is the oldest European royal residence and is not one building, but a group of buildings from different time periods and each building has a different architectural styles (Muslim, *Mudejar*, Gothic, Renaissance, Baroque, Neoclassic) ranging over 11 centuries.

In the 9th century, the Moors built a military fort on top of a Visigothic basilica, and this was during the reign of the Emir Abdul Rahman II. Later Caliph Abdul Rahman II expanded it to make it a residence for the governor. In the 11th century, King Al-Mutamid of the Abbadid dynasty expanded the palace enormously.

The Christians then reconquered Seville and Don Pedro (Peter the Cruel) renovated the palace to his tastes in 1364. The Puerta del Leon (Lion Gate) became the main entrance to the palace.

There is a heraldic lion on top of the gate, in a tiled panel. This gate leads to the Patio del Leon (Courtyard of the Lion). The Alcazar is one of the most beautiful palaces in Spain.

Actually it has a Mudejar Palace and a Gothic Palace by its side. The Mudejar Palace has the most beautiful patio, the Patio del Yeso with its reflecting pool and sunken gardens. It is surrounded by columns supporting Moorish arches. The pillars support a very decorative mesh of stucco. The tile work in the palace has some of the most beautiful tiles in Andalusia and the stucco work on the walls is impressive. The Mudejar ceilings also call attention for their beauty and color. There is a three tier gallery that is on the southern side.

The Alcazar was the official residence of the Kings of Spain (Spanish Habsburgs and Bourbons) during their visits to Seville over the XVI-XX centuries, so they further enlarged it with Renaissance and Baroque Palaces.

Congress Dinner

Wednesday, September 9th from 21.00 to 23.00 h

The Congress organizers would like to invite you to join for the Congress Dinner which will take place at the ***Restaurante La Raza,*** *Av. Isabel la Católica, 2, 41013 Sevilla.* This place is at a walking distance from ***Reales Alcazares*** (see map below).

Tickets for Congress gala dinner can be purchased separately at the registration counter at the latest by Monday (Ticket price: 35 Euro).

The restaurant *La Raza* is located in the Maria Luisa Park, former gardens of the Palace of the Dukes of Montpensier, opposite the Casino Exhibition and *Teatro Lope de Vega,* surrounded by extensive greenery and outdoor terraces, privileged place in the city that has become a landmark of Seville restoration.

The history of Seville is that of a melting pot of cultures, with a fertile balance of arts, sciences and commerce. Legend has it that the city was founded by Hercules, and from its foundation onwards, three thousand years of history have left a spirit of tolerance and a rich legacy from cultures as diverse as Phoenician, Roman, Muslim, Jewish and Christian.

The Romans initially came to Spain to fight Hannibal's Carthaginian armies but ended up staying for over six centuries. The period of Roman dominance left not only a language, but also numerous architectural remains. The town of Italica, built as a home for battle-weary troops, houses a Roman theatre so well preserved that it is still used for performances. It was also the town where two great emperors were born: Trajan and Adrian. By the end of the fifth century AD, Spain had been overrun by barbarians, but it was not long before they too succumbed to Andalusia's civilizing influence, and Seville became an important city in the Visigoth Kingdom. However, in 712 Seville was suddenly engulfed in the northward push of Muslim armies. The Moorish occupation was to last for over five centuries, a period that saw the largely peaceful coexistence of Muslims, Jews and Christians.

Islam has left many indelible marks in the city: the winding alleys of the city center, the cool courtyards of the numerous mansions and the tranquil fountains of the Santa Cruz quarter. Another excellent example of the Muslim heritage is the Alcazar, a fabulous palace and gardens, in the city center, similar to the Alhambra in Granada.

All the defences built by the Muslims could not stem the reconquest that began in the north of Spain: in 1248 the city fell to the Christian armies of King Ferdinand III. Now the city's patron saint (San Fernando), he maintained a policy of religious tolerance, dividing the city into zones for each of the three faiths. Inspired by faith the Christians built many impressive buildings: the City Hall, numerous churches and palaces and, of course, the magnificent Cathedral.

Christopher Columbus planned his expeditions in the Monastery of *Santa Maria de las Cuevas,* in the *Isla de la Cartuja,* now restored to be a Modern Art museum. Trade with the New World made Seville the richest city in Europe. However, the monopoly of trade was lost in the eighteenth century and with it the wealth, which had created scores of magnificent churches and had supported painters like Velázquez, Murillo and Zurbarán, as well as many other artists.

With the loss of the American colonies, the nineteenth century was a time of decline for Spain. Nevertheless, it was precisely this mixture of orange blossom and decay that so enticed the romantics of northern Europe. They came south looking for mystique and innocence that had been smudged out by the smoke of industrial revolution, and Seville came to represent a key venue in the Grand Tour itinerary.

Lord Byron wrote of his admiration for the city's dark-eyed damsels, some of who feature in his reworking of the traditional story of "Don Juan Tenorio".

Mozart's "Don Giovanni" is drawn from the same local legend, and his "Les Nozzes di Figaro" is also set in Seville. With Rossini's "Il Barbiere di Siviglia", Verdi's "La Forza del Destino", Beethoven's "Fidelio" and over twenty other works all based in the city, Seville can consider itself to be the opera capital of the world. This claim has recently been enhanced by the construction of a fabulous new opera house, the *Teatro de la Maestranza,* which frequently hosts productions from leading international companies.

Across the river from the opera house lies the old sailors' quarter of Triana, with the beautiful Betis Street, and it was there that the gypsy chants of a girl inspired the young French writer Prosper Merimée during his stay in the 1840's. After him, Bizet, with an adaptation of the work "Carmen", created one of the most beautiful operas ever written.

Useful Information

Currency:
Euro.

Language:
Spanish (actually Castilian) throughout the country.

Visa:
No visa is necessary for citizens of other EU countries staying a maximum of three months. For citizens of other countries, a visa may be required.

Emergency Numbers (dial toll-free from any phone):

Emergency services Tel: 112.

Fire service/ Bomberos Tel: 080.

National Police/Policía Nacional Tel: 091(in case of serious crime).

Local Police/Policía Municipal Tel: 092 (for towns and cities).

Telephone Calls: To make an international call from Spain, dial 00 and then add the country code followed by the telephone number. To call Spain from abroad, the country code is 34.

Business hours.
The normal opening hours for shops:

Monday to Saturday: 10am - 1.30pm and 5pm - 8pm.

Large stores usually stay open all day from 10am to 10pm.

Most shops are closed on Sundays.

Most museums are open from Tuesday to Sunday, except public holidays.

Banking facilities:
All Spanish and most international banks have braches in the city.

Opening times:
Monday to Friday: 9am - 2pm. Saturday: 9am - 1pm.

Taxis:
Taxis are easily identified in the cities as white cars with a green light on the roof. The light is lit when they are available. Prices are reasonable but make sure the meter is reset when you start your journey. Surcharges apply for airport pick-ups, items of luggage, journeys outside of city boundaries and after 10pm and at weekends.

Tipping:
Typically Spanish people leave a few coins after a meal but there is no 15% rule.

Tap water:
Tap water is of excellent quality and safe to drink. Most locals drink tap water.

- ▲ Bus stop.
- Line to de airport.
- Line 34.

1. *Silken Al-Andalus Palace* Hotel – Conference Center.
2. Faculty of Computer Engineering (*ETSII*).

PROGRAMME OVERVIEW **VII European Congress of Protistology in partnership with ISOP,** 5-10th Sept. 2015, Seville, Spain.

Time	Saturday, 5 Sept.	Sunday, 6 Sept.	Monday, 7 Sept.	Tuesday, 8 Sept.	Wednesday, 9 Sept.	Thursday, 10 Sept.
8.00		Registration at the HOTEL	Poster set-up Registration at UNI			
				Poster set-up	Poster set-up	
9.00			PL III Jan Pawlowski	PL V (Hutner Lect.) Ross Waller	PL VII Weibo Song	PL VIII Roberto Docampo
		Opening Ceremony				
10.00		PL I Graham Clark	S7M W7M GO-1	S8M W8M GO-3	S9M W9M GO-6	S10M W10M GO-8
11.00		*Coffee Break HOTEL*				
		S6M (ISOP sponsored Symposium)	*Coffee Break UNI*	*Coffe Break UNI*	*Coffe Break UNI*	*Coffe Break UNI*
12.00			S7A W7A GO-2	S8A W8A GO-4	S9A W9A Mini Symposium and GO-7	S10A W10A GO-9
13.00						
		Lunch HOTEL	*Lunch UNI*	*Lunch UNI*	*Lunch PICNIC*	Farewell
14.00						
15.00		PL II Pierangelo Luporini	PL IV Nancy Guillen	PL VI Wilhelm Foissner	Poster Session III	
16.00		*Coffee Break HOTEL*	SS7E-1, SS7E-2, SS7E-3 w7E	Keynote Lecture and S8E W8E GO-5	Free time	
		S6E (ISOP sponsored Symposium)				
17.00	Registration					
			Coffe Break UNI	*Coffe Break UNI*		
18.00			Poster Session I	Poster Session II	19.30-20.30 h Guided Visit to *Reales Alcázares* (Royal Palace, Seville)	
		Meetings of Societes				
19.00	Welcome Reception Hotel		Meetings of Societes	FEPS Meeting		
					Congress Dinner	

VENUES. HOTEL: Hotel Silken Al-Andalus Palace (***5 and 6 Sept.***); UNI: University of Seville, Reina Mercedes Scientific Campus (***7-10 Sept.***)
Plenary Lectures (8) // Symposia (11) // Workshops (10) // General (9) and Special (3) Oral Sessions // Poster Sessions (3)

OPENING CEREMONY

Al-Andalus Silken Conference Center Sunday 6th, 9.30-10.00 h.
Aurelio Serrano, Sevilla, Spain. ***Chair.***
Ana Martín, Madrid, Spain. ***Welcome by President of GEP – SEM.***
Antonio Ventosa, Sevilla, Spain. ***Welcome by President of SEM.***
Frederick W. Spiegel, Fayetteville, USA. ***Welcome by President of ISOP.***
Aurelio Serrano, Sevilla, Spain. ***Welcome by President of the Organizing Committee.***

PLENARY LECTURES

Plenary Lectures will take place in the **Al-Andalus Silken Conference Center** (Sunday 6th) and the *Auditorium Maximum* (*Salón de Actos*) of the **Faculty of Computer Engineering** (***Escuela Técnica Superior de Ingeniería Informática, ETSII***) of the ***Reina Mercedes* Scientific Campus** (Monday 7th to Thursday 10th):

- **Sunday 6th, 10.00-11.00 h.**

PL I

Chair: Andrew J. Roger, Halifax, Canada.
Cryptic Diversity in Organisms and Organelles.
Graham Clark.
London School of Medicine and Tropical Hygiene, London, UK .
(***ISOP sponsored lecture***).

- **Sunday 6th, 15.00-16.00 h.**

PL II

Chair: Maria Cristina Angelici, Rome, Italy.
Pheromone Signalling in Protists.
Pierangelo Luporini.
University of Camerino, Italy.

- **Monday 7th, 9.00-10.00 h.**

PL III

Chair: Ramon Massana, Barcelona, Spain.
Protist Metabarcoding and its Applications.
Jan Pawlowski.
University of Geneva, Switzerland.

- **Monday 7th, 15.00-16.00 h.**

PL IV

Chair: Eduardo Villalobo, Sevilla, Spain.
Molecular and Cell Biology of *Entamoeba histolytica* Pathogenesis.
Nancy Guillen.
Institut Pasteur, Paris, France.

- **Tuesday 8th, 9.00-10.00 h.**

PL V

Chair: Alastair Simpson, Halifax, Canada.
Hutner Award Lecture 2015.
Evolution and Devolution in Alveolates: Invasion, Organelles and Chromatin.
Ross Waller.
Cambridge University, UK.
(***ISOP sponsored lecture***).

- **Tuesday 8th, 15.00-16.00 h.**

PL VI

Chair: Thomas Weisse, Mondsee, Austria.
Protists as Bioindicators in Wastewater Treatment: Identification, Ecology, and Further Needs.
Wilhelm Foissner.
University of Salzburg, Austria.
(***Lecture sponsored by Grupo Bioindicación Sevilla - EMASESA***).

- **Wednesday 9th, 9.00-10.00 h.**

PL VII

Chair: Alan Warren, London, UK.
Ciliate Researches in China: Active Groups, Chance for Collaboration and the On-going Studies.
Weibo Song.
Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao, China.

- **Thursday 10th, 9.00-10.00 h.**

PL VIII

Chair: Aurelio Serrano, Sevilla, Spain.
Functional Analysis of Acidocalcisomes: Organelles Conserved from Bacteria to Human Cells.
Roberto Docampo.
University of Georgia, USA.

SYMPOSIA and WORKSHOPS

These sessions will take place in the **Al-Andalus Silken Conference Center** (Sunday 6th) and in three different buildings of the ***Reina Mercedes* Scientific Campus** (Monday 7th to Thursday 10th):

SYMPOSIA

Faculty of Computer Engineering.
***Escuela Técnica Superior de Ingeniería Informática* (*ETSII*).**
Reina Mercedes Ave., 41012 Sevilla.
Auditorium Maximum (*Salón de Actos*).

WORKSHOPS

Faculty of Biology – Red Building.
Facultad de Biología – Edificio Rojo.
Reina Mercedes Ave. 6, 41012 Sevilla.
Conference Classroom (*Salón de Grados*), ground floor.

Celestino Mutis building (CITIUS II).
***Edificio Celestino Mutis* (*CITIUS II*).**
Reina Mercedes Ave., 41012 Sevilla.
Conference Classroom (*Salón de Grados*).

SYMPOSIUM S6M

(ISOP sponsored symposium) (Sunday 6th, 11.30-13.30 h).

Sex in Protists

Organizers and Chairs:
Micah Dunthorn.
University of Kaiserslautern, Germany.
Thomas Weisse.
University of Innsbruck, Austria.

Speakers.

Sex on the Beach, but not in Open Oceans.
Colomban de Vargas.
Station Biologique de Roscoff, France.

Sex in Diatoms: Insights and Inspiring Questions.
Marina Montresor.
Stazione Zoologica Anton Dohrn, Napoli, Italy.

Sex in Foraminifera.
Jere Lipps.
University of California Museum of Paleontology, USA.

***Tetrahymena* Genome Architecture Provides the Benefits of Sex in the Absence of Sex.**
Rebecca Zufall.
University of Houston, USA.

SYMPOSIUM S6E

(ISOP sponsored symposium) (Sunday 6th, 16.30-18.30 h).

Evidence of Taxa-, Clone-, and Kin-discrimination in Protists: Ecological and Evolutionary Implications

Organizers and Chairs:
Avelina Espinosa.
Roger Williams University, USA.
Guillermo Paz-y-Miño-C.
University of Massachusetts Dartmouth, USA.

Speakers.

Mechanisms of Discrimination and Kin Recognition: from Unicellular to Multicellular Eukaryotes.
Guillermo Paz-y-Miño-C.
New England Center for the Public Understanding of Science, Roger Williams University, USA.

Exploration of the Developmental Program of the Social Life Cycle in *Copromyxa protea* (Tubulinea, Amoebozoa) using Ultra Low Input RNAseq.
Matthew W. Brown.
Mississippi State University, USA.

Recognition Genes, Population Density, Sorting, and Cheating in the Social Amoeba *Dictyostelium discoideum*.
Joan E. Strassmann.
Biology Department, Washington University in St. Louis, USA.

Intercellular Signalling, Aggregative Behaviour and Experimental Challenges in *Entamoeba* Discrimination Trials.
Avelina Espinosa.
Department of Biology, Roger Williams University, USA.

SYMPOSIUM S7M

(Monday 7th, 10.00-11.30 h).

Leaving "Everything is Everywhere" Behind: Recent Advances in the Biogeography of Marine Protists.

Organizers and Chairs:
John R. Dolan.
Université Paris6/CNRS Laboratoire d'Océanographie de Villefranche-sur-Mer, Villefranche-sur-Mer, France.
David J.S. Montagnes.
University of Liverpool, Institute of Integrative Biology, Liverpool, University of Liverpool, UK.

Speakers.

Parasitic Dinoflagellates over Time and Space
D. Wayne Coats
Smithsonian Institution, Edgewater, MD, USA

Beyond Everything is Everywhere – The Burgeoning Field of Landscape Genetics and its Application for Understanding Protist Biogeography.
Chris Lowe.
University of Exeter, Exeter, UK.

Perceptions of Biogeography: Correspondence between Molecules and Morphologies in Tintinnid Ciliates.
Luciana Santoferrara.
University of Connecticut, Groton, CT, USA.

Ciliates in the Oligotrophic Ocean: Do Transient Dynamics Determine Long-term Patterns?
Stephen Wickham.
University of Salzburg, Austria.

WORKSHOP W7M

(Monday 7th, 10.00-11.30 h).

Free-living Amoebae Infections: Are They Rare Pathogens or an Emerging Threat?

Organizer and Chair:
Jacob Lorenzo Morales.
Universidad de La Laguna, Spain.

Speakers.

Genome Assembly and Annotation of *Balamuthia mandrillaris*.
Albrecht F. Kiderlen.
Robert Koch Institute, Berlin, Germany.

Natural Products as a Source of Potential Therapeutics against *Acanthamoeba* Infections.
Ines Sifaoui.
University of Cartague, Tunisia.

***Acanthamoeba* keratitis: Update on Current Treatment Options.**
Jacob Lorenzo Morales.
Universidad de La Laguna, Spain.

Anti-*Acanthamoeba* Activity of Different Artificial Tears Used in the Treatment of Amoeba Keratitis.
Angela Magnet.
San Pablo CEU University, Madrid, Spain.

Detection of *Acanthamoeba* Strains in the Ocular Surface of Contact Lens Wearers Using the Schirmer Strip Test.
María Reyes-Batlle.
Universidad de La Laguna, Spain.

SYMPOSIUM S7A

(**Monday 7th, 12.00-13.30 h**).

Molecular Cross-talk between Parasitic Protists and their Host Cells.

Organizer and Chair:
Helena Soares
Escola Superior de Tecnologia da Saúde de Lisboa, IPL & Faculdade de Ciências, Universidade de Lisboa, Portugal

Co-chair:
Alexandre Leitão
Instituto de Investigação Cientifica Tropical, Universidade Técnica de Lisboa, Portugal

Speakers.

Apicomplexan Parasites: Intracellular Life Style Specialists and their Astonishing Adaptive Potential to Anti-proliferative Drugs.
Andrew Hemphill.
Cell Biology and Parasitology, Institute of Parasitology, Universität Bern, Switzerland.

Interactions between *Theileria* and the Host Cell Cytoskeleton.
Kerry Woods.
Division of Molecular Pathobiology, Vetsuisse Faculty, University of Bern, Switzerland.

The two *Apicomplexa Besnoitia besnoiti* and Toxoplasma *gondii* Differentially Alter Intrinsic Host Cell Polarity by Manipulating Centrosome and Golgi Apparatus.
Helena Soares.
Universidade de Lisboa and Escola Superior de Tecnologia da Saúde de Lisboa, Portugal.

WORKSHOP W7A

(**Monday 7th, 12.00-13.30 h**).

Symbioses in Microbial Eukaryotes.

Organizers:
Genoveva F. Esteban (Bournemouth University, UK).
Martin T. Embley (Newcastle University, UK).

Speakers.

Endosymbiotic Algae of the Ciliates *Euplotes daidaleos, Frontonia* sp. and *Paramecium bursaria.*
Undine Achilles-Day.
Scottish Association for Marine Science, Culture Collection of Algae and Protozoa, Scottish Marine Institute, Oban, Argyll, UK.

Organelle Evolution in Anaerobic Ciliates.
William Lewis.
Institute for Cell and Molecular Biosciences, The Medical School, Newcastle University, UK.

Genomics of the Methanogenic Endosymbionts of Anaerobic Ciliates.
Anders E. Lind.
Department of Cell and Molecular Biology, Biomedical Centre, Uppsala University, Uppsala, Sweden.

Kitchen Garden or Recycling Center? Comparative Genomics of the *Kentrophoros* Ciliate-Bacteria Symbiosis.
Brandon Seah.
Symbiosis Department, Max Planck Institute for Marine Microbiology, Germany.

Intracellular Bacterial Symbiosis in Genus *Arcella* (Arcellinids: Amoebozoa): a Key Player for Adaptation to Hostile Environments.
Fatma Gomaa.
Department of Organismic and Evolutionary Biology, Biological Laboratory, Harvard University, Cambridge, Massachusetts, USA.

WORKSHOP W7E

(Workshop sponsored by the COST Action BM1102) (Monday 7th, 16.00-17.30 h).

Ciliate genome evolution and adaptation.

Organizer and Chair:
Cristina Miceli.
University of Camerino, Italy.

Speakers.

Ciliates as Model Systems to Study Molecular Adaptation and Environmental Responses.
Cristina Miceli.
University of Camerino, Italy.

How Many Cell Polarity Related Genes Are Conserved from *Tetrahymena* to Metazoa?
Helena Soares.
Escola Superior de Tecnologia da Saúde de Lisboa, IPL & Faculdade de Ciências, Universidade de Lisboa, Portugal.

Ciliates as Natural Reservoir of Potentially Pathogenic Bacteria: State of the Art After a Four Year Networking Project.
Giulio Petroni.
University of Pisa, Italy.

Control of Splicing in *Paramecium.*
Julia Contreras.
Universidad de Sevilla, Spain.

SYMPOSIUM S8M

(**Tuesday 8th, 10.00-11.30 h**).

Ecological and Evolutionary Significance of Novel Protist Lineages.

Organizer and Chair:
Ramon Massana.
Institut de Ciències del Mar, CSIC, Barcelona, Spain.

Co-chair:
Javier del Campo.
University of British Columbia, Canada.

Speakers.

(Re)-discovery of Marine ALVeolate Protistan Pineages (MALV, Syndiniales) in the Plankton and their Relevance in Marine Ecology.
Laure Guillou.
Station Biologique de Roscoff, France.

Exploring the Diversity of Divergent Protist Lineages in Freshwater Ecosystems.
Purificación López-García.
UMR CNRS 8079, Université Paris-Sud. Orsay, France.

Investigation of a Novel Opisthokont with a Predatory Lifestyle in the Context of the Evolution of Multicellularity.
Elisabeth Hehenberger.
University of British Columbia, Canada.

Exploring the Marine Pico-eukaryotic Dark-matter Using Single-cell Genomics.
Ramiro Logares.
Institut de Ciències del Mar, CSIC, Barcelona, Spain.

WORKSHOP W8M

(**Tuesday 8th, 10.00-11.30 h**).

Biology of Anaerobic Protists: Adaptation to Anaerobiosis and Parasitic Style of Life.

Organizer and Chair:
Jan Tachezy.
Charles University in Prague, Czech Republic.

Speakers.

Variations in Anaerobic Metabolism of Protists.
Aloysius G.M. Tielens.
Dept. Medical Microbiology and Infectious Diseases, Erasmus MC, / University Medical Center Rotterdam, Rotterdam, The Netherlands.

***Trichomonas* MIF: molecular mimic of host MIF?**
Patricia Johnson.
Department of Microbiology, Immunology & Molecular Genetics, UCLA, USA.

Cholesterylsulfate Synthesized via the Mitosome-compartmentalized Sulfate Activation Pathway is Required for Encystation of *Entamoeba*.
Tomoyoshi Nozaki.
Department of Parasitology, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo, Japan.

Imaging *Giardia* in vivo Metabolism and Infection Dynamics.
Scott Dawson.
University of California Davis, Davis, USA.

N-acetyl-L Ornithine Deacetylase is an Essential Factor for Adaptation of the Parasite *Entamoeba histolytica* to Nitrosative Stress.
Serge Ankri.
Department of Molecular Microbiology, B. Rappaport Faculty of Medicine, Haifa, Israel.

***Mastigamoeba balamuthi* and *Entamoeba histolytica:* So Similar Yet So Different.**
Jan Tachezy.
Department of Parasitology, Charles University in Prague, Czech Republic.

SYMPOSIUM S8A

(Tuesday 8th, 12.00-13.30 h).

Stress and Protists: No Life without Stress.

Organizer and Chair:
Juan-Carlos Gutierrez.
Universidad Complutense, Madrid, Spain.

Speakers:

Redox-Based Sensing of Environmental Stress: From Organelle Signalling to Cell fate Decisions.
Assaf Vardi.
Weizmann Institute of Science, Rehovot, Israel.

Engineered Nanoparticles and Oxidative Stress in Aquatic Protists.
Vera Slaveykova.
University of Geneva, Switzerland.

Stress Responses and Photoprotective Strategies of Ciliates Exposed to Ultraviolet Radiation.
Bettina Sonntag.
University of Innsbruck, Austria.

Adaptations to High-Salt Environments in Two Bacterivorous Halophiles.
Tommy Harding.
Dalhousie University, Halifax, Canada.

WORKSHOP W8A

(Tuesday 8th, 12.00-13.30 h).

Atypical Metabolism in Protists.

Organizer and Chair:
Michael Ginger.
Lancaster University, UK.

Speakers.

The Diversity and Origins of Anaerobic Metabolism in Mitochondria and Related Organelles.
Andrew J. Roger.
Dalhousie University, Halifax, Canada.

Evolution of Thylakoid Membrane Complexes in Eukaryotes and Functional Implications in *Chromera velia*.
Heather Esson.
Institute of Parasitology, Academy of Sciences of the Czech Republic.

The Calvin Cycle of Non-Photosynthetic *Euglena longa:* a Role in Central Energy Metabolism?
Zoltan Fussy.
Biology Centre of the Czech Academy of Sciences, Institute of Parasitology, Ceské Budejovice, Czech Republic.

Inorganic Pyrophosphate Metabolism in Protists: Metabolic and Evolutionary Implications.
Jose Roman Perez-Castiñeira.
IBVF, CSIC-Universidad de Sevilla, Spain.

KEYNOTE LECTURE

(Tuesday 8th, 16.00-16.30 h).

Malaria Parasite Resistance to the Common Drug Atovaquone is Unable to Transmit via Mosquitoes.
Geoff McFadden.
School of BioSciences, University of Melbourne, Australia.

SYMPOSIUM S8E

(Tuesday 8th, 16.30-17.30 h).

New Challenges in Microalgae Biotechnology.

Organizers and Chairs:
Federico Valverde and Aurelio Serrano.
Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Spain.

Speakers.

Biofuel from Microalgae?
Miguel G. Guerrero.
Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla y CSIC, Spain.

Development of New Molecular Tools for the Genetic Engineering of Eukaryotic Microalgae.
Rosa M. Leon.
Universidad de Huelva, Spain.

A *Chlamydomonas* Gene Co-expression Network reveals Global Properties of its Transcriptome and the early Establishment of Key Co-expression Patterns in the Green Lineage.
Francisco J. Romero-Campero.
Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Spain.

WORKSHOP W8E

(**Workshop sponsored by Grupo Bioindicación Sevilla – EMASESA**)
(**Tuesday 8th, 16.00-17.30 h**).

Protist Relevance in Wastewater Treatment.

Organizers:
Eva Rodriguez.
Asociación Científica Grupo Bioindicación Sevilla, Seville, Spain.
Humbert Salvadó.
Universitat de Barcelona, Spain.
Susana Serrano.
Universidad Complutense, Madrid, Spain.

Chair:
Wilhelm Foissner.
University of Salzburg, Austria.

Speakers.

User-friendly Methods for the Identification of Ciliate Species in Biological Aerobic Wastewater-treatment Processes.
Allan Warren.
The Natural History Museum, London, UK.

Relevance of Microscopical Analysis in Wastewater
Marina Ettl.
Yara Industrial GmbH, Germany.

Protists: Tireless Informants of Wastewater Treatment Systems. The Shortcut Biological Nitrogen Removal and Partial Nitritation Processes.
Oriol Canals.
Universidad de Barcelona, Spain.

Understanding the Eukaryotic Microbial Community of Slow Sand Filters and the Implications for Bacterial Pathogen Removal from Wastewater
Joseph Gibbs.
National University of Ireland, Galway, Ireland.

Effect of high *Lecane inermis* rotifers abundance on activated sludge biocenosis.
Fyda Janusz.
Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland.

SYMPOSIUM S9M

(Wednesday 9th, 10.00-11.30 h).

Functional Ecology of Aquatic Protists.

Organizer and Chair:
Thomas Weisse.
University of Innsbruck, Research Institute for Limnology, Mondsee, Austria.

Speakers.

Functional Ecology of Aquatic Protists – Key Issues and Open Questions.
Thomas Weisse.
University of Innsbruck, Research Institute for Limnology, Mondsee, Austria.

Evaluating Predator-Prey Dynamics: Making the Most out of Functional and Numerical Response Data.
David J.S. Montagnes.
Institute of Integrative Biology, University of Liverpool, Liverpool, UK.

Numerical/functional Response and Prey Selectivity in Heterotrophic vs. Mixotrophic Protists.
Ruth Anderson.
University of Copenhagen, Dept of Biology, Marine Biological Section, Helsingør, Denmark.

On the Functional Ecology of Heterotrophic Flagellates and the Complexity Behind.
Hartmut Arndt.
University of Cologne, Zoological Institute, General Ecology, Cologne, Germany.

Opening the Marine Microzooplankton Black Box: Sources of Variability in Protozoan Grazing Impacts.
Albert Calbet.
Dept. Biologia Marina I Oceanografia, Institut de Ciències del Mar (CSIC), Barcelona, Spain.

WORKSHOP W9M

(Workshop sponsored by Red Iberoamericana sobre Pneumocystosis – RED TEMÁTICA 212RT0450 CYTED)
(Wednesday 9th, 10.00-11.30 h).

Frontier-of-knowledge in *Pneumocystis* Infection.

Organizer and Chair:
Enrique J. Calderon.
CIBER de Epidemiología y Salud Pública, Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Spain.

Speakers.

New High-throughput Methodologies in Epidemiology and Diagnosis of *Pneumocystis jirovecii* Pneumonia.
Olga Matos.
Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Portugal.

Airborne Spread of Pneumocystis jirovecii and Nosocomial Infection.
Gilles Nevez.
Brest University hospital, University of Brest, France.

***Pneumocystis jirovecii* in Chronic pulmonary Diseases.**
Carmen de la Horra.
CIBER de Epidemiología y Salud Pública, Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Spain.

SYMPOSIUM S9A

(ISOP sponsored symposium) (Wednesday 9th, 12.00-13.30 h).

Emergent Harmful Protists in the Globalization Era.

Organizer and Chair:
Maria Cristina Angelici.
National Institute of Health (ISS), Department of Environment and Primary Prevention, Rome, Italy.

Speakers.

Waterborne Protozoan Infections: Emerging and Reemerging Pathogens in the Globalization Era.
Panagiotis Karanis.
Medical School, University of Cologne, Germany / Center for Biomedicine and Infectious Diseases-The Qinghai Medical School, Qinghai University, Xining, China.

Opportunistic Protists: What we Know and What we Have to Know.
Olga Matos.
Instituto de Higiene e Medicina Tropical, Universidade NOVA de Lisboa, Portugal.

A Still Not Well-known Parasite: *Balantidium* sp., Epidemiology and Diagnostic Tools.
Francisco Ponce Gordo.
Department of Parasitology, Complutense University Madrid, Spain.

Toxic Protists and Water Microbioma: a Feedback to Environmental Change.
Andrea Curti.
Department of Environment and Primary Prevention, National Institute of Health (ISS), Rome, Italy.

WORKSHOP W9A

(Wednesday 9th, 12.00-13.30 h).

Community Ecology through the Lens of High-throughput Sequencing.

Organizers and Chairs:
Ramiro Logares and Ramon Massana.
Institut de Ciències del Mar, CSIC, Barcelona, Catalonia, Spain.

Speakers.

Freshwater Plankton Community Ecology under High Throughput Sequencing Perspective.
Enrique Lara.
University of Neuchâtel, Switzerland.

High-throughput Sequencing Reveals Strongly Season-dependent Diversity and Dynamics of Haptophytes in North Atlantic Coastal Waters.
Egge Elianne Sirnæs.
University of Oslo, Norway .

Harvesting Phylogenetic Information to Unveil the Global Marine Diversity and Biogeography of the Mamiellophyceae Lineage of Eukaryotic Phytoplankton.
Adam Monier.
University of Exeter, UK.

Single Cell Transcriptomics of Two Uncultivated Radiolarian Species
Anders Krabberød.
University of Oslo, Norway.

Molecular and Morphology Methods for the Assessment of Marine Dinoflagellates Diversity: Do they Agree?
Albert Reñé.
Institut de Ciències del Mar, CSIC, Barcelona, Spain.

Genetic and Phenotypic Diversity Characterization of Natural Populations of the Parasitoid *Parvilucifera sinerae*.
Rosa I. Figueroa.
Instituto de Investigaciones Marinas de Vigo, IEO, Spain, and Department of Biology, Lund University, Sweden.

Special Mini-Symposium

(Wednesday 9th, 12.00-12.30 h, in the frame of General Oral Session 7 Evolution/Phylogeny III).

Towards an integrated taxonomic and morpho-genetic system for eukaryotes.

Chairs:
Sina Adl.
Colomban de Vargas.

Speakers.

EukRef: Phylogenetically informed, bottom-up curation of eukaryotic 18S rDNA sequences.
Javier del Campo.
University of British Columbia, Canada.

UniEuk: A universal, expert validated taxonomic framework integrating reference gene databases for eukaryotic biology, ecology, and evolution.
Cedric Berney.
Station Biologique de Roscoff, France.

SYMPOSIUM S10M

(Thursday 10th, 10.00-11.30 h).

Genome Editing in Protists.

Organizer and Chair:
Roberto Docampo.
Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA, USA.

Speakers.

Unlocking the *Toxoplasma gondii* Genome through CRISPR/Cas9.
Sebastian Lourido.
Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA.

Revolutionizing Functional Studies in *Plasmodium falciparum* with CRISPR/Cas.
Jessica M. Bryant.
Unité de Biologie des Interactions Hôte–Parasite – INSERM U1201- CNRS, Institut Pasteur, Paris, France.

CRISPR/Cas9 Genome Editing in *Trypanosoma cruzi* Reveals the Role of Paraflagellar Rod Proteins in Flagellar Attachment and Motility.
Noelia Lander.
CTEGD, University of Georgia, Athens, GA, USA, and Department of Clinical Pathology, State University of Campinas, Campinas, S.P., Brazil.

WORKSHOP W10M

(**Thursday 10th, 10.00-11.30 h**).

Soil Protist Diversity and Ecology.

Organizers and Chairs:
Enrique Lara.
University of Neuchâtel, Switzerland.
Stefan Geisen.
Netherlands Institute of Ecology (NIOO), Wageningen, The Netherlands.

Speakers.

Introduction to the Workshop on Soil Protists and First Question: Can we Trust Data on Protist Distribution and Ecology?
Edward A. D. Mitchell.
University of Neuchâtel, Switzerland.

Protists Hold a Central Regulator Role in Soil Ecosystem Nutrient Cycling.
Sina Adl.
University of Saskatchewan, Canada.

Biodiversity of Protists in Soils: What we Know, What we Miss.
Anna Maria Fiore-Donno.
University of Cologne, Germany.

Soil Micro-eukaryotes: Different Perspectives on a Diverse and Highly Partitioned Biome.
David Bass.
The Natural History Museum/CEFAS, London, UK.

Roundup: Diversity and Key Roles of Protists in Soils.
Stefan Geisen.
Netherlands Institute of Ecology (NIOO), Wageningen, the Netherlands.

SYMPOSIUM S10A

(**Thursday 10th, 12.00-13.30 h**).

Novel Therapies against Parasitic Protists.

Organizer and Chair:
José Manuel Bautista.
Universidad Complutense, Madrid, Spain.

Speakers.

Trypanocidal Activity and Mode of Action of Carbohydrate binding Agents.
Dolores González-Pacanowska.
Instituto de Parasitología y Biomedicina López-Neyra, CSIC, Granada, Spain.

Molecular Approaches against Malarial *Plasmodium*.
Irene Díaz Moreno.
Instituto de Bioquímica Vegetal y Fotosíntesis, University of Sevilla-CSIC, Sevilla, Spain.

Miltefosine as an Anti-*Acanthamoeba* Drug.
Julia Walochnik.
Medizinische Universität Wien, Vienna, Austria.

WORKSHOP W10A

(**Thursday 10th, 12.00-13.30 h**).

Autophagy-related Processes in Unicellular Eukaryotes.

Organizer and Chair:
José L. Crespo.
IBVF, CSIC-Universidad de Sevilla, Spain.

Speakers.

Acidification-dependent Defects in Membrane Traffic and Autophagy in Yeast Sterol Mutants.
Aurelio Serrano.
Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Spain.

Eco-physiological Context for the Conserved Autophagy Pathway during Algal Bloom Dynamics.
Assaf Vardi.
Weizmann Institute of Science, Rehovot, Israel.

Activation of Autophagy by Redox Unbalance in the Model Green Alga *Chlamydomonas reinhardtii*.
José L. Crespo.
Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Spain.

FAREWELL SESSION

Auditorium Maximum (*Salón de Actos*).
Faculty of Computer Engineering.
(**Thursday 10th, 13.30-14.00 h**).

SPECIAL SESSIONS

Oral Presentations by PhD Students and Young Postdocts.
Monday 7th (16.00-17.30 h).
Three parallel sessions (12 min long presentations).
These sessions will take place in three different buildings of the *Reina Mercedes* Scientific Campus:

Faculty of Computer Engineering.
Escuela Técnica Superior de Ingeniería Informática.
Reina Mercedes Ave., 41012 Sevilla.
Auditorium Maximum (*Salón de Actos*).

Faculty of Biology – Red Building.
Facultad de Biología – Edificio Rojo.
Reina Mercedes Ave. 6, 41012 Sevilla.
Conference Classroom (*Salón de Grados*), ground floor.

Celestino Mutis building (CITIUS II).
***Edificio Celestino Mutis* (*CITIUS II*).**
Reina Mercedes Ave., 41012 Sevilla.
Conference Classroom (*Salón de Grados*).

SS7E-1

Chair: Jacob Lorenzo Morales, La Laguna, Spain.

1. **Genomes of monoxenous species shed light on the evolution of parasitism in trypanosomatids.**
Anzhelika Butenko · *Genomics/Molecular Biology.*

2. **Genetic regulation of sporocarp development in a protosteloid amoeba, *Protosteliopsis fimicola* (Vanellidae, Amoebozoa).**
Alexander Tice · *Genomics/Molecular Biology.*

3. **Uncovering the ancestral state of mitochondrial targeting.**
Sriram Garg · *Cell Biology.*

4. **Urea vs. nitrate: concurrent uptake of nutrients by dinoflagellates *Prorocentrum minimum* at a population and single-cell level.**
Olga Matantseva · *Physiology and Metabolism.*

5. **High prevalence of the 'bovine genotype' *T. foetus* in domestic pig faecal samples and transcriptomic comparisons of the porcine, bovine and feline *T. foetus* isolates.**
Victoria Morin-Adeline · *Parasitology.*

6. **Hexaazatrinaphthylenes with apoptosis-like activity against *Leishmania donovani.***
Atteneri López-Arencibia · *Parasitology.*

7. **Promiscuous and conservative symbiont acquisition in the genus *Nuclearia.***
Sebastian Dirren · *Evolution/Phylogeny.*

SS7E-2

Chair: Alastair Simpson, Haifax, Canada.

8. **Evolutionary history of the arginine deiminase pathway among eukaryotes.**
Lukáš Novák · *Evolution/Phylogeny.*

9. ***Tetrahymena thermophila*** **shows increased evolvability following sexual reproduction.**
Jason Tarkington · *Evolution/Phylogeny.*

10. **Strain PAP020, a novel anaerobic microeukaryote branching at the base of Fornicata.**
Euki Yazaki · *Evolution/Phylogeny.*

11. **Hunting for agile prey: two novel Leptophryid amoebae (Vampyrellida, Cercozoa) devouring planktonic freshwater algae.**
Sebastian Hess · *Evolution/Phylogeny.*

12. **Single-cell multigene and transcriptomics-based cataloging of phagotrophic euglenids: towards multigene phylogenetics.**
Gordon Lax · *Evolution/Phylogeny.*

13. **Cytoplasmic double-infection - *Paramecium biaurelia* infected by two novel *Rickettsia*-like bacteria.**
Franziska Szokoli · *Environmental Microbiology.*

14. **Combined culture-based and culture-independent approaches provide insights into diversity of jakobids, extremely plesiomorphic eukaryotic lineage.**
Tomáš Pánek · *Environmental Microbiology.*

SS7E-3

Chair: Ana Martín, Madrid, Spain.

15. **Functional diversity of peatland testate amoebae: finding relevant traits to assess the response of communities to ecological stress.**
Isabelle Koenig · *Ecology.*

16. **Water-energy balance, past ecological perturbations and evolutionary constraints shape the latitudinal diversity gradient of soil testate amoebae in southwestern South America.**
Leonardo D. Fernández · *Ecology.*

17. **The response of Myxomycete communities to 14 years of N, P, and K addition in a lowland tropical rain forest.**
Laura Walker · *Ecology.*

18. **Environmental diversity of cryptic species from the *Nebela collaris* complex is strongly correlated with environmental filters.**
David Singer · *Ecology.*

19. **Accessing the ecology of uncultured picoeukaryotes through a high-throughput automatic cell enumeration approach.**
Jean-François Mangot · *Ecology.*

20. **Protozoan diversity in activated sludge from MBR systems.**
Julián Andrés Parada-Albarracín · *Ecology.*

21. **Impact of the conversion of tropical lowland rainforests on soil testate amoebae community composition.**
Valentyna Krashevska · *Ecology.*

GENERAL ORAL SESSIONS

(Morning, afternoon and evening sessions, Monday 7th to Thursday 10th).

A number of abstracts have been selected for presentation in nine General Oral Sessions (GO), which will take place in parallel to Symposia and Workshops. Due to the large number of applications for oral presentation received these presentations will have 15-min long formats.

The Organizing Committee decided to allocate some oral presentations to particular sessions, always in agreement with the organizers and presenters, and when the thematic affinities permitted it.

General Oral Sessions will take place in:

Celestino Mutis building (CITIUS II).
***Edificio Celestino Mutis* (*CITIUS II*).**
Reina Mercedes Ave., 41012 Sevilla.
Conference Classroom (*Salón de Grados*).

Faculty of Biology – Red Building.
Facultad de Biología – Edificio Rojo.
Reina Mercedes Ave. 6, 41012 Sevilla.
Conference Classroom (*Salón de Grados*), ground floor.

GO-1

(Monday 7th, 10.00-11.40 h).

Cell Biology

Chair: Juan C. Gutiérrez, Madrid, Spain.

Molecules and molecular coordination during phagocytosis of *Entamoeba histolytica*.
Esther Orozco et al.

An ancestral bacterial division system is widespread in eukaryotic mitochondria.
Michelle Leger et al.

Climacostol, a ciliate secondary metabolite with anticancer activity: results from in vitro and in vivo studies.
Federico Buonanno et al.

The evolution of the mitochondrial protein import in protists.
Pavel Dolezal et al.

How to build an Invasion Machine.
Ke Hu et al.

Meckelin guides basal body location.
Judith Van Houten et al.

Morphological versus molecular phylogenies: a debate.
Komal Kamra.

GO-2

(**Monday 7th, 12.00-13.30 h**).

Taxonomy - Environmental Microbiology (I)

Chair: John R. Dolan, Villefranche-sur-Mer, France

Unexpected diversity of marine ciliates from coastal wetlands of South China Sea.
Xiaozhong Hu et al.

Current and future perspectives on the systematics, taxonomy and nomenclature of testate amoeba.
Anush Kosakyan et al.

***Paramecium chlorelligerum* Kahl, 1935 and its *Holospora* endosymbiont.**
Sergei Fokin et al.

Morphology versus DNA – The taxonomic status of *Paramecium buetschlii* sp. nov. and its novel cryptic congeners.
Krenek Sascha et al.

New bioremediation technique for radioactive cesium-contaminated soil using *Paramecium bursaria.*
MD Shafiqul Islam et al.

Identification and characterization of *Vernalophrys algivore* n. g. n. sp. (Rhizaria: Cercozoa: Vampyrellida), a new algal predator isolated from outdoor mass culture of *Scenedesmus dimorphus.*
Yingchun Gong et al.

GO-3

(**Tuesday 8th, 10.00-11.30 h**).

Genomics/Molecular Biology – Parasitology (I)

Chair: Federico Valverde, Sevilla, Spain.

Epigenetic regulation of transposable elements in *Tetrahymena thermophila.*
Shan Gao et al.

Alternative splicing and the evolution of chlorarachniophyte algae.
Cameron Grisdale et al.

A new species of *Ripella* Smirnov et al., 2007 (Amoebozoa, Discosea) and intragenomic variation of the SSU rRNA gene within this genus.
Anna Gladkikh et al.

Complete nuclear genome sequence of *Goniomonas avonlea,* a plastid-lacking cryptomonad.
Ugo Cenci.

New intracellular symbionts of Paramecia.
Maria Rautian et al.

In vitro effect of several olive leaf extract molecules on *Acanthamoeba castellanii* Neff.
Ines Sifaoui et al.

GO-4

(**Tuesday 8th, 12.00-13.30 h**).

Parasitology (II)

Chair: Jacob Lorenzo Morales, La Laguna, Spain.

Apicomplexan diversity across environments. From the crimson blood to the shining sea.
Javier del Campo et al.

Expanding our knowledge about parasites using PATHOS-DB (The PArasiTe–HOSt-DataBase).
Marit F. M. Bjorbækmo et al.

Pee, poo and parasitic protists - what is living inside the fish we eat?
Janina Fuss et al.

New therapy strategies to fight against malaria.
Isabel G. Azcárate et al.

Phylogenetic position of metchnikovellids (Microsporidia: Metchnikovellidae).
Elena Nassonova et al.

Morphology, life cycle and molecular phylogeny of parasitic dinoflagellates of marine plankton.
Fernando Gomez et al.

GO-5

(**Tuesday 8th, 16.00-17.30 h**).

Evolution/Phylogeny (I)

Chair: Javier del Campo, British Columbia, Canada.

The cyanobacterial ancestor of eukaryotic chloroplasts pinpointed.
David Moreira et al.

Phylogenetic position of Nephridiophagidae at the fungal root and description of a new species of *Nephridiophaga*.
Renate Radek et al.

Diversity and phylogenetic relationships within the genera *Paramoeba* and *Neoparamoeba* (Amoebozoa, Dactylopodida).
Ekaterina Volkova et al.

Opisthosporidia, a new deep lineage of opisthokonts at the border of Holomycota and Holozoa.
Sergey Karpov et al.

Baikal plankton dinoflagellates as cases of recent diversification and radiation.
Nataliia Annenkova et al.

Morphology based cladistic analysis of selected dinophysoid dinoflagellate species.
David U. Hernández-Becerril.

GO-6

(**Wednesday 9th, 10.00-11.30 h**).

Evolution/Phylogeny (II)

Chair: Ramon Massana, Barcelona, Spain.

Are Amoebozoa ancestrally amoeboid?
Frederick Spiegel et al.

Discordant morphological and molecular evolution in testate amoebae.
Daniel Lahr et al.

Single cell transcriptomics of Oxymonads.
Martin Kolisko et al.

Phylogenomic investigation of the centrohelid heliozoans.
Fabien Burki et al.

There and back again: coverings evolution in centrohelid heliozoans.
Vasily Zlatogursky.

Reconstruction of chloroplast proteome of the earliest branching phototrophic euglenid, *Rapaza viridis* based on transcriptomic data.
Naoji Yubuki et al.

GO-7

(**Wednesday 9th, 12.00-13.30 h**).

Evolution/Phylogeny (III)

Chair: Sina Adl, Saskatchewan, Canada.

Special Mini-Symposium (**two talks**).

***Towards an integrated taxonomic and morpho-genetic reference system for eukaryotes* - 1. EukRef: Phylogenetically informed, bottom-up curation of eukaryotic 18S rDNA sequences.**
Javier del Campo et al.

***Towards an integrated taxonomic and morpho-genetic reference system for eukaryotes* - 2. UniEuk: A universal, expert validated taxonomic framework integrating reference gene databases for eukaryotic biology, ecology, and evolution.**
Cedric Berney et al.

New lineages of deep-branching predatory flagellates and their evolutionary significance.
Denis Tikhonenkov et al.

Evolution and Cellular Localization of Rhodoquinone Biosynthesis in *Pygsuia biforma* and Other Anaerobic Eukaryotes.
Courtney Stairs et al.

A phylogenomic framework for stramenopiles.
Romain Derelle et al.

The evolutionary origin of mitochondrial cristae from alpha-proteobacteria.
Sergio A. Muñoz-Gómez et al.

GO-8

(**Thursday 10th, 10.00-11.30 h**).

Environmental Microbiology (II)

Chair: Thomas Weisse, Mondsee, Austria.

Parasites diversity and abundance in the Tara-Oceans dataset challenge classic views on marine plankton ecology.
Cedric Berney et al.

A House for Two – Double Bacterial Infection in *Euplotes woodruffi* Sq1 (Ciliophora, Euplotia) Sampled in Southeastern Brazil.
Marcus V. X. Senra et al.

UV induces transfer of 16S rRNA fragments of the micronucleus-specific bacterium *Holospora undulata* to the host Paramecium nucleoli.
Masahiro Fujishima et al.

Soil protist diversity and community structure along a gradient of forest productivity in the temperate rainforest of British Columbia (Canada).
Thierry Heger et al.

Symbiosis in the cold: identification and characterization of a new *Francisella* endosymbiont from the polar ciliate, *Euplotes petzi*.
Adriana Vallesi et al.

***Paramecium* and its motile endosymbionts: for better and for worse.**
Elena Sabaneyeva et al.

GO-9

(**Thursday 10th, 12.00-13.30 h**).

Ecology - Barcoding

Chair: David J.S. Montagnes, Liverpool, UK.

Latitudinal gradient is of types and redundancy in planktonic protists.
John Dolan et al.

Three-dimensional structure of river biofilms in its importance for protozoans in biofilms.
Anja Scherwass et al.

Nature and specificity of diatom-bacteria interactions in marine intertidal sediments.
Koen Sabbe et al.

Chemotaxis response of phytoplankton to the exudates of ciliates.
Zhuo Shen et al.

The genetic structure of amoebae morphospecies – pattern in space and time.
Alexey Smirnov et al.

Diversity and abundance of diplonemids, a major planktonic component of the the world oceans, as revealed by the Tara Oceans meta-barcoding dataset.
Olga Flegontova et al.

POSTER SESSIONS

Due to the large number of posters communications received, three evening Poster Sessions of 1 h will take place:

- **Poster Session I, Monday 7th (18.00-19.00 h).**
- **Poster Session II, Tuesday 8th (18.00-19.00 h).**
- **Poster Session III, Wednesday 9th (15.00-16.00 h).**

Each poster will be assigned to a specific session of 1 h. It is the responsibility of the presenting author to ensure that at least one of the authors is present during that time, and that the poster is removed at the end of the session. Posters will be displayed in the lobby of the **Faculty of Biology – Red Building** and the entrance hall of the *Auditorium Maximum* (**Faculty of Computer Engineering**) throughout the duration of all meeting activities of the day.

TOPICS

Genomics/Molecular Biology	**Poster Session I**
Evolution/Phylogeny	**Poster Session I**
Cell Biology	**Poster Session II**
Taxonomy	**Poster Session II**
Ecology	**Poster Session II**
Physiology and Metabolism	**Poster Session III**
Barcoding	**Poster Session III**
Environmental Microbiology	**Poster Session III**
Parasitology	**Poster Session III**

POSTER SESSION I

(Monday 7th, 18.00-19.00 h).

Genomics/Molecular Biology

(***Entrance hall Auditorium Maximum, Faculty of Computer Engineering***)

1. **Gene expression analysis of metallothioneins and AP-1 transcription factors in experimentally adapted *Tetrahymena thermophila* strains to extreme metal stress. A model of gene expression coordination.**
Juan Carlos Gutiérrez et al.

2. **A draft genome of the anaerobic flagellate *Carpediemonas membranifera,* a free-living relative of metamonad parasites.**
Dayana Salas-Leiva et al.

3. **Complete nuclear genome sequence of *Goniomonas avonlea,* a plastid-lacking cryptomonad.**
Ugo Cenci.

4. **CRISPR/Cas9 gene disruption in *Trypanosoma cruzi:* an approach to study proteins involved in calcium homeostasis.**
Noelia Lander et al.

5. **Comparative analysis of chlorarachniophyte mitochondrial genomes; evolutionary insights from genome architecture and endosymbiotic gene transfer.**
Goro Tanifuji et al.

6. **Effects of global warming on growth and genetic diversity of marine ciliated protists.**
Rao Fu et al.

7. **Studies on nuclear and genomic biology of the dinoflagellate *Oxyrrhis marina.***
Susana Breglia et al.

8. **Intestinal epithelial cell-parasite cross-talk during giardiasis.**
Staffan Svärd et al.

9. **The dynamics of mitochondrial metabolism in a cercozoan capable of growth in aerobic and low-oxygen conditions.**
Ryoma Kamikawa et al.

10. **The guided entry of tail-anchored proteins pathway in *Giardia intestinalis.***
Vladimira Najdrova et al.

11. **Investigating miniSOG as a protein localization tool in oxygen-sensitive protozoan parasites.**
Victoria Morin-Adeline et al.

12. **Adaptation to a free-living lifestyle via gene acquisitions in the diplomonad *Trepomonas* sp. PC1.**
Feifei Xu et al.

13. **Genome Annotation and Analysis of *Holospora curviuscula*.**
Alexandra Beliavskaia et al.

14. **The chloroplast genome of Euglena *mutabilis* and evolutionary implications.**
Nadja Dabbagh et al.

15. **Searching for genes involved in mating type determination in selected species of *Paramecium aurelia* complex.**
Natalia Sawka et al.

16. **Getting inside in the *Acanthameoba – Legionella* relationship.**
Angela Magnet et al.

17. **Bacterial-like mitochondrial genome for planktonic foraminifera *Globigerinella aequilateralis.***
Chienhsun Chen et al.

18. **The kinome of the giant ciliate *Stentor*: over 2000 kinases and novel domain architectures.**
Sarah Reiff et al.

19. **Construction and expression of vector with *mip*/*pilE* advantages epitope genes of *Legionella pneumophila.***
Jinlei He et al.

20. **Comparative analysis of the *Monocercomonoides* sp. and *Trimastix pyriformis* - insight into the evolution of Metamonada.**
Anna Karnkowska et al.

21. **Diversification in diplomonads: reduction, acquisitions and genomic complexity.**
Jan Andersson et al.

22. **Comparative genomics of *Nephromyces* communities from tunicate renal sacs.**
Sergio A. Muñoz-Gómez et al.

Evolution/Phylogeny

(***Lobby, Faculty of Biology – Red Building***).

23. **Integrating morphological, ontogenetic and molecular data to evaluate the phylogeny of ciliates: a case study on the highly controversial order Urostylida (Protista, Ciliophora).**
Xumiao Chen.

24. **Resolving an ongoing debate whether subclass Peritrichia (Ciliophora, Oligohymenophorea) is monophyletic based on a multi-locus analysis.**
Zifeng Zhan et al.

25. **The genus *Cochliopodium* Hertwig et Lesser, 1874 (Amoebozoa, Discosea): phylogenetic relationships, current state of taxonomy and further challenges.**
Alexander Kudryavtsev et al.

26. ***Amoeboradix* spp. represent a highly divergent lineage of parasitic eukaryotes potentially related to fungi.**
Purificacion Lopez-Garcia et al.

27. **Systematics and evolution of the cell coat in amoebae of the genus *Korotnevella* (Amoebozoa, Discosea).**
Ilya Udalov.

28. **Pinpointing the root of extant eukaryotic diversity: advances, challenges and consequences.**
Laura Eme et al.

29. **Specific composition of the genus *Paramecium*.**
Ewa Przyboś et al.

30. **Development of new nuclear gene markers in Ciliates using gene capture and next-generation sequencing.**
Jiamei Jiang et al.

31. **Interclonal variability, autogamy, clonal life history, senescence and cohesion of histophagous species : *Tetrahymena rostrata*.**
Andrzej Kaczanowski et al.

32. **Exploring the mitochondrial genomes of Amoebozoa in search of novel molecular markers: the emergence of a new barcode for Arcellinida.**
Quentin Blandenier et al.

33. **Evolutionary significance of free-living *Preaxostyla*.**
Petr Táborský et al.

34. **Comparative genome analysis of pedinophyte plastids and the pedinophyte-derived plastids in two dinoflagellates *Lepidodinium chlorophorum* and strain MRD-151.**
Kounosuke Morita et al.

35. **The protein pheromone family of *E. petzi,* a psychrophilic and early branching *Euplotes* species.**
Adriana Vallesi et al.

36. **The phylogenetic analyses of animal pathogens in Enterobacteriaceae.**
Yanxia He.

37. **Expansion of the 'reticulosphere': diversity of novel branching and network-forming amoebae helps to define Variosea (Amoebozoa).**
Cedric Berney et al.

38. **Expanding the *Entamoeba* universe: new hosts yield novel ribosomal lineages.**
Alison Jacob.

39. **"Protist X": a novel anaerobic sister lineage to metamonads.**
Yana Eglit et al.

40. **Revising Amoebozoa systematics using phylogenomics from broad set of taxa.**
Seungho Kang et al.

41. **Protists with eye-like organelles: the dinoflagellate *Erythropsidinium*.**
Fernando Gómez et al.

42. **Comparative proteomics of perisymbiont and digestive vacuoles in *Paramecium bursaria*.**
Toshinobu Suzaki et al.

43. **Secondary structure of ITS transcripts in Spirostomatid Ciliates (Ciliophora, Heterotrichea): implications for structural evolution and phylogenetic reconstruction.**
Mann Kyoon Shin et al.

44. **Investigating the diversity and evolution of *Neoparamoeba* species and their kinetoplastid endosymbionts.**
Shannon Sibbald et al.

45. **Evolution of the "unconventional" O2-scavaging system in Diplomonads.**
Alejandro Jimenez-Gonzalez et al.

46. ***Phytomonas nordicus*: the monoxenous trypanosomatid descended from plant parasites.**
Alexei Kostygov et al.

47. **Molecular phylogeny of the family Ophryoscolecidae with emphasis on validity of the genus *Eodinium* (Entodiniomorphida, Ophryoscolecidae).**
Franciane Cedrola et al.

48. **Resting cyst morphology as a generic marker in the Tovelliaceae: a reassessment.**
Mariana Sofia Pandeirada et al.

49. **Multigene analysis of Archamoebae (Amoebozoa: Conosa) shows that Entamoebidae represents a deep lineage of the group.**
Tomáš Pánek et al.

50. **A combined 18S-28S rDNA dataset to elucidate higher-level relationships within Rhizaria, with focus on Endomyxa.**
Cedric Berney et al.

51. **Mapping the diversity of Metopida and revealing new marine anaerobic ciliates hosting prokaryotic symbionts.**
Johana Rotterová et al.

52. **Taxon-rich multigene phylogeny of the photosynthetic euglenoids (Euglenophyceae).**
Jong Im Kim et al.

53. **Tintinnids (Ciliophora, Spirotrichea, Choreotrichida) from China Seas: phylogenies based on three rDNA loci.**
Qianqian Zhang et al.

54. **Phylogenomic analysis of *Nassula* sp., *Nassula citrea,* and *Pseudomicrothorax* dubius provides high support for a nassophorean clade.**
Denis Lynn et al.

55. **Phylogenetic relationships within the class Heterotrichea (Ciliophora, Postciliodesmatophora) inferred from five molecular makers and morphological data.**
Noemi Fernandes et al.

56. **Comparison of ultrastructure and chemical composition of the cell wall of *Chlorella* in free-living and endosymbiotic conditions.**
Rina Matsumoto et al.

57. **Elucidating evolutionary relationships within Physaraceae (Amoebozoa) through a multilocus approach.**
Joaquina M. García-Martín et al.

58. **A lineage-defining cytoskeletal structure of parabasalian parasites makes use of proteins that resemble intermediate filament proteins.**
Harald Preisner.

59. **Genome annotation of *Acrasis kona*.**
Sanea Sheikh et al.

60. **Ultrastructure and phylogenetic position of a novel deep-branching kinetoplastid.**
Naoji Yubuki et al.

61. **Iron Sulphur cluster assembly systems in Cryptophyta, Haptophyta, Stramenopila, Alveolata and Rhizaria share common features.**
Jan Pyrih et al.

62. **Molecular phylogeny of peritrich ciliates (Ciliophora: Peritrichia), with emphasis on the rhabdostylids (Epistylididae).**
Roberto Júnio Dias et al.

POSTER SESSION II

(**Tuesday 8th, 18.00-19.00 h**).

Cell Biology

(***Entrance hall Auditorium Maximum, Faculty of Computer Engineering***).

63. **Toxicology research on ultrastructure of ciliates.**
Ying Chen et al.

64. **Study about the toxicity effects of the Hg^{2+} and Cd^{2+} on *Stentor coeruleus*.**
Xuan Wang et al.

65. **The actin network as a structural basis of the *Amoeba proteus* nucleus.**
Mariia Berdieva et al.

66. **Cytological characterization of *Euplotoides octocarinatus* (Carter, 1972) from Mexico, with data of its world geographic distribution and 18S rDNA sequence.**
Daniel Méndez Sánchez et al.

67. **Uncovering the mating locus in diatom *Seminavis robusta*.**
Petra Bulankova.

68. **Release of *Holospora*-like bacteria in different ciliate species.**
Sergei Fokin.

69. **The colorless cortical granule of oxytrichids (Ciliophora, Hypotrichida) represents a new extrusive organelle.**
Xinpeng Fan et al.

70. **Ion channels in dinoflagellates revealed by patch-clamping and analysis of transcriptomes.**
Ilya Pozdnyakov et al.

71. **Morphological Changes In The Cytostome-Cytopharynx Complex Of *Trypanosoma cruzi* Epimastigotes During Cell Division.**
Carolina de Lima Alcantara et al.

72. **3-dimentional analysis of dinoflagellate nucleus by electron microscopy.**
Chihong Song et al.

73. **Participation of ESCRT-III proteins in erythrophagocytosis of *Entamoeba histolytica*.**
Yunuen Avalos-Padilla et al.

74. **Investigation of the *Euglena gracilis* transcriptome and plastid proteome with a focus on plastid membranes evolutionary history and protein-targeting.**
Anna Vanclova et al.

75. **Twin-arginine translocase in the mitochondria of the eukaryotic organism *Naegleria gruberi*.**
Markéta Petru et al.

76. **Reduced version of bacterial secretion system in the mitochondrion of excavates.**
Lenka Horváthová et al.

77. **High-resolution DAPI-banded chromosomes of dinoflagellate *Prorocentrum minimum*.**
Mariia Berdieva et al.

78. **Anaerobic CIA pathway in *Trichomonas vaginalis*.**
Darja Stojanovová et al.

79. **Targeting of C-tail anchored proteins into hydrogenosomes and endoplasmic reticulum of *Trichomonas vaginalis*.**
Petr Rada et al.

80. **Synchronized and ER-associated division of *Giardia intestinalis* mitosomes.**
Luboš Voleman et al.

81. **The flagellar apparatus of *Cyanophora*.**
Aaron Heiss.

Taxonomy

(***Lobby, Faculty of Biology – Red Building***).

82. **The diatom flora of Naoli River Wetland in Northeast China.**
Fan Yawen et al.

83. **SEM studies on the morphology of certain marine planktonic dinoflagellates (Dinophyta) from Mexican Waters, including new species.**
David Hernández-Becerril.

84. **Comparison of cell division patterns in the Tintinnid genera *Favella* and *Schmidingerella* (Alveolata, Ciliophora, Spirotricha).**
Sabine Dr. Agatha et al.

85. **Diversity of *Pleuronema* spp. (Ciliophora, Scuticociliatia) in the Hangzhou Bay estuary, with reporting four new species.**
Hongbo Pan et al.

86. **Dinoflagellate taxonomy and classification, a proposal.**
Mona Hoppenrath.

87. **Diversity of the diatom genus *Frustulia* in northern Europe.**
Vojtech Scharfen et al.

88. **New taxa refresh the phylogeny and classification of Pleurostomatid ciliates (Protozoa, Ciliophora, Litostomatea).**
Lei Wu et al.

89. **Taxonomy and phylogeny of Karyorelictean ciliates.**
Yuan Xu et al.

90. **Molecular systematics of marine gregarine apicomplexans from Pacific tunicates: Linking surface ultrastructure and molecular phylogeny.**
Sonja Rueckert et al.

91. **Morphological taxa delimitation of Dinophysales (Dinophyceae), with emphasis on *Dinophysis* and *Phalacroma*.**
Carmen Zinssmeister et al.

92. ***Paulinella longichromatophora* sp. nov., a new marine photosynthetic testate filose amoeba containing the Chromatophore.**
Myung Gil Park et al.

93. **pH preferences in a diatom species complex.**
Pavla Urbankova et al.

94. **Morphology and molecular phylogeny of two new *Zoothamnium* species (Ciliophora, Peritrichia, Zoothamniidae) and a suggestion of guideline to describe Zoothamniidae.**
Ji Hye Kim et al.

95. **Phylogenetic species delimitation in the *Eunotia bilunaris|flexuosa* species complex (Bacillariophyta).**
Pieter Vanormelingen et al.

96. **Description and phylogenetic position of *Corlissina maricaensis* gen. nov., sp. nov. (Karyorelictea, Geleiidae), a new interstitial ciliate from Brazil, with redefinition of the family Geleiidae.**
Pedro Henrique Campello Nunes et al.

97. **Some details of the lorica aperture of *Lagenophrys discoidea* (Peritrichia: Lagenophryidae) with scanning electron microscopy, and notes on its geographic distribution.**
Rosaura Mayén-Estrada et al.

98. **Taxonomy, morphology and phylogeny of a new oligotrich ciliate - *Strombidium hongkongensis* n. sp. (Protozoa: Ciliophora) from Clear Water Bay, Hong Kong.**
Zhuo Shen et al.

99. **On the morphology of a new *Spirostomum* species (Ciliophora, Heterotrichea) from Brazil.**
Inácio D. da Silva-Neto et al.

100. **Vital species of *Flamella* (Amoebozoa: Variosea) isolated from ancient Arctic permafrost sediments.**
Lubov Shmakova et al.

101. **Comparison of morphological and molecular data between two population of *Strombidinopsis* minima (Choreotrichia:Ciliophora) of Korea.**
Sun Young Kim.

102. **Diversity and systematics of thecamoebid amoebae (Amoebozoa: Discosea: Thecamoebidae).**
Yelisei Mesentsev.

103. **Experimental and observational evidence of phenotypic plasticity on leidy's butterfly, *Hyalosphenia papilio*.**
Matthieu Mulot et al.

104. **Monograph of the family Euplotidae Ehrenberg, 1838 (Ciliophora, Spirotrichea).**
Helmut Berger.

105. **Rumen ciliates in Brazilian sheep, with new records and redescription of *Entodinium contractum* (Entodiniomorphida: Ophryoscolecidae).**
Roberto Júnio Dias et al.

106. **Diversity of ciliates in two Wastewater Treatment Plants in Rio de Janeiro, Brazil.**
Luiggia Girardi Bastos Reis de Araújo et al.

Ecology

(***Lobby, Faculty of Biology – Red Building***).

107. **Control of foraminifera by temperature, salinity and depth in the Yellow Sea sediments: a cross system comparison from intertidal zone to continental shelf.**
Yanli Lei et al.

108. **Response of benthic protist communities to macroalgal and giant jellyfish blooms: regime shift in seafloor ecosystems.**
Kuidong Xu et al.

109. **Is the altitude important for ciliates from tank bromeliads in Mexico?**
Victor Romero-Niembro et al.

110. **Gause was wrong: a practical use of functional and numerical responses.**
David Montagnes et al.

111. **Response of testate amoebae and plant communities to peatland restoration: implications for community concordance.**
Emmanuela Daza Secco et al.

112. **First record for the Americas of the giant ciliate *Loxodes rex*.**
Hunter N. Hines et al.

113. **Response of soil micro-eukaryotes to cadaver decomposition as assessed by high throughput sequencing.**
Monika K. Reczuga et al.

114. **Assessing the responses of peatland micro-eukaryotes to climate change using next generation sequencing.**
Monika K. Reczuga et al.

115. **Metabarcoding of soil eukaryotes – multiple applications for biodiversity assessment to applied ecological research.**
Christophe Seppey et al.

116. **Characterization of protist communities from granite weathering pits in a Spanish national park.**
Blanca Pérez-Uz et al.

117. **Diversity of ciliate communities in a human-impacted river at a Spanish national park.**
Pablo Quintela-Alonso et al.

118. **Obligate mixotrophy of the pigmented dinoflagellate *Polykrikos lebourae* (Dinophyceae, Dinoflagellata).**
Sunju Kim et al.

119. **Small freshwater ecosystems harbour complex protist communities characterised by seasonal dynamics and resilience.**
Ludwig Jardillier.

122. **MicroPolar - Diversity and dynamics of microbial eukaryotes in the Arctic.**
Egge Elianne Sirnæs et al.

123. **Exploring the health risk of pathogenic protists contamination in recreational waters of a protected area.**
Pablo Quintela-Alonso et al.

124. ***Trichodina domerguei cf. diaptomus.* in a warm-monomictic maar-crater lake: A vegetarian ectoparasite?**
Miroslav Macek et al.

125. **The impact of environmental changes on the diversity of symbiotic bacteria associated with ciliated protists.**
Jun Gong et al.

126. **When a lake stops mixing – the fatal effects of warming on the protistan community.**
Gianna Pitsch et al.

127. **Microaerobic scuticociliates in a saline monomictic maar-crater lake Alchichica (Mexico).**
Miroslav Macek et al.

128. **Ciliates community in the assessment of impacts on Neotropical streams.**
Luiz Felipe M Velho et al.

129. **Divergent patterns of common and rare taxa of planktonic ciliates and the influence of flood events in Neotropical floodplains.**
Luiz Felipe M Velho et al.

130. **Structure and dynamic of planktonic ciliates community along the only remaining dam-free stretch of a great tropical river.**
Luiz Felipe M Velho et al.

131. **The importance of herbivory by protists in a Neotropical floodplain system.**
Bianca Ramos Meira et al.

132. **Hydrological connectivity determining metacommunity structure of planktonic heterotrophic flagellates.**
Fernando Miranda Lansac-Tôha et al.

133. **Distribution of soil free-living amoebae' trophic groups around roots of *Zea mays* micorrized by *Glomus intrarradices*.**
Sandra Cortes Perez et al.

134. **Host-parasitoid interactions: *Parvilucifera sinerae* infecting toxic marine dinoflagellates.**
Elisabet Alacid et al.

135. **Epibiont ciliates on neotropical limnic gastropods: new records, composition and structure of the community assembly.**
Sthefane D`ávila et al.

136. **Changes in ciliate populations in an intertidal microbial community with chronic exposure to petroleum hydrocarbons on Prudence Island in Narragansett Bay, Rhode Island.**
Gaytha Langlois et al.

POSTER SESSION III

(**Wednesday 9th, 15.00-16.00 h**).

Physiology and Metabolism

(***Entrance hall Auditorium Maximum, Faculty of Computer Engineering***).

137. **Presence of three antioxidant-systems (GSH/GR, TRX(SH_2)/TRXR and TRY(SH_2)/TRYR) in *Tetrahymena thermophila:* an integrated view of the stress response to metal(oid)s.**
Ana Martín-González et al.

138. **Erythrins, new toxic metabolites from the eurialine ciliate *Pseudokeronopsis erythrina* used as chemical defense against predators.**
Andrea Anesi et al.

139. **Physiological role of mitochondrial calcium uniporter (MCU) in the causative agent of Chagas Disease, *Trypanosoma cruzi*.**
Miguel Angel Chiurillo et al.

141. **Chlorophyll catabolism generating cyclopheophorbide enols generated by autotrophic and heterotrophic euglenoids.**
Yuichiro Kashiyama et al.

142. **Mitochondrial pyruvate carrier in *Trypanosoma brucei*.**
Zdenek Verner et al.

143. **Similarities and differences of proteins involved in inorganic polyphosphate metabolism in bacteria and photosynthetic protists.**
Tomás Albi et al.

144. **Sodium-translocating membrane pyrophosphatases, a novel strategy for ionic homeostasis in photosynthetic marine protists.**
Juan Manuel Madroñal et al.

145. **Evaluation of biomass and fatty acid productivity of three microalgae for biodiesel production in continuous culture.**
Esperanza del Río et al.

147. **Mitochondrial respiratory chain of an oyster parasite *Perkinsus marinus*.**
Motomichi Matsuzaki et al.

Barcoding

(***Entrance hall Auditorium Maximum, Faculty of Computer Engineering***).

148. **Protistan nanofauna distribution in the mesoscale: species richness in 150 grassland soil samples.**
Paul Venter et al.

149. **Amoebozoa-specific genes as potential DNA barcodes to study environmental diversity of amoebae.**
Natalya Bondarenko et al.

Environmental Microbiology

(***Lobby, Faculty of Biology – Red Building***).

151. **Experimentally adapted *Tetrahymena thermophila* strains to extreme metal stress: differential and reversible *CdMT* gene amplification.**
Juan Carlos Gutiérrez et al.

152. ***Chlamydomonas acidophila:* a polyextremophile photosynthetic protist isolated from Tinto river with a high resistance to heavy metal(oid)s.**
Ana Martin Gonzalez et al.

153. **Candidate protozoa barcode genes in ecological research.**
Yan Zhao.

154. **Use of protozoa for assessing water quality in a mid-subtropical urban wetland ecosystem, southern China.**
Xinlu Shi.

155. **Land-use and climate factors drive soil ciliate diversity.**
Susana S. Santos et al.

156. **Apusomonad environmental surveys establish new clades in both marine and fresh water environments.**
Guifré Torruella et al.

157. **Accumulation of cesium in lipid droplets of *Paramecium bursaria*.**
Kyoko Nakata et al.

158. **Proteomic analysis of atrazine stress response in *Chlamydomonas reinhardtii*.**
Ángeles Cid et al.

159. **Morphology, phisiology and molecular phylogeny of two new halophilic heterolobosean amoebo-flagellates.**
Andrey Plotnikov et al.

160. **Centrohelid heliozoa from saline and brackish inland water bodies of Russia.**
Andrey Plotnikov et al.

161. **Benthic foraminifera as an indicator of environmental stress.**
Sergei Korsun et al.

162. **A novel bio-monitoring system with the heliozoon *Raphidiophrys contractilis* for continuously detecting toxic substances in water.**
Chisato Yoshimura et al.

163. **A comparison of some methods to quantify heterotrophic flagellates of different taxonomic groups.**
Alexandra Jeuck et al.

164. **Evaluation of the sensitivity to zinc of three most common benthic ciliates and their naturally associated bacteria from a polluted tropical bay.**
José Augusto Pires Bitencourt et al.

165. **Grazing of *Blepharisma americanum* on toxic and non-toxic *Microcystis aeruginosa* cells.**
Genoveva Esteban et al.

166. **Swarm v2: highly-scalable and high-resolution amplicon clustering.**
Micah Dunthorn et al.

167. **Proteome profiles of phytoplanktonic protist species discriminated by MALDI-TOF mass spectrometry to assess the aquatic ecological quality.**
Lucía Arregui et al.

168. **Biodiversity of protists and prokaryotes of two playa-lakes from Central Spain.**
Oscar Cabestrero et al.

169. **Testate amoebae associated to biological soil crust of an intertropical desert in Mexico.**
Horacio Perez et al.

170. **Free-living amoebae of an intertropical Mexican desert: driving the distribution of these communities in the soil?**
Horacio Perez et al.

172. **Global distribution and vertical patterns of a prymnesiophyte-cyanobacteria obligate symbiosis.**
Ana M Cabello et al.

173. ***Crytosporidium* and *Giardia* in raw and treated sludge from wastewater treatment plants.**
Yolanda Moreno et al.

174. **Free-living amoebae in water sources by PCR and sequencing in Spain.**
Yolanda Moreno et al.

Parasitology.

(***Lobby, Faculty of Biology – Red Building***)

175. **Alterations in the cytoplasmic structures and cyst wall during differentiation of *Giardia intestinalis.***
Victor Midlej et al.

176. **Intestinal parasitosis in relation to CD4+T cells levels and anemia among HAART initiated and HAART naïve pediatric HIV patients in Model ART Center, Addis Ababa, Ethiopia.**
Hylemariam Mihiretie Mengist.

178. **Characterization of the biological role of the multifunctional EhURE1-BP protein of *Entamoeba histolytica,* different to the transcription factor.**
Javier Cázares et al.

179. **Identification and subcellular localization of a putative sodium-calcium exchanger of *Entamoeba histolytica.***
Martha Iris Valle Solis et al.

180. **Functional characterization of the ABCG2 transporter from the protozoan parasite *Leishmania.***
Francisco Gamarro et al.

181. **Molecular and phylogeographic characterization of the infection by *Trypanosoma* spp, in cattle of Colombia.**
Jeiczon Jaimes.

182. **The prevalence of canine oral protozoa and their association with periodontal disease.**
Niran Patel et al.

183. ***Toxoplasma gondii* Mob1 sub-cellular localization and assessment of its potential role in parasite replication.**
Alexandre Leitão et al.

184. **Genetic diversity and classification of pathogenic bovine *Eimeria* species from Central Anatolia Region of Turkey based on 18S rRNA, ITS-1 and mt-COI genes.**
Alparslan Yildirim et al.

185. **Prevalence of bovine coccidiosis in Central Anatolia Region of Turkey and development of a real time PCR assay for detection of pathogenic *Eimeria* species.**
Onder Duzlu et al.

186. **Molecular characterization and expression of the apical membrane Antigen-1 (AMA-1) from *Babesia bigemina* Kayseri/Turkey strains.**
Arif Ciloglu et al.

187. **Possible phylogenetic position of the parasitic dinoflagellate *Syltodinium listii* Drebes (Dinophyceae, Gymnodiniales).**
Carmen Zinssmeister et al.

188. ***Plasmodium* (*Novyella*) *unalis* cf. and Plasmodium (*Haemamoeba*) *lutzi* in *Turdus* spp. (Passeriformes) of the Atlantic Forest in southeastern of Brazil: morphological and molecular characterization.**
Marta D´Agosto et al.

189. **Paramyxida: emergence of an enigmatic order of invertebrate parasites.**
David Bass et al.

190. ***Acanthamoeba*** **genetic diversity inside Drinking Water Treatment Plants.**
Angela Magnet Dávila et al.

191. **Oral trichomonads in cats and dogs.**
Pavlina Voborilova et al.

192. **A new reproducible method for fast and efficient conversion of Chinese *Leishmania* SC10H2 promastigote forms into amastigote forms *in vitro*.**
Jiao Li.

193. **Influence of *Mycoplasma hominis* on pathobiology of *Trichomonas vaginalis*.**
Pier Luigi Fiori et al.

194. **Kinetics of circulating antibody response to *Trichomonas vaginalis:* clinical and diagnostic implications.**
Paola Rappelli et al.

195. **Development of a drug selection system for transfection of the oyster parasite *Perkinsus marinus* (Alveolata).**
Hirokazu Sakamoto et al.

196. **Selection and characterization of scfv antibodies against *Pneumocytis jirovecii* from phage display libraries.**
Olga Matos et al.

197. **Testicular Myxosporidiasis and ultrastructural characteristics of *Myxobolus bufonis* (Myxobolidae) infecting the Egyptian Toad *Bufo regularis* (Bufonidae). A Light and Electron Microscopic Study.**
Kareem Morsy et al.

198. **Accumulation of some heavy metals in *Hysterothylacium aducum* (Nematoda, Anisakidae) infecting the common sole *Solae solae* (Soleidae) and its role as a biological indicator of pollution from Mediterranean sea, Egipt.**
Rewaida Abdel-Gaber.

ABSTRACTS

Plenary Lectures

CRYPTIC DIVERSITY IN ORGANISMS AND ORGANELLES

Graham C. Clark (London School of Hygiene and Tropical Medicine).

Over the past 35 years, the methodology available to investigate genetic variation between and within species has evolved dramatically. The application of these tools has been particularly beneficial to those of us who work on organisms that have limited morphological characters on which to base comparisons. In this lecture, I will use my own experiences to illustrate the changes in methods over this time period and the impact this has had on our understanding of protist relationships, concentrating on the genera *Naegleria, Entamoeba* and *Blastocystis.*

PHEROMONE SIGNALLING IN PROTISTS

Pierangelo Luporini (University of Camerino, Italy).

As it is common among multi-cellular life forms, also single-cell organisms use pheromones to communicate among members of the same species. In protists, pheromones have more extensively been studied for their biology and structure in ciliates, in which these signalling molecules have been identified in functional association with mating systems. Consistently with this association, they have for long time been regarded only as non-self sexual signals committed to elicit a mating response of cells to which they bind in heterologous (paracrine) fashion. Their spectrum of activity has however revealed wider borders. It also includes a self activity directed to promote the growth of the same cells from which pheromones are constitutively secreted throughout the cell life cycle and to which they continuously bind in autocrine fashion. This double self and non-self activity is made possible by the pheromone ability to compete with one another in cell binding reactions. In species of *Euplotes*, which synthesize pheromone families under the control of multiple series of alleles at a single locus, this ability is ensured by the relationships of the structural homology that link these signaling molecules into species-specific globular, disulfide-rich protein families. These relationships, extensively studied by means of NMR and crystallographic analyses of native protein preparations, are the main topic of this presentation.

PROTIST METABARCODING AND ITS APPLICATIONS

Jan Pawlowski (Department of Genetics and Evolution, University of Geneva).

High-throughput amplicon sequencing of environmental DNA and/or RNA offers a powerful tool to describe protist diversity. This new approach called also the eDNA metabarcoding has totally transformed our view of protist diversity, revealing a large number of novel lineages and expanding the range of protist phylogenetic diversity at almost every taxonomic level. However, the vast majority of metabarcoding studies have purely scientific objectives and the practical applications of environmental DNA surveys of protist communities are very limited. This may appear surprising given that several groups of protists are commonly used as bioindicators of environmental changes in freshwater or marine ecosystems.

It is well known that the traditional monitoring based on morpho-taxonomic inventories of protist communities is time-consuming, expensive and requires excellent taxonomic expertise. Yet, the capacity of eDNA metabarcoding approach to meet the quality standards of environmental bioindication is subject of controversy. Here, I will present our studies comparing the biotic indices inferred from morpho-taxonomic and molecular data for two groups of protists (diatoms and foraminifera). The results of these studies show that despite some biological and technical biases, molecular data quite faithfully reflect the morphology-based indices and provide a similar assessment of ecosystem status. Protist metabarcoding appear as a rapid and accurate tool for the evaluation of the quality of aquatic ecosystems that should be integrated in the future biomonitoring projects.

MOLECULAR AND CELL BIOLOGY OF ENTAMOEBA HISTOLYTICA PATHOGENESIS

Nancy Guillen (**Institut Pasteur, Paris, France**), **Roman Thibeaux**(**Institut Pasteur, Paris, France**), **Christian Weber** (**Institut Pasteur, Paris, France**), **Chung-Chau Hon** (**Institut Pasteur, Paris, France**).

Entamoeba histolytica is a single celled organism and the ethological agent of amoebiasis, an infectious disease targeting the human intestine and liver. Virulent and non-virulent *E. histolytica* strains colonize the human intestine. Deciphering the molecular bases accounting for virulence variability of *E. histolytica* is a crucial challenge to understand the molecular bases of amoebiasis. The genome of this parasite is marked by lateral gene transfert from bacteria; in particularly genes encoding important enzymes of metabolic pathways. To characterize the largely unexplored transcriptome of the unicellular parasite *E. histolytica,* we performed a comprehensive census on its RNA populations using high throughput sequencing. In this study, we focused on four aspects, including gene model revision, alternative splicing, antisense transcripts and small RNAs. We demonstrated the pervasive existence of antisense and small RNA transcripts that map to the 3' end of their sense counterpart. We then demonstrated the differential expressions of mRNA between two strains (virulent and non-virulent) are negatively correlated with that of the antisense small RNAs, suggesting the potential roles of these small RNAs in regulation of gene expression. In particular we highlighted enzymes involved in carbohydrate metabolism, glycolysis and in the regulation of glycosylated residues processing which were specifically upregulated in the pathogen strain only upon contact with human mucus. Gene expression blockage by RNA interference of one of these (β-amylase) abolishes mucus depletion by the virulent strain, suggesting a crucial role of this enzyme during mucus glycan foraging process. The pathogenicity of *E. histolytica* seems highly conditioned by parasite capacity to cleave and to use sugar moieties during invasion of the mucus layer barrier.

* This work is supported by a grant from the French National Research Agency (ANR-2010-GENM-011-01, GENAMIBE).

EVOLUTION AND DEVOLUTION IN ALVEOLATES: INVASION, ORGANELLES AND CHROMATIN

Ross Waller (University of Cambridge, UK).

Infrakingdom Alveolata comprises major, but seemingly disparate, eukaryotic lineages including apicomplexans, dinoflagellates and ciliates. Together these groups represent a wide array of cell forms and metabolic lifestyles, and occupy diverse environmental niches. Molecular phylogenies have crystallised our understanding of the relatedness of these organisms, and increasingly a wide range of structural, molecular and biochemical characters substantiate their common ancestry. More interestingly, these features provide an opportunity to study the transition between lifestyles such as predation, autotrophy and parasitism. These transitions often involve adaptation and repurposing of pre-existing cell features. The alveolate pellicle, for example, is a key innovation of this group that has provided a malleable platform for cell adaptation. Comprised of an inner membrane complex of vesicles and proteinaceous skeletal elements beneath the plasma membrane, in apicomplexans this structure has been adapted to enable gliding motility and invasion of host cells during the transition to parasitism. Some transitions result in redundancy, such as the presence of plastids after loss of photosynthesis. This transition has occurred multiple times in alveolates, and thus they provides independent opportunities to examine plastid reductive evolution. Finally, within Alveolata dramatic cases of divergent evolution are found where eukaryotic norms are seemingly discarded, although the driving force for such changes can be unclear. Dinoflagellate nuclei are highly unusual in that histones have apparently been abandoned as the major instruments for organising and regulating chromatin. Thus these cells provide a remarkable opportunity to consider alternative mechanisms of eukaryotic function, in this case chromatin function in the absence of histones.

PROTISTS AS BIOINDICATORS IN WASTEWATER TREATMENT: IDENTIFICATION, ECOLOGY, AND FURTHER NEEDS

Wilhelm Foissner (University of Salzburg, Austria).

Historically, one might recognize three periods for using protists as indicators in the activated sludge process. The age of Discovery and Exploitation may be set between 1914 and 1950 when Ardern & Lockett (1914) created the term activated sludge and several scientists recognized the importance of protists in the cleaning process. The age of Bloom may be set between 1950 and 2000. It commenced with the revision of the saprobic system and peaked with an experimental study by Curds et al. (1968), who showed with a few photographs the need of protists for a clean plant effluent. Now the field developed rapidly because several protistological groups dealt with activated sludge and practical indices were developed classifying the performance of sewage plants (Madoni 1994). Since the turn of the century, we are in a Period of Decline mainly beacuse most of the young biologists don´t like identification of microscopic organisms although very useful keys are available (Berger & Foissner 2003). Thus, molecular tools should be developed for identification. Further, I provide a Table showing what is indicated by certain species and communities. This field should be improved both by a more reliable identification of the organisms and statistical tools. Continuous control of the protozoan community can prevent sludge bulking and may greatly save money for sludge oxygenation because many protist species are excellent indicators for the amount of oxygen present in the sludge plant. Activated sludge is a unique habitat for particular species, often undescribed, to develop in considerable numbers so that their morphology can be studied easily. As an example, I present two new species. The first is a new *Phialina* that is unique in having the contractile vacuole slightly posterior to mid-body. The second is a new, minute (~30µm) *Metacystis* that makes an up to 500µm-sized mucous envelope mimicking a sludge floc. (Supported by the FWF, Project 26325-B16 and the Spanish organizers of ECOP VII.)

For Literature, see review in the European Journal of Protistology.

CILIATE RESEARCHES IN CHINA: ACTIVE GROUPS, CHANCE FOR COLLABORATION AND THE ON-GOING STUDIES

Weibo Song (Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao, CHINA).

The Chinese Society of Protozoology (CSOP) was set up in 1981 and has currently over 350 members who belong to over 50 groups covering almost all directions of protozoological fields. Among them, about 3/5 are working on ciliate studies which can be tracked back to early 20th century, a long history compared to the short period of scientific researches in China. However, the bloom of the development regarding their membership and activities only started from the middle 1980's. From then a conspicuous development has been achieved: the number of research groups arose from 4 to the current over 25 teams with more than 40 PIs working on different disciplines of ciliatology, e.g. biodiversity and taxonomy, cytology and physiology, genetics, ciliate-based molecular biology, evolution and systematics, parasitology, ecology and environmental biology. Yearly over 100 papers have been published in international peer-reviewed journals. As a summary, the most significant achievements in last decades and on-going work performed by Chinese colleagues are: (1) the fauna and biodiversity studies on free-living ciliates in all kinds of habitats off China seas, including the current work on the tropical and subtropical biotopes, which documents totally over 900 species with over 20% new taxa identified including many new orders, families and genera; (2) studies on the cell development and morphogenesis of hypotrichs which make up over 2/3 of reports all over the world; (3) setting up the DNA bank of ciliates with over 1000 marine species/populations in last ten years; together with submission of over 2500 new sequences of 4 mark genes (18S, 5.8S, 28S, alpha-tubulin) from about 900 species representing over 23 orders; (4) field ecology and environmental biology along the coastal area, sedimentary sites, pelagic as well as maricultural waters in China seas; (5) phylogenetic analyses on main ciliate lineages at about order/class levels; (6) evolutionary genomics & phylogenomics, gene function/evolution, transcriptomic analyses of *Tetrahymena* and other test or non-test organisms; (7) epi-genetics, genome re-arrangement and nuclear development in *Tetrahymena*; (8) ultrastructure, cell physiology & nuclear apoptosis of *Paramecium*, and (9) studies on parasitic forms in marine and fresh water fishes and other economic organisms.

FUNCTIONAL ANALYSIS OF ACIDOCALCISOMES: ORGANELLES CONSERVED FROM BACTERIA TO HUMAN CELLS

Roberto Docampo (University of Georgia, USA).

Acidocalcisomes are acidic calcium stores that have been found from bacteria to human cells. They are rich in phosphorus compounds in the form of orthophosphate, pyrophosphate, and polyphosphate (polyP) and their acidity are maintained by proton pumps such as the vacuolar proton pyrophosphatase, the vacuolar proton ATPase, or both. It has been claimed that acidocalcisomes could be present in all domains of life, including archaea, and may thus date back as far as to the last universal common ancestor. On the other hand, acidocalcisomes of trypanosomes share characteristics with organelles known as lysosome-related organelles (LROs), such as human platelets dense granules and mast cell granules, which are also considered acidocalcisome-like organelles. This does not necessarily suggest a different origin of acidocalcisomes in eukaryotes but a potential further adaptation in these cells. The point here is that similar membrane-bounded polyP-containing acidic calcium stores are present in both prokaryotes and eukaryotes and they could have appeared either autogenously or by convergent evolution. Recent studies in trypanosomatids and in other species have revealed their role in phosphate metabolism, and cation and water homeostasis, as suggested by the presence of novel pumps, transporters, and channels. The presence of calcium uptake (calcium ATPase) and calcium release (inositol 1,4,5-trisphosphate receptor) mechanisms suggests an active role of acidocalcisomes of *Trypanosoma brucei* in calcium signaling. An important role in autophagy has also been described. The study of the biogenesis of acidocalcisomes as well as of the interactions of these LROs with other organelles have uncovered important roles in calcium signaling and osmoregulation.

ABSTRACTS

Oral Presentations

ENDOSYMBIOTIC ALGAE OF THE CILIATES EUPLOTES DAIDALEOS, FRONTONIA SP. AND PARAMECIUM BURSARIA

Undine Achilles-Day (SAMS / Bournemouth University).

The ciliates *Paramecium bursaria, Euplotes daidaleos* and *Frontonia* sp. are well known for their endosymbiotic relationships with small coccal green algae. These relationships are mutually beneficial to the partners and many attempts have been made to unravel how these develop. Endosymbiosis is a strategy in which novel biochemical functions can be acquired; in this case the photoautotrophic capacity to fix carbon dioxide is enhanced (Kamako & Imamura, 2006). In recent years there has been a resurgent increase of interest in these relationships, as endosymbiosis is now seen as a major driver in evolution through horizontal gene transfer.

However, it is challenging to establish pure cell lines of the endosymbiont(s) in culture. In addition, it has been extremely difficult to find a reliable method to identify these organisms and to confirm whether they are true photobionts or facultative symbionts. Using micromanipulation and enrichment approaches algae cell lines have been established for some taxa. A polyphasic approach has been employed using phenotypic characters combined with genotypic analyses employing a barcoding method using SSU and ITS genes. This approach has confirmed that relationships are significantly more complex than was initially thought and a range of taxa belonging to different genera of green algae have been identified in this study.

Kamako S & Imamura N (2006) Effect of Japanese *Paramecium bursaria* extract on photosynthetic carbon fixation of symbiotic algae. Journal of Euk. Microbiol, Vol. 53: 2: pp 136-141.

PROTISTS HOLD A CENTRAL REGULATOR ROLE IN SOIL ECOSYSTEM NUTRIENT CYCLING

Sina Adl (University of Saskatchewan).

Protists in soil are very diverse and the dominant bacterivores in the system. However, they are competing for resources in a variety of other functional groups as well. These include fungivory, cytotrophy, and predation on invertebrates. Studies with functional response curves and stable isotopes, or respiration studies, have indicated they hold a central role in the soil microbial food web as they are known to do in marine systems. Few modern studies exists that have quantified these contributions due to technical problems caused by the soil environment. However modern approaches are beginning to show us a glimpse of the diversity of functions and to quantify these trophic interactions.

NUMERICAL/FUNCTIONAL RESPONSE AND PREY SELECTIVITY IN HETEROTROPHIC VS MIXOTROPHIC PROTISTS

Ruth Anderson (**Marine Biological Section, University of Copenhagen**), **Per Juel Hansen** (**Marine Biological Section, University of Copenhagen**).

`Black box´ approaches, which group organisms into functional units assuming uniform behavior, are widely used to understand predation processes at a community level in the study of aquatic systems. For strictly heterotrophic protists feeding on bacteria or other protists, ignoring interspecies predation variability has long been known to be problematic, especially when making ecosystem level predictions based on the obtained rates. However, an equally concerning aspect, which has received considerably less attention, is the assumption that the phagotrophic activity of mixotrophic protist can be treated as equal to that of their heterotrophic counterparts. For example, is it valid to extrapolate their daily impact on prey standing stocks from hourly ingestion rates as is routinely done for heterotrophic protists, disregarding any potential day/night variability in mixotrophic predation? To be able to answer such questions we need to know whether mixotrophic protists deviate consistently and predictably from heterotrophs in such aspects as their functional and numerical responses (the change in ingestion and growth rates with increasing prey abundance, respectively); how they select and impact their prey; or how their predation activity responds to changes in environmental parameters. Here, we will take a close look at what we know from cultured mixotrophic and heterotrophic representatives to assess how close we are to being able to properly answer these questions and we will pinpoint vital avenues for future studies.

N-ACETYL-L ORNITHINE DEACETYLASE IS AN ESSENTIAL FACTOR FOR ADAPTATION OF THE PARASITE ENTAMOEBA HISTOLYTICA TO NITROSATIVE STRESS

Preeti Shahi (**Technion**) , **Meirav Gefen** (**Technion**), **Shruti Nagaraja** (**Technion**), **Shai Vanunu** (**Technion**), **Amit Avrahami** (**Technion**), **Serge Ankri** (**Technion**).

Entamoeba histolytica is a protozoan parasite responsible for amebiasis, a disease which is characterized by acute inflammation of the colon. As part of host innate immune response, the release of reactive nitrogen species at micromolar concentration by phagocytes exerts it toxic effect by S-nitrosylating key metabolic enzymes and by fragmentation of endoplasmic reticulum (ER). Adaptation to this toxic level of nitric oxide (NO) is an important feature for successful survival of parasite within the host. To obtain insight into NO adaptation in *E. histolytica,* trophozoites were selected in vitro by stepwise exposures to increasing amounts of NO donor S-Nitrosoglutathione (GSNO) up to 110 µM concentration. Phenotypic characterization of these adapted trophozoites (GSNO trophozoites) showed more resistance to acute exposure of GSNO (350µM), to activated macrophages and better capability to invade porcine colon explants compared to wild type trophozoites. Among the 208 genes that were up-regulated in GSNO-trophozoites was a gene that encodes acetyl ornithine deacetylase (NAOD). NAOD catalyzes the deacylation of N2-acetyl-L-ornithine to yield ornithine and acetate. Immunofluorescence confocal microscopy using NAOD antibody showed that NAOD has a cytoplasmic location in wild type trophozoites. However, NAOD accumulates within cytoplasmic vacuoles that are reminiscent to ER in GSNO trophozoites. We found that overexpression of NAOD confers a selective advantage to trophozoites exposed to increasing amount of GSNO. This selective advantage was also observed when a mutated form of NAOD, devoid of its catalytic activity, was overexpressed. Taken together, these results link the ability of the parasite to adapt to toxic amount of NO and to colonize colon tissue. In addition, this work identifies NAOD as a virulence factor involved in the parasite adaptation to nitrosative stress. This new function represents a potential target for the chemotherapy of amoebiasis.

BAIKAL PLANKTON DINOFLAGELLATES AS CASES OF RECENT DIVERSIFICATION AND RADIATION

Nataliia Annenkova (**Limnological Institute Siberian Branch of Russian Academy of Science**), **Gert Hansen** (**Marine Biological Section, Department of Biology, University of Copenhagen**), **Øjvind Moestrup** (**Marine Biological Section, Department of Biology, University of Copenhagen**), **Karin Rengefors** (**Aquatic Ecology, Department of Biology, Lund University**).

Mechanisms which promote the diversification of free-living protists have to date been insufficiently studied. How fast the speciation can be, how fast microeukaryotes disperse, what type of isolation is more important? Studies of recently diverged free-living protists can help with understanding of these questions. We observed plankton dinoflagellates, in particular two endemic species, from the largest lake in the wold (Lake Baikal). Both single-cell PCR analyzes of various DNA markers and morphological observations were used. We found that *Gymnodinium baicalense* diverged relatively recently from the Arctic *Gymnodinium corollarium*. More complex story is associated with *Peridinium baicalense.* This dinoflagellate is included in "aciculiferum/hangoei complex" with two other Baikal species, cosmopolite freshwater P. *aciculiferum,* Arctic *Scrippsiella hangoei* and Antarctic *Scrippsiella aff. hangoei.* The complex appears to originate via recent adaptive radiation. Its members are mostly genetically identical but have different morphology. Laboratory tests shown that while freshwater species (*G. baicalense, P. aciculiferum*) do not survive at more than 3 ppt, their marine relatives (*G. corollarium, S. hangoei*) have wide salinity tolerance. Moreover we found *S. hangoei*-like dinoflagellate in freshwater Baikal, though it was known as marine-brackish. Marine-freshwater boundary is an important colonization barrier and for the majority of species such transitions occurred a long time ago. Certain Baikal features are similar to marine, but it is freshwater and located at the center of Eurasia. We suggested that the existence of the Baikal protists with close marine relatives is explained by geological history of the region. Dramatic changes have occurred in the lake during the last cooling period and many pelagic species were extinct and certain ecological niches in the Baikal plankton may therefore have been empty. This "ecological factor" may have helped marine/brackish ancestors adapt to the Lake Baikal habitat even if the salinity was not optimal for them. Deeper investigations of these dinoflagellates will help in understanding protist local adaptations and why some protists radiated (like aciculiferum/hangoei comlex) while others are not (like *G. corollarium*). More attention should be paid on benthic Baikal protists: to determine if they belong to endemic species flocks and what is the age of their diversification.

ON THE FUNCTIONAL ECOLOGY OF HETEROTROPHIC FLAGELLATES AND THE COMPLEXITY BEHIND

Hartmut Arndt (University of Cologne, Biocenter, Cologne, Germany).

Heterotrophic flagellates are known as most important grazers on bacteria in aquatic ecosystem. Studies in the course of the last decades have shown that heterotrophic flagellates cannot be treated as a black box. Flagellate communities generally contain very diverse species significantly differing in their feeding behaviour and other ecological properties. The differences regarding the feeding mechanisms can be tremendous and conceptual models describing general phenomena might be suitable for one group but not applicable for other groups. The dominant taxonomic groups among heterotrophic nano- and microflagellate communities within different marine, brackish and limnetic pelagic communities (heterokont taxa, dinoflagellates, choanoflagellates) and benthic communities (euglenids, bodonids, bicosoecids, cercomonads) will be compared. Recent studies of flagellate feeding processes indicated that there are significant species-specific differences regarding the food uptake and food selection. Individual and temporal variability will be discussed as well as the prevailing feeding modes, the considerable importance of slight deviations in the time budgets of feeding phases, the ingestion rates and the feeding microhabitat. The consequences of non-linear dynamics will be illustrated. Finally, the variable complex top-down pressure exerted by flagellates on bacteria and other food items will be considered.

SOIL MICRO-EUKARYOTES: DIFFERENT PERSPECTIVES ON A DIVERSE AND HIGHLY PARTITIONED BIOME

David Bass (NHM/Cefas), Alessandra duPont (NHM), Serena Thomson (NHM/Warwick University), Sigrid Neuhauser (University of Innsbruck), Jeff Silberman (University of Arkansas), Matthew Brown (Mississippi State University), Robert Griffiths (CEH Wallingford), Thomas Bell(Imperial College London).

Microbial eukaryotic diversity in soils remains relatively understudied, but abounds with a large functional diversity of organisms, including lineages with strong positive associations with different substrate types, plants, and with other life forms. Many interesting groups are genetically divergent and/or heterogeneously distributed so are often overlooked by general molecular diversity studies. Targeted and functionally-inspired molecular studies, including high throughput sequencing (HTS) methods constitute a flexible toolkit for understanding these systems without the need for cell isolation or culturing. This talk focuses on three systems: an investigation of indicators of micro-eukaryote community differences between soils of high, medium, and low pH; fruiting amoebae and flagellates in a divergent and diverse radiation of rhizarian coprophiles, including *Helkesimastix, Guttulinopsis,* and *Rosculus;* and plasmodiophorid-plant interactions in rhizosphere and bulk soil samples from wheat – oilseed rape rotations.

PARASITES DIVERSITY AND ABUNDANCE IN THE TARA-OCEANS DATASET CHALLENGE CLASSIC VIEWS ON MARINE PLANKTON ECOLOGY

Cedric Berney (CNRS / UPMC Paris 6, UMR7144, Station Biologique de Roscoff, France), Nicolas Henry (CNRS / UPMC Paris 6, UMR7144, Station Biologique de Roscoff, France), Stephane Audic (CNRS / UPMC Paris 6, UMR7144, Station Biologique de Roscoff, France), Patrick Wincker (CNRS / Université dEvry, UMR8030, Genoscope, Evry, France), Colomban de Vargas (CNRS / UPMC Paris 6, UMR7144, Station Biologique de Roscoff, France).

Surveys of marine plankton biodiversity have so far been geographically restricted or have not accounted for the full range of plankton size. As part of the Tara-Oceans project, ~800 million eukaryotic metabarcodes (the V9 region of 18S rDNA) were sequenced from >300 size-fractionated plankton communities collected across tropical and temperate oceans, allowing for the first time an assessment of the entire eukaryotic biodiversity across a whole planetary biome. Eukaryotic V9 rDNA diversity was shown to saturate at ~150,000 operational taxonomic units, the large majority of which (85%) belonged to protists. About one-third of the OTUs could not be assigned to known eukaryotic groups, but diversity emerged at all taxonomic levels, both within the groups comprising the ~11,200 catalogued morphospecies of eukaryotic plankton and among twice as many other deep-branching lineages of hitherto unappreciated importance in plankton ecology studies. Using a reference 18S rDNA database derived from the Protist Ribosomal Reference (PR2) database, we assigned broad ecological functions and basic trophic and/or symbiotic modes to taxonomically assigned metabarcodes. Strikingly, two thirds of these belong to poorly known groups of heterotrophic protists, including a huge variety of taxa known to live in some form of symbiosis, from mutualism to parasitism. Eukaryotes appear to play a fundamental role in structuring the plankton network, and, overall, biotic and positive interactions (in particular through parasites) are significantly more prevalent than abiotic and/or negative interactions. This talk will present our current work to better understand this surprisingly high diversity of parasites in the plankton. In particular, we are using OTU abundance profiles for known parasitic lineages and metabarcodes co-occurrence networks to identify new candidate parasitic lineages among the taxonomically poorly assigned eukaryotic plankton diversity. The overarching goals of our effort are (i) to generate an exhaustive database of putative parasite lineages and OTUs in the worlds marine plankton, their distribution and abundance, and their predicted hosts, and (ii) using our extensive experience in the design of lineage-specific PCR primers for divergent eukaryotes, to create protocols of molecular detection for selected taxa of key ecological or economical importance.

TOWARDS AN INTEGRATED TAXONOMIC AND MORPHO-GENETIC REFERENCE SYSTEM FOR EUKARYOTES – 2. UNIEUK: A UNIVERSAL, EXPERT VALIDATED TAXONOMIC FRAMEWORK INTEGRATING REFERENCE GENE DATABASES FOR EUKARYOTIC BIOLOGY, ECOLOGY, AND EVOLUTION

Cedric Berney (**Station Biologique de Roscoff**), **Sina Adl** (**University of Saskatchewan**), **Stephane Audic**(**Station Biologique de Roscoff**), **Guy Cochrane** (**EMBL-EBI, Cambridge**), **Frank-Oliver Glöckner** (**Max Planck Institute for Marine Microbiology**), **Eunsoo Kim** (**American Museum of Natural History**), **Laura Wegener-Parfrey** (**University of British Columbia**), **Pelin Yilmaz** (**Max Planck Institute for Marine Microbiology**), **Colomban de Vargas** (**Station Biologique de Roscoff**).

We present EukRef and UniEuk, two highly complementary, community-based initiatives to address one of the greatest challenges faced by protistology in the new age of environmental meta-barcoding/-genomics/-transcriptomics. Current massive DNA sequencing of our planet's ecosystems, from the smallest cells to animals, is teaching us a fundamental lesson: most of the Earths biodiversity belongs to the least studied compartment of life, microbial eukaryotes. Yet this novel genetic information can only be understood from a functional, ecological, and evolutionary point of view if it is linked to the phenotypic (morphological, physiological, ecological) information of the organisms it comes from, and integrated into a coherent, morpho-genetic taxonomic framework serving as a universal language for protistologists. The next few years represent a critical time to build up a bridge between the centuries-old body of morphological and physiological knowledge of microbial eukaryotic diversity and the current deluge of novel environmental eukaryotic sequence data.

We propose to achieve, within the next three years, a morpho-genetic reference system for eukaryotic biology, ecology and evolution. It includes two major components, fully integrated but representing separate, stand-alone end-products that will be made publicly available to the scientific community and other end-users. On one hand, a standardized curation process realized by active members of the protistology community (predominantly PhD students and post-docs) will generate phylogenetically-informed reference databases of curated genetic markers with reference alignments and trees. The EukRef initiative is driving this bottom-up process for the 18S rRNA gene. On the other hand, the UniEuk initiative will structure the generated knowledge into a 'universal' taxonomic framework, integrating classical morphology-based data and information from relevant genetic markers, with top-down validation by a comprehensive network of taxonomy experts. The system will be self-sustainable and its broad use and long-term preservation will be achieved by direct implementation into the EMBL-EBI data portal, initially as a complementary choice to the NCBI taxonomy.

Overall, the EukRef/UniEuk endeavor will provide a much-needed common language for the fast-growing protistology community, bridging the profusion of novel protistan genetic data from environmental -omics to classical protistology knowledge that has effectively linked taxon names to morphological, physiological, behavioral, and ecological information.

EXPANDING OUR KNOWLEDGE ABOUT PARASITES USING PATHOS-DB (THE PARASITE–HOST-DATABASE)

Marit F. M. Bjorbækmo (**University of Oslo**), **Ralf S. Neumann** (**University of Oslo**), **Arthur A. B. Haraldsen** (**University of Oslo**), **Kamran Shalchian-Tabrizi** (**University of Oslo**).

In order to identify and gain knowledge about protistan parasites we have developed the ParasiTe-HOSt-DataBase (PATHOS-DB). This database can also be used to obtain information about what hosts protistan parasites have been found associated with – or the other way around, to obtain information about different parasites associated with a specific host. We have applied PATHOS-DB to examine what is known about protistan parasites (and their host-associations) in general, and also to analyse environmental sequence datasets.

The massive numbers of sequences generated through various high-throughput sequencing projects over the recent years have revealed that there is a huge diversity of protists in all environments investigated. To expand our knowledge beyond the mere number of 'species' occurring in an environment and to interpret ecological functions is a demanding task.

Here we demonstrate how we used PATHOS-DB to obtain information about the abundance and distribution of protistan parasites in environmental samples from marine and lacustrine sediments in the inner Oslofjord. In addition, we show that using PATHOS-DB in combination with a phylogenetic approach revealed unknown putative parasites and can be used to infer host-association for several OTUs in selected taxonomic groups.

EXPLORATION OF THE DEVELOPMENTAL PROGRAM OF THE SOCIAL LIFE CYCLE IN COPROMYXA PROTEA (TUBULINEA, AMOEBOZOA) USING ULTRA LOW INPUT RNASEQ

Alexander Tice (Department of Biological Sciences and Institute for Genomics, Biocomputing, & Biotechnology, Mississippi State University, MS, USA), Matthew Brown (Department of Biological Sciences and Institute for Genomics, Biocomputing, & Biotechnology, Mississippi State University, MS, USA).

The evolutionary innovations of multicellularity have occurred many times on the eukaryotic tree of life. In Amoebozoa, two distinct, distantly related, lineages contain socially multicellular organisms, the well-studied dictyostelids and the lesser-known copromyxids. *Copromyxa protea* is a dung inhabiting sorocarpic amoeba that forms simple but macroscopic fruiting structures composed of a single cell type. The formation of sorocarps is induced by one or a few founding amoebae, which by an unknown mechanism entice nearby trophozoites to crawl upon them and subsequently encyst causing apical growth of the sorocarp. Using time-lapse microscopy as well as the most cutting edge methods in single/few cell transcriptomics, we are now able to begin to unlock the developmental program in this social slime mold. In this study, cells at the discrete developmental stages were picked using platinum needles and lysed. Messenger RNA was reverse transcribed from the whole cell lysate and an Illumina primed library was constructed and sequencing using the Illumina HiSeq platform. We sampled three distinct life stages with three replicates of each stage. We identified ~2,200 differentially expressed transcripts at a p-value cutoff of 0.001 using differential expression profiling techniques. Of these transcripts, ~100 represent transcripts unique to early sorocarpic formation and ~200 are uniquely expressed in the apical tips of maturing sorocarps. Further, a large set of transcripts is up regulated in both the early sorocarp and maturing sorocarp samples as would be expected given the life cycle of *C. protea*. Gene ontology enrichment analyses show a significant upregulation of transcripts associated with signal transduction in this set of shared transcripts between early sorocarps and sorocarp tip stages. These analyses also illustrate numerous transcripts are down regulated in the transition from trophozoites to aggregating and encysting amoebae. These data demonstrate the effective use of low input RNAseq library production for use in examination of developmental stages in protists.

REVOLUTIONIZING FUNCTIONAL STUDIES IN PLASMODIUM FALCIPARUM WITH CRISPR/CAS

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Plasmodium falciparum pathogenesis relies on monoallelic expression of one of 60 *var* genes, which encode the antigenic surface molecules that decorate infected erythrocytes. *P. falciparum* erythrocyte and chronic infection depend on immune evasion via antigenic variation. Studying the regulation of this process has proven difficult, as conventional methods for direct genetic manipulation of *P. falciparum* were inefficient. To elucidate mechanisms of transcription regulation, we employ the newly developed CRISPR/Cas9 system of genome editing to perform gene knockouts, epitope tagging, and marker-free single amino acid mutations. We are applying this system to the investigation of genetic elements that could potentially regulate *var* gene transcription. Each *var* gene contains a conserved intron, which has been implicated in previous studies in both activation and repression of transcription via several epigenetic mechanisms including interaction with the *var* promoter, production of lncRNAs, or localization to repressive perinuclear sites. However, functional studies have relied on artificial expression constructs. Using the CRISPR/Cas9 system, we directly delete the var2csa endogenous intron, resulting in an intron-less *var* gene in a natural, marker-free chromosomal context. Deletion of the var2csa intron resulted in stable monoallelic expression of the var2csa gene in ring stage parasites but did not affect the normal temporal regulation and subsequent transcriptional silencing of the *var* gene in trophozoites or schizonts. These data suggest that the intron is required for silencing in ring stages, but not for maintaining the counting mechanism of monoallelic expression as was suggested previously. Ongoing studies apply the versatile CRISPR/Cas9 system to additional *var* gene introns as well as other genetic elements implicated in *var* gene regulation. In addition, we are developing inducible and enzymatically dead Cas9 experimental systems. These Cas9-based tools have the potential to greatly advance the study of parasites and other non-model organisms.

CLIMACOSTOL, A CILIATE SECONDARY METABOLITE WITH ANTICANCER ACTIVITY: RESULTS FROM IN VITRO AND IN VIVO STUDIES

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Climacostol is a natural toxic secondary metabolite isolated from the freshwater ciliate *Climacostomum virens* and belongs to the group of resorcinolic lipids (or alkylresorcinols). Climacostol is stored in ejectable organelles (extrusomes) and it is naturally used by the ciliate for its chemical defense against predators. In addition, it was recently shown that climacostol exerts a potent antimicrobial activity against a panel of bacterial and fungal pathogens, and that it inhibits the growth of mammalian tumor cell lines in a dose-dependent manner by inducing apoptosis via intrinsic pathway. Climacostol is also able to exert a prooxidant effect, inducing plasmid DNA strand breakage and eukaryotic DNA damage in presence of Cu(II) ions. In order to expand these data and due to the availability of the synthetic toxin, we have further analyzed different tumoral and non-tumoral cell lines of the anticancer action of this compound. Cytometry analyses on the B16-F10 mouse melanoma cells indicated that cell proliferation was effectively inhibited by climacostol, with a significant increase of apoptotic cells. The data collected prompted us to investigate the effects of climacostol on in vivo melanoma progression using a B16-F10 allograft transplantation tumor model. The results indicate that climacostol decreased tumour growth and increased the content of apoptotic cells, suggesting that the toxin may be considered for the design of cytotoxic and pro-apoptotic new drugs for melanoma therapy.

PHYLOGENOMIC INVESTIGATION OF THE CENTROHELID HELIOZOANS

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Together with Radiolaria, Heliozoa is a diverse group of protists characterized by radiating cellular projections called axopodia. Both groups are polyphyletic, with almost all main radiolarian and heliozoan lineages having been placed in different parts of the eukaryotic tree. One notable exception is the centrohelid heliozoans, the last large axopodia-bearing assemblage that remains of enigmatic evolutionary origin. Centrohelids are predatory protists very common in freshwater and soil habitats, and can also occur widely in marine environments. Phylogenies based on the 18S rRNA have notoriously failed to infer the evolutionary relationships of centrohelids to other eukaryotes, even the use of multiple protein-coding genes was unsuccessful at pinpointing a robust origin. Thus far, however, only one small transcriptome dataset for centrohelids is available, leading to 2 main shortcomings in the phylogenetic reconstructions: 1) the centrohelid diversity was not captured in earlier multigene-based inferences; 2) earlier multigene alignments remained very gappy for the centrohelids. To address the important question of the phylogenetic origin of centrohelids, we have generated large transcriptome datasets for 4 species, namely *Raphidiophrys heterophryoidea, Raineriophrys erinaceoides,* and 2 yet undescribed species *Acanthocystis* sp. and *Choanocystis* sp. Importantly, these 4 species correspond to 4 of the main centrohelid lineages, thus dramatically increasing both the diversity and the size of the genomic-scale datasets. Here, I will present the result of our eukaryote-wide phylogenomic investigation including 150 operational taxonomic units (OTUs) and 250 genes. In addition to the four newly sequenced species, we took advantage of the recent release of transcriptome datasets for a very large diversity of marine microbial eukaryotes (MMETSP) to fill important sampling gaps across the tree. Our analyses unambiguously demonstrate that centrohelids share a common origin with haptophytes, thus providing a long-awaited answer to an evolutionary enigma and bringing us one step closer to a fully resolved eukaryotic tree of life. I will discuss the implications of the phylogenetic position of centrohelids, notably in the broader context of plastid evolution.

GENOMES OF MONOXENOUS SPECIES SHED LIGHT ON THE EVOLUTION OF PARASITISM IN TRYPANOSOMATIDS

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Trypanosomatidae is a large group of parasitic protists within the class Kinetoplastea, including *Trypanosoma* and *Leishmania* species pathogenic for humans. Trypanosomatids can be restricted to one host (monoxenous) or have a life cycle involving two hosts (dixenous). We have sequenced genomes of 4 monoxenous species: *Blechomonas ayalai, Leptomonas seymouri, Leptomonas pyrrhocoris* and *Paratrypanosoma confusum.* The genome of *L. pyrrhocoris,* parasite of Pyrrhocoridae bugs, was assembled almost to chromosome level. In addition, we generated differential gene expression data for *L. seymouri* (genes upregulated at 35°C vs. 23°C), *Leishmania major* (upregulated in a virulent isolate vs. avirulent one) and *Leishmania mexicana* (upregulated in amastigotes and/or metacyclics vs. procyclic promatigotes). In order to gain an insight into evolution of gene content in trypanosomatids, we mapped gene family gains and losses on the established phylogenetic tree of kinetoplastids (27 genomes in total). Gene gains dominate at the basal nodes of: trypanosomatids, Leishmaniinae, *Leptomonas-Crithidia,* American trypanosomes, *T. cruzi,* and *T. brucei;* the other internal nodes and leaves are either dominated by losses or have almost equal counts of gains and losses. Genomes of the monoxenous species provide essential outgroups for studying genome evolution in human-pathogenic species, especially in *Leishmania.* We identified 99 gene families gained at the basal *Leishmania* node, and most frequent GO terms are associated with them are connected to proteolysis. Then we overlapped gene gains/losses patterns and differential gene expression data. As a result, we compiled a list of 39 novel candidates for *Leishmania* virulence factors, which represent targets for future knock-out and knock-down experiments. The most interesting candidate has no known domains, is around 380 amino acids in length, and is a single-copy gene in *Leishmania* spp. Selection analysis using genomes of 13 *L. pyrrhocoris* isolates was performed and 1,318 genes showing signs of positive selection were identified. Synteny analysis on a dataset of 18 trypanosomatid species reveals high levels of synteny between *L. pyrrhocoris* and *C. fasciculata* as well as between *L. pyrrhocoris* and *Leishmania* spp., which is consistent with current phylogeny of trypanosomatids. Monoxenous-specific genes are mostly located within syntenic blocks and do not interrupt large-scale synteny.

OPENING THE MARINE MICROZOOPLANKTON BLACK BOX: SOURCES OF VARIABILITY IN PROTOZOAN GRAZING IMPACTS

Albert Calbet (Institut de Ciències del Mar-CSIC).

Recent research has revealed that microzooplankton play a key role in marine ecosystems as primary grazers of phytoplankton. This insight comes primarily from techniques that measure microzooplankton grazing at the integrated or net community level. However, little is known about the importance of functional biodiversity and behaviorally driven interactions among microzooplankton. We are far from a species-level understanding similar to that of other planktonic groups, such as mesozooplankton. Moreover, most of our knowledge on this subject comes from studies based on very few, easy to culture, and many times not representative species. Can the actual information on the functional diversity and behavior of microzooplankton species be incorporated into plankton food-web models? Can we build yield predictive models (e.g., global change related) based on our present understanding of the group? In this talk I will present an overview of the present information on microzooplankton grazing rates and impacts in the oceans, and on the factors that may influence their rates and patterns of ingestion. I will also try to identify major gaps of knowledge and propose future directions for successful research on the subject.

PROTISTS: TIRELESS INFORMANTS OF WASTEWATER TREATMENT SYSTEMS. THE SHORTCUT BIOLOGICAL NITROGEN REMOVAL AND PARTIAL NITRITATION PROCESSES

Oriol Canals (**Animal Biology Department, University of Barcelona**), **Humbert Salvadó** (**Animal Biology Department, University of Barcelona**).

Nowadays, the reduction of the energy demand without a diminution of the effluent quality has become one of the main objectives in wastewater treatment. In this regard, several technologies have been developed and implemented in order to adjust the waste of energy to more sustainable practices. Among these technologies, the Shortcut Biological Nitrogen Removal (SBNR) and the combined Partial Nitritation – Anaerobic Ammonium Oxidation (PN-Anammox) are two promising processes for treating high ammonium loaded wastewater by focusing on accumulate the oxidised N-forms as nitrite instead of nitrate. In advantage terms, compared to conventional nitrification-denitrifaction process, the SBNR and PN-Anammox technologies imply less sludge production in addition to the reduction of the 25 and 50-60% of the oxygen demand and of the 40 and 100% of requirement of external organic carbon for denitrification, respectively.

Both processes are ecologically characterised by its extreme physicochemical particularities regarding the ammonia and nitrite concentrations, compounds adverse for the eukaryote microorganisms. Indeed, although eukaryotes already colonised both treatment processes, the number of eukaryote taxa were considerably lower than the taxa from a conventional nitrification-denitrification process. Despite this, the study of the eukaryote community revealed some potential Ciliophora bioindicators for the process performance.

In the SBNR, *Epistylis camprubii* was positively related to ammonium removal efficiency and negatively to ammonium concentration (N-NH4+ ranged between 0.54 to 267 mg L-1). *Epistylis camprubii* also appeared inhabiting the biofilm of the PN, where it did not show correlation with the nitrogen-related parameters but it was negatively affected by the soluble COD. Still regarding the biofilm-associated species of the PN, *Opercularia coarctata* showed a positive relationship with the soluble COD. *Vorticellides microstoma*-complex only appeared during the start-up period of the PN reactor, and this species showed a negative correlation with the biofilm colonisation (biofilm solids). Finally, *Cyclidium glaucoma* showed negative correlation with increases of organic matter and ammonia concentrations of the influent and with the organic matter of the PN reactor.

The study of the protist community in new and extreme wastewater technologies will surely provide the finding of new bioindicator species in order to optimise the performance of the innovative processes.

COMPLETE NUCLEAR GENOME SEQUENCE OF GONIOMONAS AVONLEA, A PLASTID-LACKING CRYPTOMONAD

Ugo Cenci (Dalhousie University, Canada).

The cryptomonads are eukaryotes comprising both photosynthetic and non-photosynthetic species. While cryptomonads such as *Guillardia theta* harbor a plastid of secondary endosymbiotic origin, members of the so-called Goniomonadea lack plastids. A long-standing question in the field of plastid evolution is whether the Goniomonadea are ancestrally non-photosynthetic or whether they lost their plastid secondarily. To address this and other issues, we have sequenced the genome of the newly described species, *Goniomonas avonlea,* and compared it to that of *Gu. theta*. The draft genome of *Go. avonlea* is ~96 Mbp in size, encoding ~32,000 proteins. Interestingly some metabolic pathways present in the *Gu. theta* plastid and periplastidial compartment are also present in *Go. avonlea,* suggesting that these cytosolic pathways were relocated during the course of secondary plastid integration. In contrast, other cytosolic pathways found in *Go. avonlea* are not in *Gu. theta;* these pathways could have been lost in *Gu. theta* or recently gained in *Go. avonlea*. The *Go. avonlea* genome is a valuable tool for elucidating the physiology of heterotrophic cryptomonads, as well as the metabolic 'rewiring' that took place during plastid integration.

PARASITIC DINOFLAGELLATES OVER TIME AND SPACE

D. Wayne Coats (Smithsonian, retired).

Approximately 120 species of dinoflagellates (ca. 6% of extant species) are known to parasitize marine protists, invertebrates, and vertebrates. Many of these species are parasitoids, as completion of their life requires death of the host. Host mortality resulting from dinoflagellate parasitism can have significant impacts on fisheries and influence food-web structure/function. Literature published over the past 140 yrs. shows pan-global distribution of parasitic marine dinoflagellate, with the majority of records from coastal habitats at temperate latitudes. Latitudinal distribution in parasite species richness reflects this sampling bias. Molecular studies exploring marine microbial diversity indicate much higher diversity (20-30% of extant species) and broader distribution of parasitic dinoflagellate. Population studies show marked seasonal signals, with peak infection prevalence associated with declining host populations. Community level assessments of parasitism by dinoflagellates provided differing perspectives. Sequential epidemics produced by host-specific parasites of dinoflagellate assemblages are seen as promoting successional shifts in species composition. By contrast, ciliate communities show simultaneous infection of multiple host taxa encompassing common and rare species. Nonetheless, dinoflagellates that infect ciliates do form epidemics that can impact host populations. Evaluating community and ecosystem level patterns in dinoflagellate host-parasite associations is hampered by our lack of knowledge of parasite diversity, a relative ignorance of host specificity, and a general lack of data.

CONTROL OF SPLICING IN PARAMECIUM

Julia Contreras (Departamento de Microbiologia, University of Seville) Victoria Begley (IBIS, University of Seville), Eduardo Villalobo (Departamento de Microbiologia, University of Seville).

Most eukaryotes have mRNAs disrupted by introns, which are removed during splicing. Spliced mRNAs are marked in the nucleus by the EJC, which consists of Y14, MAGO, and IF4A3.

Miss-splicing occasionally gives rise to PTC-mRNAs, which could be translated into truncated proteins. To avoid this latter phenomenon, cells possess a surveillance system called NMD, which rapidly detects and degrades PTC-mRNAs. In most eukaryotes NMD consists of UPF1-3. In some eukaryotes, PTCs are detected when NMD binds to EJC.

In *Paramecium tetraurelia* there are about 90,000 introns and miss-splicing leads to intron retention, which often gives rise to PTC-mRNAs. Paramecia deal with PTC-mRNAs thanks to NMD; the three UPFs participate in this surveillance.

The aim of our research is to determine whether EJC participates in the recognition of PTC-mRNAs in Paramecium. We have identified orthologues coding for Y14, MAGO, and IF4A3. To get insight into the function of these genes, we have knocked-down their expression (KD) by RNAi, and sought after changes in expression of endogenous PTC-mRNAs, stemming from intron retention events.

Y14 or MAGO KD leads to a decrease in cell number in cultures, while IF4A3 KD causes dead cells in cultures. This suggests that IF4A3 is essential. To corroborate this phenomenon, we have searched for a partner of IF4A3, namely CWC22, and we have knocked-down its expression. CWC22 KD also leads to dead paramecia in cultures.

Ferrochelatase is in *Paramecium* a single-intron gene producing a PTC-mRNA due to intron retention. Previous works have shown that the KD of UPFs causes an increase in ferrochelatase PTC-mRNA, among other PTC-mRNAs. When we have knocked-down Y14 or MAGO, we have not observed the above mentioned increment, suggesting that, at least for ferrochelatase, PTC recognition is independent of EJC. We have analysed intron retention of other intron-mRNAs (with or without PTC) and, we have observed that Y14 or MAGO KD produce different changes in expression depending on the targeted intron-mRNA. These results suggest that EJC controls intron splicing. If NMD controls splicing of PTC-mRNAs at the translation level, EJC seems to control a wider type of mRNAs at a higher level.

ACTIVATION OF AUTOPHAGY BY REDOX UNBALANCE IN THE MODEL GREEN ALGA CHLAMYDOMONAS REINHARDTII

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Autophagy is a membrane-trafficking process by which eukaryotic cells degrade and recycle intracellular material. This catabolic process is upregulated in response to specific types of stress such as nutrient limitation or oxidative stress, and its primary role is to allow cells to properly respond and adapt to these stress conditions. Autophagy is mediated by ATG proteins that are highly conserved through evolution, from yeasts and algae to plants and mammals. Our laboratory has demonstrated that autophagy is conserved in algae using *Chlamydomonas reinhardtii* as model system. Our results indicate that this degradative process is activated in response to different stress conditions including nutrient limitation, oxidative stress, accumulation of unfolded proteins in the endoplasmic reticulum (ER stress), photo-oxidative damage or metal excess (1, 2, 3, 4). These data suggest that there is a strong correlation between the formation of reactive oxygen species (ROS) and autophagy activation. Indeed, we have reported that the presence of ROS scavengers significantly prevents the activation of autophagy in response to high concentrations of metals (4). Here, I will discuss on the role of the intracellular redox state in the control of autophagy in *Chlamydomonas.*

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TOXIC PROTISTS AND WATER MICROBIOMA: A FEEDBACK TO ENVIRONMENTAL CHANGE

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Globalization increasing, climatic warming and pollution are dramatically changing several ecosystems. One of the most influenced environment is the marine one, coasts especially where anthropic pressure is higher. The focus on new colonizing species and their impact on human health is increasing and in this scenario microbes have a central role representing the main part of the total biomass. We have studied the harmful eukaryotic microbe *Ostreopsis ovata* well known because more than ten years ago caused several outbreaks of toxicity in hospitalized patients in the Mediterranean area. It is a new emerging species of Dinoflagellates protists producing a palytoxin-like molecule, isolated and chemically characterized as an ovatoxin typical of this species. *O. ovata* is a benthic and supposed mixotrophic organism producing mucus that help it in its habitat colonisation. Actually a wide range of bacteria genera and species lives in the marine benthos in direct contact with other microbes and micro-invertebrates and this fact could be interesting in the knowledge of the biocenosis where *O. ovata* lives. We have focused this close connection of the protist with the microbiome that lives within its mucous monitoring the in vitro growing both in xenic and axenic culture. We observed *O. ovata* on different stressful condition and at the same time we collected data over the prokaryotes living in the colony, characterizing them with molecular and cultural techniques. The collected data suggested a condition similar to a network of microbes. In fact *Ostreopsis* seems to be unable to live in axenic condition for a long period; moreover Vibrio and Pseudomonas are two of the most common prokaryotic genera living in contact with the studied dinoflagellate. Since the density of living microbes in a benthic network is 2 magnitude grades greater than of the planktonic one, we cannot leave out of consideration this relationship between bacteria and the harmful protist to well understand the periodic so called algal bloom caused by *O. ovata* in the Mediterranean Sea. This shows how a continuous study of the marine microbiome has a key role for human health, especially in the era of the global environmental changes.

PNEUMOCYSTIS JIROVECII IN CHRONIC PULMONARY DISEASES

Carmen de la Horra (IBIS, Sevilla, Spain).

Pneumocystis jirovecii is an atypical opportunistic fungus that causes pneumonia in inmunosuppresed individuals. The last years it has been demonstrated in respiratory samples of inmunocompetents subjects without signs and symptoms of pneumonia that is possible to detect *Pneumocystis* DNA, which represents colonization status.

Current detection of *Pneumocystis*-colonization by PCR allows identifying the microorganism in patients with several chronic pulmonary diseases. The prevalence of colonization is described in COPD, interstitial lung diseases and cystic fibrosis which varies with the underlying diseases.

Pneumocystis-colonization in COPD was among 16-55%, showing geographical differences. In our area, colonization is described in all stages of the disease, being more prevalent in severe COPD. In colonized patients, *Pneumocystis* modifies systemic inflammatory response by increasing circulating cytokines as IL-6, IL-8 and TNF-α. Recently, colonization is associated with increased expression of genes related to activation of Th-1 T-lymphocytes as INF-g and chemokine ligands. To date, we have developed studies in COPD-patients to elucidate the role of *Pneumocystis*-colonization in the pathophysiology of disease.

Pneumocystis-colonization rate in ILDs ranged from 30-34%. Colonized ILDs-subjects showed higher peripheral leukocyte and eosinophil levels, due to an increase of CD8, suggesting that *Pneumocystis* could be related to exacerbation of disease. Unpublished data also shows that could inhibit surfactant proteins A and D. A recent report demonstrates the antagonistic relation among *P. jirovecii* colonization and the bacterial microbiota.

Colonization in CF-patients is being investigated in several countries, reporting range varies from 1.3% up to 41.5% in CF-patients with lung transplantations. Colonization does not appear to be a risk factor to develop a PCP, but those are suffering continuous cycles of colonization and clearance that could act as a co-morbidity factor and may stimulate pulmonary inflammation. *Pneumocystis* could interact with other fungus and bacterial populations than frequently colonized the respiratory tract of CF patients, and play a role in worsening the progression of CF. In this way, we have develop a metagenomic approach to identify the CF-microbiome.

CONCLUSIONS: There is a high prevalence of *Pneumocystis*-colonization in patients with chronic pulmonary diseases and several studies suggest a role of *P. jirovecii* in the natural course of these pathologies.

SEX ON THE BEACH, BUT NOT IN OPEN OCEANS

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One of the biggest questions in biology is why do organisms have sex. Sex has a high energy cost and can disrupt good gene combinations, so why do it? A related question is why do organisms have life cycles, changing from one form to another. Why not just stick with the best form and a single ploidy level? Using *Emiliania huxleyi* as a haplo-diplontic model system, we demonstrate that high genome content variation correlates with the apparent irreversible loss of the flagellated haploid phase and reduced meiotic recombination in this globally important calcifying eukaryotic phytoplankton. Many individual *Emiliania* strains appear to have lost the genes necessary for completing the life cycle, appearing stuck in just the diploid calcified phase. In particular, these *Emiliania* lost highly conserved genes for making the eukaryotic flagellum. In these strains that have lost the ability to swim, the two versions of the genome are seen drifting apart structurally, a sign of absence of meiotic recombination. *Emiliania* that keep the ability to form swimming haploid cells don't show this effect. These major physiogenomic changes occurred multiple times within this relatively young (290 kYa) taxon and associate with open ocean habitats of lower biomass and lower spatio-temporal ecological variability. In particular, *Emiliania* never lost sex in dynamic ocean environments where it is attacked by a deadly, giant lytic virus (EhV). This fits the Cheshire Cat evolutionary hypothesis, according to which *Emiliania* can escape EhV by turning into haploid cells which are invisible to EhV. The dramatic spread of viral infection is only observed where *Emiliania* regularly reaches dense populations. Where hosts maintain steady low-density populations, the low contact rates between EhV particles and *Emiliania* cells limit viral prevalence, and it is in those regions that *Emiliania* starts to lose the haploid phase. Thus the presence of parasites and viruses in dense populations might directly drive life cycles and sex in protists. Importantly, the loss of sex in open oceans might inhibit long term capacity for adaptation to environmental change, a factor that is particularly relevant for this ecologically and biogeochemically key species threatened by ocean acidification.

TOWARDS AN INTEGRATED TAXONOMIC AND MORPHO-GENETIC REFERENCE SYSTEM FOR EUKARYOTES – 1. EUKREF: PHYLOGENETICALLY INFORMED, BOTTOM-UP CURATION OF EUKARYOTIC 18S RDNA SEQUENCES

Javier del Campo (University of British Columbia), Matthew Brown (Mississippi State University), Colomban de Vargas (Station Biologique de Roscoff), Laura Wegener-Parfrey (University of British Columbia).

We present EukRef and UniEuk, two highly complementary, community-based initiatives to address one of the greatest challenges faced by protistology in the new age of environmental meta-barcoding/-genomics/-transcriptomics. Current massive DNA sequencing of our planet's ecosystems, from the smallest cells to animals, is teaching us a fundamental lesson: most of the Earths biodiversity belongs to the least studied compartment of life, microbial eukaryotes. Yet this novel genetic information can only be understood from a functional, ecological, and evolutionary point of view if it is linked to the phenotypic (morphological, physiological, ecological) information of the organisms it comes from, and integrated into a coherent, morpho-genetic taxonomic framework serving as a universal language for protistologists. The next few years represent a critical time to build up a bridge between the centuries-old body of morphological and physiological knowledge of microbial eukaryotic diversity and the current deluge of novel environmental eukaryotic sequence data.

We propose to achieve, within the next three years, a morpho-genetic reference system for eukaryotic biology, ecology and evolution. It includes two major components, fully integrated but representing separate, stand-alone end-products that will be made publicly available to the scientific community and other end-users. On one hand, a standardized curation process realized by active members of the protistology community (predominantly PhD students and post-docs) will generate phylogenetically-informed reference databases of curated genetic markers with reference alignments and trees. The EukRef initiative is driving this bottom-up process for the 18S rRNA gene. On the other hand, the UniEuk initiative will structure the generated knowledge into a 'universal' taxonomic framework, integrating classical morphology-based data and information from relevant genetic markers, with top-down validation by a comprehensive network of taxonomy experts. The system will be self-sustainable and its broad use and long-term preservation will be achieved by direct implementation into the EMBL-EBI data portal, initially as a complementary choice to the NCBI taxonomy.

Overall, the EukRef/UniEuk endeavor will provide a much-needed common language for the fast-growing protistology community, bridging the profusion of novel protistan genetic data from environmental -omics to classical protistology knowledge that has effectively linked taxon names to morphological, physiological, behavioral, and ecological information.

APICOMPLEXAN DIVERSITY ACROSS ENVIRONMENTS. FROM THE CRIMSON BLOOD TO THE SHINING SEA

Javier del Campo (University of British Columbia), Thierry Heger (University of British Columbia), Thomas A Richards (University of Exeter), Ramon Massana (Institut de Ciències del Mar - CSIC), Patrick Keeling (University of British Columbia).

Apicomplexans are well-known and widespread animal parasites, from cnidarians to mammals. They have been studied deeply because of their impact on human and animal health. However, to date the diversity and environmental distribution of the group as a whole has never been studied from a molecular perspective. We aim to unveil the diversity and distribution of the apicomplexans and related lineages (ARL) using high-throughput environmental sequencing (HTES) metabarcoding of the 18S rDNA. We built an exhaustive phylogenetic framework using all the 18S rDNA data for apicomplexans and ARL available in NCBIs GenBank. The retrieved sequences taxonomy was annotated based on phylogeny creating a curated database and a backbone phylogenetic tree, the most extensive to date. Our meta-analysis revealed that apicomplexans and ARL present a larger diversity than previously observed, including the existence of novel apicomplexan groups, and we were able to picture their distribution within hosts and free-living environments. Using our backbone reference tree we successfully placed and annotated HTES reads obtained from different environments. The depth of the sequencing effort allowed us to take a step further and from the HTES data emerged more novel apicomplexan diversity and revealed previously unnoticed distribution patterns of well-studied groups. Our results will help to better understand the apicomplexans and related lineages ecology and to explore putative new roles of these organisms in the environment.

A PHYLOGENOMIC FRAMEWORK FOR STRAMENOPILES

Romain Derelle (Paris-Sud University) , Purificacion Lopez-Garcia (Paris-Sud University), David Moreira(Paris-Sud University).

Stramenopiles embrace a large diversity of protists including ecologically important algal groups such as diatoms or large multicellular seaweeds, as well as heterotrophic (e,g. Bicosoecid, MAST groups) and parasitic (e.g. *Blastocystis,* oomycetes) species. So far, phylogenetic studies based on 18S ribsomoal RNA have failed to clarify deep relationships between stramenopile lineages. In this study, we have built a large phylogenomic dataset composed of 341 protein alignments. Taking advantage of the recently released transcriptomes, genomes and the genomic assembly of *Incisomonas marina* obtained in our lab, our phylogenomic matrix includes 41 stramenopiles representing most lineages of this group.

I will present our phylogenetic results, with a particular attention to the position of the stramenopile root and deep relationships among ocrophytes (i.e. photosynthetic stramenopiles).

We believe that the phylogenetic results obtained in this study will serve as a robust framework to study the evolution of cytological and genomic characters within stramenopiles.

MOLECULAR APPROACHES AGAINST MALARIAL PLASMODIUM

Irene Díaz-Moreno (IBVF – cicCartuja, Seville, Spain), Isabel Cruz-Gallardo (IBVF – cicCartuja, Seville, Spain), Antonio Díaz-Quintana (IBVF – cicCartuja, Seville, Spain), Miguel A. De la Rosa (IBVF – cicCartuja, Seville, Spain).

The discovery of effective new antimalarial agents is urgently needed and demands to decipher molecular interfaces in malarial protein complexes that provide valuable information for drug design.

One of the most frequently-studied molecules anchored to the parasite surface is the merozoite surface protein-1 (MSP1). At red blood cell invasion MSP1 is proteolytically processed and the 19-kDa C-terminal fragment (MSP119) remains on the surface and is taken into the red blood cell, where it is transferred to the food vacuole and persists until the end of the intracellular cycle. Given the structural homology of cupredoxins with the Fab domain of monoclonal antibodies that inhibit erythrocyte invasion and parasite growth, an approach combining NMR and ITC measurements with docking calculations based on BiGGER is employed on MSP119-cupredoxin complexes. Among the cupredoxins tested, rusticyanin forms a well-defined complex with MSP119 at a site that overlaps with the surface recognized by the inhibitory antibodies. The addition of holo-rusticyanin to infected cells results in parasitemia inhibition, but negligible effects on parasite growth can be observed for apo-rusticyanin and other proteins of the cupredoxin family.

Beyond the molecules at the *Plasmodium* surface, malaria parasites harbor an essential vestigial plastid-like organelle, the apicoplast, acquired by a secondary endosymbiotic event. This organelle contains a ~35-kb circular genome whose transcription mechanisms are still not well understood. The annotation of the *P. vivax* genome revealed the presence of a putative nuclear encoded RNA binding protein (PvRBP) predicted to be trafficked into the apicoplast. Herein we describe the structural model, sub-cellular localization and essentiality of PvRBP. The 3D-structure model predicted the presence of a single and canonical RNA recognition motif (RRM) with the $\beta1\alpha1\beta2\beta3\alpha2\beta4$ topology, whose secondary structure was further validated by CD spectroscopy. The expression of the PvRBP in *P. falciparum* showed an apicoplast localization of the *P. vivax* protein and significant increase in gametocyte production. Together, these results revealed that this protein could represent a reminiscent of the bacterial transcription control. The prokaryotic nature of PvRBP, along with its essentiality, makes this protein an attractive therapeutic target.

PROMISCUOUS AND CONSERVATIVE SYMBIONT ACQUISITION IN THE GENUS NUCLEARIA

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Intimate associations between organisms have been observed for a long time. Knowing that such associations are manifold, we use the term symbiosis in a very general manner. We simply call the phenomenon of a close living together of dissimilar organisms a 'symbiosis'. We focused on members of the amoeboid genus *Nuclearia* (Opisthokonta, Nucleariidae) which often live in symbiosis with ecto- and endosymbiotic bacteria. We isolated 16 *Nuclearia* strains from five different Swiss lakes, of which 7 strains were associated with symbionts. The isolated amoebae were characterized morphologically as well as by their 18S rDNA. Phylogenetic analyses resulted in four already established monophyletic branches (made up by the six so far sequenced species of the genus) and an additional cluster formed by two new isolates. A very heterogeneous picture emerged by highlighting *Nuclearia* strains with associated symbionts. Apart from one cluster which included only *Nuclearia* spp. with symbiotic bacteria and two clusters with no symbionts, we also found mixed clusters which were composed of amoebae with and without symbionts. By analysing 16S rRNA genes of symbiotic bacteria, the picture got even more 'obscure'. Although already seven different symbiotic bacterial strains have been identified, it seems that we still are only scratching the surface of the symbionts' diversity. Furthermore, the characters of the symbioses seem to be different depending on the host species. *Nuclearia thermophila* harboured the same endosymbiont even when isolated from different lakes. This points to a rather conservative and obligate interaction. However, we also found two isolates of *Nuclearia delicatula* to be associated with different endosymbiotic bacteria. Here the symbiont acquisition seems to be more promiscuous. As far as we know there are no other documented cases of opisthokont protists with prokaryotic symbionts. This is especially remarkable considering the importance of symbiotic interactions for higher opisthokonts. Thus, Nucleariidae represents an ideal model group to study the basic principles of symbioses.

LATITUDINAL GRADIENT IS OF TYPES AND REDUNDANCY IN PLANKTONIC PROTISTS

John Dolan (CNRS, France), Eun Jin Yang (KOPRI, Korea), Sung-Ho Kang (KOPRI, Korea), Tae Siek Rhee (KOPRI, Korea).

Declines in species richness with latitude is a well-known biogeographic pattern of both multicellular and microbial taxa. Surprisingly, rarely considered are likely concomitant differences in the characteristics of species assemblages. There can be fewer rare species of perhaps low ecological relevance, or fewer distinct ecological types, or declines in redundancy, species functionally similar to one another, thus impacting adaptability of an assemblage. We focused on tintinnid ciliates of the microzooplankton in which prey size is closely linked to morphology (specifically lorica oral aperture) allowing identification of functionally similar species. We sampled from 39°N in the East Sea/East Sea/Sea of Japan to 82°N in the High Arctic Sea. We determined abundance distributions of biological species and also ecological types by grouping species in size-classes, sets of species which presumably exploit similar size prey. Assemblages differed considerably. While the overall size range of the community was unchanged, there were declines in both the number of size-classes present, the portion of size-classes containing multiple species, and numbers of rare species. In lower latitudes, dominant species are accompanied by many apparently ecologically similar species, presumably able to replace the dominant species, at least with regard to the size of prey exploited. Such redundancy appears to decline markedly with latitude in assemblages of tintinnid ciliates.

THE EVOLUTION OF THE MITOCHONDRIAL PROTEIN IMPORT IN PROTISTS

Martin Kolisko (University of British Columbia, Canada), Eva Martincova (Charles University in Prague, Czech Republic), Jan Pyrih (Charles University in Prague, Czech Republic), Andrew Roger (Dalshousie University, Canada), Pavel Dolezal (Charles University in Prague, Czech Republic).

The protein import into mitochondria is orchestrated by several elaborate molecular machines.

These protein complexes known as TOM, SAM, OXA, TIM23 and TIM22 enable different protein populations to reach and assemble in the correct mitochondrial subcompartment. During the course of evolution the core subunits of these molecular machines have been perfected by accessory subunits, which may differ among eukaryotic lineages. We have been interested in the evolution of the mitochondrial protein import and, particularly, in the identification of the protein translocases of the simplest mitochondrial forms knowns as mitosomes. *Giardia intestinalis* mitosomes have been considered to lack the SAM and the TIM complexes of the outer and the inner membranes, respectively. Recently, we have identified core component of the mitosomal TIM complex, which tethers the molecular motor to the translocase. Currently, we are working on the characterization of the putative mitosomal protein translocase. We combine bioinformatics, in vivo and in vitro functional studies to finally characterize the long time elusive mitosomal TIM complex. Our experimental data will be put in the context of the origin and the evolution of the mitochondrial protein translocation machines.

INTERCELLULAR SIGNALING, AGGREGATIVE BEHAVIOR AND EXPERIMENTAL CHALLENGES IN ENTAMOEBA DISCRIMINATION TRIALS

Avelina Espinosa (Roger Williams University, USA), Guillermo Paz-y-Miño-C (New England Center for the Public Understanding of Science, USA).

Competition between kin should be minimized via the ability to discriminate and/or recognize conspecifics' distinct levels of genetic proximity. In recent studies on discrimination in the *Entamoeba* lineage we highlighted a potential methodological problem. Laboratory strains customarily classified within single taxonomic lineages might belong to distinctive taxa and, therefore, generate confounding interpretations of results in discrimination tests. *E. invadens* IP-1 and VK-1:NS, illustrate this scenario. Both differ in a single nucleotide of the small subunit ribosomal RNA (ssrRNA) and are considered strains of the same species, even though they have been isolated from phylogenetically distant hosts. When grown in mixed cultures, each strain aggregates only with self and maintains separation from clusters of the non-alike amoebae. All seven *Entamoeba* varieties showed comparable results. Because these amoebic lines were isolated from multiple hosts, they most likely belong to separate taxa, possibly distinct biological species. Proteomic characterization of aggregative factors (i.e. molecules secreted by clusters of strain-specific cells) as 'recruiters' of kin members for each *Entamoeba* variety will be discussed. The studies on protistan behavior, ecology and evolution need to explore the spatio-temporal effect of kin discrimination/recognition on fitness (i.e. the kin population structure resulting from the mechanisms of discrimination/recognition).

EVOLUTION OF THYLAKOID MEMBRANE COMPLEXES IN EUKARYOTES AND FUNCTIONAL IMPLICATIONS IN CHROMERA VELIA

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The photosynthetic apparatus includes four multi-protein complexes (Photosystems I and II, ATP synthase, and cytochrome b6/f) that are located in the thylakoid membranes of cyanobacteria and eukaryotic plastids. These complexes are conserved throughout all oxygenic phototrophs; however, since plastid genomes, the descendants of cyanobacterial endosymbionts, have lost genes through nuclear transfer or gene deletion over evolutionary time, it is unclear how these processes have affected the protein content and function of photosynthetic apparatus in different eukaryotic lineages. In order to address this question, we used a combination of BLAST and perl scripts to retrieve the sequences for 59 proteins (representing 29 species of cyanobacteria and eukaryotes) associated with photosynthetic complexes from NCBI and other sequence databases. Each protein was categorized as plastid-encoded, nuclear-encoded, or absent, and results were compared amongst the 29 sampled taxa. Our data indicate that photosynthetic gene loss is more extensive in *Chromera velia*, a unicellular alga related to apicomplexan parasites, than in other taxa, with 17 missing proteins (29% of those sampled). In order to explore the structural effects of protein absences on photosystem I (PSI) in *C. velia,* we purified this complex and identified individual components by two-dimensional electrophoresis and mass spectrometry. *Chromera*'s PSI appears to be atypical. It is tightly associated with two different superoxide dismutases and the conserved PsaD, PsaE and PsaF subunits contain extra C-terminal regions. Moreover, we detected an unusually large spectrum of light-harvesting antenna attached to this PSI. These data suggest that *C. velia* rebuilt the PSI complex and copes with environmental stresses using strategies that differ from those in cyanobacteria or plants.

RELEVANCE OF MICROSCOPICAL ANALYSIS IN WASTEWATER

Ettl Marina (Yara Industrial, Germany).

A first standardised method to determine and evaluate the community of organisms living in activated sludge of wastewater treatment plants was published by the Bavarian State Office for Water Management in the 1980thin German language. The method was revised three times; the latest version that is well established in German-speaking Europe is dated from 1999. The figures presented in this study are based on the approach that is defined in this edition.

Data since 1994 from professional activated sludge analyses of Austrian, German, Suisse and Czech WTTPs were collected and evaluated [n=82]. The most abundant taxa of relevant bacteria, sessile and mobile protists as well as multicellular organisms are presented.

The working group "micro-organisms in wastewater" of the German wastewater Association is currently developing a review of the list of indicator organisms, and some of the presented results will be regarded. Some taxa, e.g. *Plagiocampa* and *Thuricola,* that are commonly occurring in municipal WWTPs will be integrated in the list of indicator organisms. Indicator values and the evaluation of the analysis are revised. A field test of the first draft of the working sheets at several WWTPs has recently started. It is planned to publish the new edition also in English language and to make it available via internet.

Key words: microscopical analysis, activated sludge, wastewater, indicators.

WATER-ENERGY BALANCE, PAST ECOLOGICAL PERTURBATIONS AND EVOLUTIONARY CONSTRAINTS SHAPE THE LATITUDINAL DIVERSITY GRADIENT OF SOIL TESTATE AMOEBAE IN SOUTHWESTERN SOUTH AMERICA

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Soil testate amoebae are water-dependent protists and do not tolerate well extremely high or low temperatures. Accordingly, these protists are highly diverse in temperate biomes (mid-latitudes) of southwestern South America (SSA) but they seem to be much less diverse in arid (lower latitudes) and cold (higher latitudes) biomes of this region. We therefore hypothesized that the need for high water inputs and mild temperatures is a phylogenetically conserved trait in these protists, and that the strength of this conservatism has limited their capacity to adapt to arid and cold conditions. We found that soil testate amoebae exhibit a robust unimodal diversity gradient in SSA. This diversity gradient peaked at mid-latitudes, coinciding with areas where there is a trade-off between water and energy inputs. A stepwise approach based on OLS regressions, SARerr models and variation partitioning confirmed that this diversity peak is indeed strongly related to ecological predictors involving water-energy dynamics. The analysis of the midpoint of the latitudinal range showed that testate amoeba species have shorter range sizes at mid-latitudes than at lower and higher latitudes. By assessing multiple-site dissimilarity measures of β-diversity along SSA, we found that climate filtering processes impose a limited dispersal of species towards lower and higher latitudes, thus producing an orderly loss of species. Furthermore, the analysis of the latitudinal variation in the taxonomic distinctness revealed that testate amoeba assemblages are phylogenetically less related at mid-latitudes, whereas the modelling of the latitudinal diversity distribution for different taxonomic levels showed that mid-latitudes harbour a higher phylogenetic diversity than expected by chance. Finally, the analysis of the degree of nestedness at different taxonomic levels revealed that assemblages in lower and higher latitudes are phylogenetically clustered subsets of the temperate pool. Our results suggest that testate amoebae species responses to present-day environmental conditions are limited by the evolution of assemblages that occupied climatically more stable areas such as those found at mid-latitudes; and by post-dispersal in lower and higher latitudes, areas that were strongly affected by historical perturbations (desertification and glaciation, respectively).

GENETIC AND PHENOTYPIC DIVERSITY CHARACTERIZATION OF NATURAL POPULATIONS OF THE PARASITOID PARVILUCIFERA SINERAE

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In this study we have worked with the host-parasite system formed by the microalgae *Alexandrium minutum* (Dinophyceae) and the intracellular parasitoid *Parvilucifera sinerae* (Perkinsozoa), which we have studied as an opportunity to advance our knowledge on the population genetic structure and impact of microparasites in the phytoplanktonic communities. DNA extracted from 73 clonal strains of *P. sinerae,* from ten different locations along the Atlantic and Mediterranean coasts, was used to genetically characterize this parasitoid at the species level. All strains showed identical small and large subunits and internal transcribed spacer of the ribosomal RNA as well as in the β-tubulin genes. However, the phenotypical characterization showed variability in terms of host invasion, zoospore success, maturation time, half-maximal infection, and infection rate. This characterization grouped the strains within three phenotypic types distinguished by virulence traits. A particular virulence pattern could not be ascribed to host-cell bloom appearance or to the location or year of parasite-strain isolation; rather, some parasitoid strains from the same bloom significantly differed in their virulence traits. Identical markers such as ITS and β-tubulin genes of *P. sinerae* strains from different geographic areas and from different years precludes their use in assessing intra-specific diversity and could indicate a recent dispersion of this species. A new approach using Illumina sequencing is now going on.

BIODIVERSITY OF PROTISTS IN SOILS: WHAT WE KNOW, WHAT WE MISS

Anna Maria Fiore-Donno (University of Cologne), Michael Bonkowski (University of Cologne).

Protists play critical roles in soils, mainly as bacterial grazers, stimulating the rates of organic matter decomposition and shaping the bacterial community structure. Our current understanding of soil protistan diversity and function is limited by our ability to precisely identify and quantify free-living species. Inventories of protists in soils mostly rely on the Most Probable Number Method (MPN), consisting of serial soil dilutions, which has a strong bias towards cultivable organisms and underestimates species richness. The advent of environmental sequencing offered potential new insights in biodiversity. However, most studies have been conducted in marine and fresh-water environments. The majority of the few conducted in terrestrial ecosystems have focussed on fungi, microfauna and prokaryotes, leaving the protists in limbo. This is because the genetic divergence observed between and within major protistan groups greatly exceeds that between animals, fungi and plants. This high variability has the direct and unfortunate consequence that molecular markers are strongly biased towards only few lineages. Most striking is the underrepresentation of the phylum Amoebozoa - including very common and widespread soil amoebae - in all studies conducted using various universal eukaryotic primers. We will review, compare and critically analyze the biodiversity assemblages obtained by recent significant studies, tentatively explaining why they differ. Finally, we will try to provide guidelines for choosing the approach that could effectively retrieve the organisms of interest.

DIVERSITY AND ABUNDANCE OF DIPLONEMIDS, A MAJOR PLANKTONIC COMPONENT OF THE THE WORLD OCEANS, AS REVEALED BY THE TARA OCEANS META-BARCODING DATASET

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Diplonemea remains an obscure group of Euglenozoa (Excavata), represented by a few benthic bacteriovorous or parasitic species living in marine or freshwater habitats (*Diplonema, Rhynchopus, Hemistasia*) and by a number of environmental 18S rRNA sequences coming primarily from marine plankton. The Tara Oceans global metabarcoding study revealed that the bulk of eukaryotic plankton diversity is concentrated in heterotrophic clades, and, unexpectedly, the Diplonemea clade emerged as the third most diverse (after Dinozoa and Metazoa) and the sixth most abundant eukaryotic component of the photic zone plankton. We analysed 287,160 diplonemid ribotypes of the V9 region of 18S rRNA gene, clustered into 62,601 OTUs and coming from 124 stations of the Tara Oceans expedition. The most abundant diplonemid OTUs were cosmopolitan, and no clear geographic pattern of diplonemid diversity emerged. However, their communities were stratified by depth and size fractions. Diplonemids were much more diverse and abundant in the mesopelagic zone (compared to the photic zone) and among pico- and nanoplankton (0.8-20 µm) rather than in larger size fractions. Full-length rRNA sequences support the conclusion that the bulk of diplonemid diversity is concentrated in the marine planktonic clade, a sister-group of classic benthic diplonemids (*Diplonema, Rhynchopus*). The phylogenetic position of *Hemistasia,* a recently revised genus of planktonic predatory protists, remains unresolved within diplonemids. A detailed study of this overlooked yet apparently very important group of heterotrophic protists is needed to unravel their role in oceanic ecosystems.

PARAMECIUM CHLORELLIGERUM KAHL, 1935 AND ITS HOLOSPORA ENDOSYMBIONT

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The number of valid morphospecies within the genus *Paramecium* (Ciliophora, Oligohymenophorea) comprises 19 items (Krenek et al., 2015). While some of these species seem to have a cosmopolitan distribution, other *Paramecium* spp. are less widely distributed or might even be considered endemic. *P. chlorelligerum,* a forgotten European species was recently redescribed from southern Germany (Kreutz et al., 2012), and in 2014 it has been found also in Peterhof, St. Petersburg district (Russia). The new strain has been molecularly characterized using different genetic markers, namely 18S rDNA, cytocrome oxydase subunits I and II, and the internal transcribed spacer, which confirmed the identity with German isolate of *P. chlorelligerum.* This is the second «green» paramecia after *P. bursaria.* The symbiotic alga in the Peterhof population looks similar to *Meyerella* alga of the German one (5.2 x7.0 µm vs. 6.2 x 7.7 µm), but has not been yet identified from a molecular point of view. We succeed in cultivating this ciliate in the laboratory on the bacterial medium added with some additional microelements as well as beta-Sitosterol. However, the ciliate has very low rate of division – about 1-2 per week. In the current population of *P. chlorelligerum* 4-7% of cells manifested macronuclear infection with some bacteria (Bt), morphologically resembling *Holospora.* The population of this *Holospora Bt* in stable infected P. chlorelligerum macronucleus includes infectious (IF) and reproductive (RF) forms. RF are short, spindleshaped, about 2.5–3.0 µm long; IF are 3.0–5.0 µm long, 0.6–0.7 µm wide, spindleshaped straight rods with tapered ends. Instead of previously described «classical» *Holospora* representatives (from *P. bursaria* and *P. caudatum* – 6 species) the current one does not manifest ability to form a «connecting piece» – a body where majority of IF concentrates during the infected nucleus division. Ultrastructure of IF (periplasmic part) reminds that of "Ca. Gortzia infectiva" (Boscaro et al., 2012). Nevertheless, according to our molecular investigation the Bt represents a new species belonging to the genus *Holospora.* The phylogeny, based on the 16S rRNA gene, places this new Bt within the clade of "classical" *Holospora* associated to *H. undulata* and *H. obtusa.*

UV INDUCES TRANSFER OF 16S RRNA FRAGMENTS OF THE MICRONUCLEUS-SPECIFIC BACTERIUM HOLOSPORA UNDULATA TO THE HOST PARAMECIUM NUCLEOLI

Masahiro Fujishima (**Yamaguchi University, Japan**), **Yuki Kawamoto** (**Yamaguchi University, Japan**).

Gram-negative *Holospora* species are endonuclear symbiotic bacteria of the ciliate *Paramecium species.* They show species-specificity and nucleus-specificity in their habitats. Infectious form of *Holospora* shows distinctive structure, one half of which contains the cytoplasm and the other half, the periplasmic lumen with an electron-translucent tip called an invasion tip. When the infectious forms of *Holospora* are mixed with aposymbiotic paramecia, the bacteria appear in the host cytoplasm through the host digestive vacuoles, migrate to their target nucleus, distinguish the nuclear envelopes of two kinds by affinity between the bacterial lipopolysaccharides of the outer membranes and unknown substance of the target nuclear envelope, invade into alternative nucleus with the invasion tip ahead, and change gene expressions of the host cell. *H. undulata* is a micronucleus-specific symbiont of *P. caudatum.* To investigate the underlying molecular mechanism on effects of the *Holospora*'s infection to the host cell, we examined fates of 16S rRNA fragments of the *H. undulata* when the bacteria in the host micronucleus were damaged by irradiation of ultraviolet (UV)-C rays. FISH with a probe correspond to a helix H22 of the 16S rRNA showed that UV-C ray induces transfer of the 16S rRNA fragments to the host macronucleus and the RNA fragments accumulate temporarily in the granular structures of the nucleus. Indirect immunofluorescence microscopy with a monoclonal antibody specific for the macronuclear nucleoli of *P. caudatum* showed that the granular structures labelled by the *Holospora* 16S rRNA probe are macronuclear nucleoli. Our observation shows that fragments of the 16S rRNA derived from UV-C ray irradiated *H. undulata* are transferred to the host macronuclear nucleoli. These results also suggest a possibility that exogenous symbiotic or parasitic bacterial RNA fragments may be utilized by the eukaryotic host cells, or affect to the host cell even if the bacteria were destructed in the host cells.

PEE, POO AND PARASITIC PROTISTS - WHAT IS LIVING INSIDE THE FISH WE EAT?

Janina Fuss (**University of Oslo**), **Estelle Gronneberg** (**University of Oslo**), **Torbjorn Gylt** (**University of Oslo**), **Marit Bjorbakmo** (**University of Oslo**), **Jean-Francois Mangot** (**Institut de Ciències del Mar, CSIC, Barcelona**), **Kamran Kamran Shalchian-Tabrizi** (**University of Oslo**), **Dag Klaveness** (**University of Oslo**).

Several studies have addressed the parasitic fauna of cod (*Gadus morhua*), focusing on macroparasitic and bacterial infections. They have shown that cod is host to a wide range of metazoan parasites and prokaryotes. One possible explanation for the diversity of infections observed can be the extraordinary immune system of gadoid fish that lack MHC II. This might increase the vulnerability of cod to infections of all kinds, especially to parasites including protists. But while metazoan parasites in the gut content have been studied quite well, very little is known about the protist diversity in the gut of cod, even though the digestive tract is a well-known habitat as well as entry and exit point for parasitic species. The same is true for the urinary tract of cod, which is an important component of the immune system in fish. In our study, we therefore address the degree and diversity of the infestation of cod with protist parasites in the gut content and the urinary tract of 50 fish of a coastal population in the Oslofjord. We used next generation sequencing (Illumina MiSeq) of the general V4 region of the small ribosomal subunit (SSU) to determine the protist fauna in the gut and the urine of these fish. Our analysis revealed a broad diversity of protists, many of them belonging to obligate parasitic taxonomic groups like the gregarines and microsporidia. In my talk I will present the outcome of the study focusing on general similarities and differences between bladder and gut content and highlight some dominating groups of protists.

THE CALVIN CYCLE OF NON-PHOTOSYNTHETIC EUGLENA LONGA: A ROLE IN CENTRAL ENERGY METABOLISM?

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Euglenas occupy a multitude of environmental niches taking advantage of their unique metabolic capabilities. *E. longa* is a saprotrophic unicellular alga retaining a remnant non-photosynthetic plastid with an unknown but essential function. In a minimal medium, *E. longa* grows efficiently if ethanol or lactate is added as carbon source, while addition of streptomycin to interfere with plastid translation leads to cessation of cell division and death with a later onset in case of lactate. Based on transcriptomic data and bioinformatic predictions, we determined that the only metabolic pathway localizing to the plastid compartment is the Calvin cycle. We present data that suggest a major role for the Calvin cycle in the central metabolism of *E. longa* under aerobic conditions, introducing CO2 as the electron acceptor for the NAD(P)H produced by cytoplasmic alcohol (ADH) and aldehyde dehydrogenases (ALDH), while electrons from lactate are transferred via the respiratory complex to molecular oxygen. After switch to anaerobic conditions, the activity of Calvin cycle further increases the efficiency of wax fermentation from carbohydrate storage. This model explains the delayed streptomycin inhibition for lactate. Notably, this is the first documented instance of Calvin cycle being an essential pathway in a non-photoautotrophic organism, adding to the diversity of biochemically unusual reduced organelles appearing throughout the evolution of eukaryotes.

Supported by Czech Science Foundation grant GA13-33039S and Czech Academy of Science fellowship (ZF).

NEW THERAPY STRATEGIES TO FIGHT AGAINST MALARIA

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Among all parasite diseases, malaria causes the highest morbidity and mortality in the world. In humans, this infection elicits a wide range of immune responses that are always incomplete, non-sterilizing and transient. Since the naturally acquired immunity is an efficient resource against severe disease or lethality in continuously malaria-exposed adults, efforts directed to prophylactic interventions based on facilitating an efficient immunological response would eventually help to control malaria disease. In our lab we have observed that anti-malarial compounds with a delayed-death effect promote effective immune responses in preclinical studies. Whereas no vaccine for this disease has yet been licensed, combined therapy including this kind of parasitostatic compounds could promote long-term immune protection in infected patients, being a candidate strategy to control malaria.

BIOFUEL FROM MICROALGAE?

Miguel G. Guerrero (Instituto de Bioquímica Vegetal y Fotosíntesis. Universidad de Sevilla-CSIC, Sevilla, Spain).

Microalgae is a polyphyletic grouping and a huge pool of biological diversity. Properties typical of higher plants are combined in microalgae with biotechnological attributes proper of microbial cells. These and other properties of microalgae (such as metabolic plasticity, tolerance to extreme environmental conditions, and amenability to genetic engineering), are valuable for bioindustry. Microalgae are a source of compounds with commercial value, such as carotenoids, phycobiliproteins, polyunsaturated fatty acids, polysaccharides, and an array of bioactive compounds for agriculture and food, feed, pharmaceutical, cosmetic, and chemical industries. Microalgae can also be used for reclamation of wastewater and carbon dioxide abatement.

Microalgae have also been proposed as a source of renewable biofuel capable of meeting the global demand for transport fuels. The mass production of liquid biofuels from plant biomass is increasingly being questioned. The "food versus fuel" dilemma and the limitations in available fertile land for a world's growing population are calling to reconsider reliance in biofuels from crop plants. Microalgae represent an alternative to land plants, since cultures could be developed in non-arable land, employing brackish, saline or even waste water, as well as carbon dioxide from flue gases as source of carbon. Expected fuel productivity per area unit for algal cultures outdoors is over 20.000 L per hectare and year.

The option "microalgae for biofuels" is currently being the subject of intense R&D activity. Significant efforts are addressed to the development of viable processes able to generate microalgal biofuels massively and at prices that can compete with those of established fuels. The biomass production step has to be considerably improved, but also harvesting and dewatering of the biomass, as well as the extraction of the biofuel precursor and its conversion into the final product still need optimization.

Selection of the most appropriate strains of microalgae is a key issue. Not just the content of the biofuel precursor (either fermentable sugars or fatty acids) should be considered, but rather the production capacity, looking for the optimal combination of product level and biomass productivity.

EPIGENETIC REGULATION OF TRANSPOSABLE ELEMENTS IN TETRAHYMENA THERMOPHILA

Shan Gao (Ocean University of China), Yifan Liu (University of Michigan), Jie Xiong (Institute of Hydrobiology, Chinese Academy of Sciences), Wei Miao (Institute of Hydrobiology, Chinese Academy of Sciences), Wen Dui (University of Michigan), et al. ()

RNA interference (RNAi) and Polycomb repression play evolutionarily conserved and often coordinated roles in transcriptional silencing. Here we show that in the protozoan *Tetrahymena thermophila,* germ line-specific sequences—many related to transposable elements (TE)—are reactivated in mutants deficient in nuclear RNAi and Polycomb repression. Importantly, transcriptional silencing and reactivation of TE-related sequences are contingent upon shunting between the noncoding RNA (ncRNA) and mRNA production pathways, which can be affected by co-transcriptional processing, nuclear RNAi, and Polycomb repression. We propose that interplay between the nuclear RNAi and Polycomb repression pathways may be a widespread phenomenon, whose ancestral role is epigenetic silencing of TE.

UNCOVERING THE ANCESTRAL STATE OF MITOCHONDRIAL TARGETING

Sriram Garg (**Institute of Molecular Evolution, Heinrich-Heine-Universität, Düsseldorf**), **Verena Zimorski** (**Institute of Molecular Evolution, Heinrich-Heine-Universität, Düsseldorf**), **Jan Stölting** (**Institute of Molecular Evolution, Heinrich-Heine-Universität, Düsseldorf**), **Tachezy Jan** (**Institute of Molecular Evolution, Heinrich-Heine-Universität, Düsseldorf**), **William Martin** (**Institute of Molecular Evolution, Heinrich-Heine-Universität, Düsseldorf**), **Sven Gould** (**Institute of Molecular Evolutions**).

The origin of protein import was a key step in the endosymbiotic acquisition of mitochondria. Though the main translocon of the mitochondrial outer membrane, TOM40, is ubiquitous among organelles of mitochondrial ancestry, neither is the electrochemical gradient across the inner membrane of the organelles, nor the N-terminal targeting sequences (NTSs) on the proteins imported. To better understand the nature of evolutionary conservation in mitochondrial protein import we investigated the reciprocal targeting behaviour of mitochondrial and hydrogenosomal NTSs in *Trichomonas vaginalis* and *Saccharomyces cerevisiae,* respectively. Hydrogenosomes import yeast mitochondrial proteins even in the absence of their native NTSs but do not import yeast cytosolic proteins. Conversely, yeast mitochondria import hydrogenosomal proteins with and without their short NTSs. Conservation of an NTS-independent mitochondrial import route from excavates to opisthokonts indicates its presence in the eukaryote common ancestor and suggests that it predates the origin of NTS-dependent targeting. We suggest that the ongoing loss of NTSs in hydrogenosomes and mitosomes results from the loss of the electrochemical gradient in these mitochondria-derived organelles, accompanied by the evolutionary reduction of the entire translocon machinery. Consistent with that view, proteomic profiling of *Trichomonas* hydrogenosomes shows that 90 % of the proteins identified lack a recognizable N-terminal leader. Our results uncover the simpler, ancestral state of mitochondrial protein import.

ROUNDUP: DIVERSITY AND KEY ROLES OF PROTISTS IN SOILS

Enrique Lara (Laboratoire de Biologie du Sol, Université de Neuchâtel, Switzerland), Stefan Geisen (Department of Terrestrial Ecology, Netherlands Institute for Ecology (NIOO-KNAW), Wageningen, The Netherlands), Christophe V.W. Seppey (Laboratoire de Biologie du Sol, Université de Neuchâtel, Switzerland), R. Claire Le Bayon (Laboratoire d'Ecologie Fonctionnelle, Université de Neuchâtel, Switzerland), David Singer (Laboratoire de Biologie du Sol, Université de Neuchâtel, Switzerland), Michael Bonkowski (Department of Terrestrial Ecology, Institute of Zoology, University of Cologne, Germany), Edward A.D. Mitchell (Laboratoire de Biologie du Sol, Université de Neuchâtel, Switzerland; Jardin Botanique de Neuchâtel, Switzerland).

Soil is certainly the least studied environment for environmental micro-eukaryotic diversity. Especially the diversity and ecological functioning of protists remains largely elusive. Here we want to provide a summary of the talks given in this session which show the enormous genetic and functional diversity soil protists. Last, we are giving an overview of the importance of protists in soil foodwebs to oppose the common misconception of lumping all protists into the single functional group of bacterivores.

Before the development of massive sequencing approaches, the overarching dominance of fungal sequences prevented the study of whole eukaryotic communities. New techniques of ultradeep sequencing such as Illumina HiSeq now allow retrieving a more complete picture of the soil protist diversity. Recent results suggest that soils may be one of the most diverse environments on Earth.

Further, we start the discussion by presenting a case study in which ultradeep Illumina HiSeq sequencing was applied targeting the eukaryotic diversity across different soil types on the volcanic island of Lanzarote (Canary Islands, Spain). Climate is characterized by stable, moderate temperatures and limited rainfall (<200 mm per year), but is influenced by episodic sandstorms originated in the neighbouring Sahara. In addition, the important volcanic activity covered some parts of the island with ashes. As a consequence, soils are thin (regosols/leptosols) and alkaline (pH around 8.5). We collected 11 samples through a gradient of organic matter content from less than 1% to 13%. We determined how organic matter content may influence microeukaryotic taxonomic and functional diversity.

UNDERSTANDING THE EUKARYOTIC MICROBIAL COMMUNITY OF SLOW SAND FILTERS AND THE IMPLICATIONS FOR BACTERIAL PATHOGEN REMOVAL FROM WASTEWATER

Joseph Gibbs (**National University of Ireland, Galway**) , **Gavin Collins** (**National University of Ireland, Galway**), **Sarah Haig**(**University of Glasgow**), **Eoghan Clifford** (**National University of Ireland, Galway**), **Christopher Quince**(**University of Glasgow**).

Slow Sand Filtration (SSF) is a low-energy, low-technology, chemical-free, simple-to-use approach for tertiary municipal waste-water treatment. The biologically-driven removal of bacteria is the key aspect of this technology, making it suitable for hospital waste-waters with antibiotic-resistant bacteria, or those municipal waste being released into water bodies used for recreation or aquaculture.

Previous work on SSF treatment of potable water discovered the central role of protist grazing in coliform removal through SIP. This study focuses on the development of the protist community and its relationship to the removal of faecal indicator organisms. Two filter configurations were tested: the traditional slow sand filter (TSSF); and the MEL-Biological Filter (MEL-BF), which is a variation on the TSSF providing efficient sludge removal, a key requirement due to turbidity (5-25 mg/l suspended solids) associated with secondary effluent.

Results from a 188-day trial of six laboratory-scale replicates of each configuration type showed removal rates of: 99.2% coliforms, 97.8% *E.coli* by TSSF; 98.5% coliforms, 98.4% *E.coli* by MEL-BF. Depth-resolved sampling from half of the filters showed the proportion of total removal in the upper 2.5cm of the filter bed was: 89.0% coliforms, 83.9% *E.coli* by TSSF; 89.4% coliform, 84.1% *E.coli* by MEL-BF. The concurrent protist colonization of the filter bed over time, and with depth, measured by both Q-PCR and direct microscopy, showed a strong correlation with improved coliform and *E.coli* removal.

Furthermore, mesocosm incubation assays indicated addition of 13C-labelled pathogenic *E.coli* to the filter bed microbial community led to increased 18S-rRNA concentration. Tracking the fate of the13C-labelled pathogen biomass into the DNA of the species consuming can help clarify the role of protist grazing in bacterial removal from waste-water, and identify the many layers of this food-web.

Whilst many previous studies have described the bacterial microbiome of SSFs, this study is the first to specifically target the eukaryotic community, and to describe its development, variability and structure. With eukaryotic microbes underpinning bacterial removal in SSF, it is a prime example of how increased understanding of these eukaryotic microbes would allow optimisation of the technology through specifically engineered environmental amendments, or augmentation with individual or symbiotic protist cultures.

A NEW SPECIES OF RIPELLA SMIRNOV ET AL., 2007 (AMOEBOZOA, DISCOSEA) AND INTRAGENOMIC VARIATION OF THE SSU RRNA GENE WITHIN THIS GENUS

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The genus *Ripella* comprises small members of the order Vannellida (Amoebozoa, Discosea). Their SSU rRNA gene has an unusually short sequence lacking several pieces in the hypervariable regions. Until now the genus has been containing only one named species, *R. platypodia,* but some previously published data on unnamed strains indicate that *Ripella* may be more diverse than considered previously. We confirm this by presenting a morphological, ultrastructural and molecular investigation of another new freshwater species of *Ripella* inhabiting the culture of *Tribonema* sp., and one more strain of *R. platypodia* isolated from a small slightly brackish lake in the Negev Desert (Israel). Being typical members of the genus *Ripella*, both strains have shown unusual features of their SSU rRNA gene also evident in the type strain CCAP 1589/2 of R. *platypodia*. Besides a small length, this gene demonstrated a significant level of intragenomic variability in several local areas located in all studied strains in the same sites of the variable regions V2 (helices 9-11), V4 (helices E23-13 – E23-14), V5 (helix 29), V7 (helices E43), V8 (helices E45, 46) and V9 (helix 49), which is evident from molecular cloning. The number of sequence variants in each particular site is finite, and the detailed sequence analysis shows that additional variability occurs by combining different sites within a particular molecular clone. Analysis of a significant number of PCR fragments and molecular clones suggests that the observed variability is not a PCR, cloning or sequencing artifact. The demonstrated intragenomic variability of the SSU rRNA suggests a large number of copies of this marker in the genome, and a qPCR study is currently on the way to obtain a precise estimate of this copy number. By contrast, no significant intragenomic variation was shown in the cytochrome C oxidase subunit 1 (Cox1) gene. The results obtained emphasize caution needed in species identification and interpretation of the estimates on the species diversity in amoebae based on the MOTUs when the SSU rRNA gene is used as a marker.

This study was partially supported by the RFBR grant 15-04-06239-a and the grant 1.50.125.2014 from the St-Petersburg State University.

INTRACELLULAR BACTERIAL SYMBIOSIS IN GENUS ARCELLA (ARCELLINIDS: AMOEBOZOA): A KEY PLAYER FOR ADAPTATION TO HOSTILE ENVIRONMENTS

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There is a growing appreciation of the roles played by microbial symbiosis in animal evolution. But, it remains unknown whether microbial symbiosis played a key role in the evolution of protists. The Arcellinid testate amoebae are "living fossils" belonging to the genus *Arcella* spp., which have been adapting to excessive environmental and climatic changes over long geological periods with very well documented fossil records back to Neoproterozoic Era (750-800 Mya). Also, *Arcella* spp. have exceptional tolerance to environmental and climatic changes, i.e., low pH values and oxygen levels, low temperatures and high concentrations of toxic heavy metals. The research aim is to elucidate whether the bacterial symbiosis in *Arcella* species is the major driving force behind their evolutionary adaptation to different environments. For this purpose, we measured the diversity of the bacterial endosymbionts in two different *Arcella* species, *A. vulgaris* and *A. hemispherica,* through 16S metabarcoding with Illumina MiSeq and confirm the identity of the endosymbionts using Fluorescent in situ hybridizations (FISH) analysis. We used both cultured and environmental samples collected from three different geographical locations.

Results revealed that *A. vulgaris* associated with Proteobacteria bacteria that are members of family Comamonadaceae, *Comamonas* testosterone, gram negative bacteria, abundant in extreme and polluted environments, and which play key metabolic roles in bioremediation and steroid degradation. We suggest that the *C. testosterone* might provide metabolic adaptation for the host cells and thus beneficial to *A. vulgaris* in polluted and hostile environments. In addition, we discovered bacteria which are closely related to *Legionella jordanis,* the human pathogen that can cause respiratory tract infections.

MORPHOLOGY, LIFE CYCLE AND MOLECULAR PHYLOGENY OF PARASITIC DINOFLAGELLATES OF MARINE PLANKTON

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The diversity and ecological significance of parasites is overlooked and parasitism needs to be taken into account when attempting to understand the aquatic ecosystems. Nearly all the basal dinoflagellates and ~90 species of 'core' dinoflagellates are parasites able to infect a broad array of protist and animal hosts. We examine the parasites of the copepods, the most abundant animal group on Earth. We report the morphology, life cycle and molecular phylogeny of the copepod endoparasites *Blastodinium* and *Syndinium*, the ectoparasite *Ellobiopsis*, and the egg-infesting *Dissodinium* and *Chytriodinium*. We also examine *Oodinium* and *Apodinium*, ectoparasites of appendicularians (*Appendicularia*, gelatinous plankton); *Amyloodinium* and *Ichthyodinium*, parasites of fishes; and *Amoebophrya* and *Euduboscquella* as endoparasites of dinoflagellates and ciliates, respectively.

IDENTIFICATION AND CHARACTERIZATION OF VERNALOPHRYS ALGIVORE N. G. N. SP. (RHIZARIA: CERCOZOA: VAMPYRELLIDA), A NEW ALGAL PREDATOR ISOLATED FROM OUTDOOR MASS CULTURE OF SCENEDESMUS DIMORPHUS

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Microbial contamination is the main cause of loss of biomass yield in microalgal cultures, especially under outdoor environmental conditions. Little is known about the identity of microbial contaminants in outdoor mass algal cultures. In this study, a new genus and species of vampyrellid amoeba, *Vernalophrys algivore*, is described from cultures of *Scenedesmus dimorphus* in open raceway ponds and outdoor flat-panel photobioreactors. This vampyrellid amoeba was a significant grazer of *Scenedesmus* and was frequently associated with a very rapid decline in algal numbers. We report on the morphology, subcellular structure, feeding behavior, molecular phylogeny and life cycle. The new amoeba resembles *Leptophrys* in the shape of trophozoites and pseudopodia and in the mechanism of feeding (mainly by engulfment). It possesses two distinctive regions in Helix E10_1 (nucleotides 117-119, CAA) and E23_1 (nucleotides 522-523, AG) of the 18S rRNA gene. It did not form a monophyleytic group with *Leptophrys* in molecular phylogenetic trees. We establish a new genus, *Vernalophrys*, with the type species *Vernalophrys algivore*. The occurrence, impact of the amoeba on mass culture of *Scenedesmus dimorphus,* and means to reduce vampyrellid amoeba contamination in *Scenedesmus* cultures are addressed. The information obtained from this study will be useful for developing an early warning system and control measures for preventing or treating this contaminant in microalgal mass cultures.

TRYPANOCIDAL ACTIVITY AND MODE OF ACTION OF CARBOHYDRATE BINDING AGENTS

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The protozoan parasite *Trypanosoma brucei* is the etiologic agent of Human African trypanosomiasis, or sleeping sickness, a disease that primarily affects the poorest rural populations in some of the least developed countries of Central Africa. Current treatments are inadequate and drugs for late-stage disease are toxic. Parasites living in the mammalian host rely on antigenic variation to evade the immune system of the host. Thus parasites are mainly covered by only one kind of a variant surface glycoprotein (VSG) that constitutes an effective barrier that protects from effectors of the host immune system. In the formation of this protective barrier the N-glycosylation of VSGs is of major importance. Surface VSGs are modified by mannose rich or complex glycans. We report a series of carbohydrate-binding agents (CBAs) that bind to surface glycoproteins and exhibit a strong trypanocidal activity against the clinically relevant bloodstream form of *T. brucei.* Analysis of the mode of action showed a rapid internalization of glycoprotein-CBA complexes and accumulation in the lysosome leading to perturbation in endocytosis and in the progression of the cell cycle. Long-term exposure to these agents yielded resistant parasites with reduced CBA binding and uptake. Resistant cell lines present modifications in the glycosylation profile of VSGs as a result of genetic rearrangements in the TbSTT3B oligosaccharyltransferase (OST) gene. The resistance phenotype was associated with the total loss of the expression of this OST, and a reduction of infectivity and virulence in mice. Carbohydrate-binding nonpeptidic compounds were also tested and exhibit pronounced antiparasitic activity both in vitro and in vivo. Thus, specific glycosylation patterns are important for CBA cytotoxicity and parasite fitness in vivo and agents binding efficiently to surface glycoproteins may provide a unique and highly novel avenue for the development of treatments against parasitic diseases.

ALTERNATIVE SPLICING AND THE EVOLUTION OF CHLORARACHNIOPHYTE ALGAE

Cameron Grisdale (Dalhousie University), John Archibald (Dalhousie University).

Eukaryotic evolution has been shaped by ancient endosymbiotic events. However, many aspects of host-endosymbiont evolution remain unclear. Following the primary endosymbiosis of alpha-proteobacteria in early eukaryotes, cyanobacteria were acquired in another primary endosymbiotic event, giving rise to the first photosynthetic eukaryotes. These primary photosynthetic organisms have themselves been taken up by heterotrophic eukaryotes in multiple secondary endosymbiotic events, leading to a large diversity of photosynthetic eukaryotes. Included in these groups of secondary photosynthetic algae are many ecologically and economically important organisms, such as diatoms, which are responsible for nearly a quarter of global photosynthetic carbon fixation. While secondary endosymbionts typically become reduced to the point of only retaining the plastid compartment and genome, two unrelated lineages of complex algae have retained remnant nuclei of the eukaryotic endosymbionts (i.e., nucleomorphs). Recent sequencing efforts have determined the DNA sequence of nuclear and organellar genomes of host and endosymbiont in two of these complex algae, the cryptomonad *Guillardia theta* and the chlorarachniophyte *Bigelowiella natans*. Unprecedented levels of genetic and biochemical mosaicism were found, with many genes encoded in the host genome whose products are targeted to various organelle compartments. Remarkably, levels of alternative splicing (AS) in the *B. natans* nuclear genome were found to be similar to those in the human cortex, much higher than expected for a unicellular eukaryote. While these efforts have progressed research of complex algae, many questions remain unanswered, such as: the phylogenetic position of species within the chlorarachniophytes, the nearest extant green algal relative of the endosymbiont, and the role of alternative splicing (AS) in transcriptome complexity and regulation of subcellular protein targeting. Using large RNA-seq datasets for several chlorarachniophytes, we are addressing these and other questions in order to gain a better understanding of the biology of complex algae and the evolutionary processes involved in eukaryote-eukaryote endosymbiotic partnerships.

(RE)-DISCOVERY OF MARINE ALVEOLATE PROTISTAN LINEAGES (MALV, SYNDINIALES) IN THE PLANKTON AND THEIR RELEVANCE IN MARINE ECOLOGY

Laure Guillou (France, Roscoff).

The discovery and widespread occurrence of novel Marine ALVeolate (MALV) lineages in marine planktonic communities raised the question of their functional roles. They are suspected to belong to the order Syndiniales (class Syndinea), for which all members known to date (sometime described since more than a century) have a parasitic live style. Syndiniales are restricted to marine habitats, where their genetic traces could be detected from estuaries to the most oligotrophic areas and from the sea surface to deep hydrothermal vents. Diversity of these parasites appears to be comparable to the species richness of their hosts, which extends from unicellular organisms to metazoans. Most of them obligatory kill their hosts to accomplish their live cycle (they are parasitoids). Syndiniales are singular by several aspects, from their cellular/genome organization, host specialization and ecological success. Some Syndiniales have been isolated in cultures, opening new avenues for experimental and physiological studies.

ADAPTATIONS TO HIGH-SALT ENVIRONMENTS IN TWO BACTERIVOROUS HALOPHILES

Tommy Harding (Dalhousie University), Matthew W. Brown (Mississippi State University), Alastair G. B. Simpson (Dalhousie University), Andrew J. Roger (Dalhousie University).

Microbial life in environments near salt saturation has evolved to withstand the osmotic stress that would otherwise kill the cells. Halobacteriaceae (the famous haloarchaea) and Halanaerobiales (anaerobic bacteria) preferentially import inorganic ions into their intracellular environment to equilibrate the osmotic balance. This adaptation has led to a molecular signature that includes a highly acidic proteome. Most other microbes, including the autotrophic protist *Dunaliella salina* and yeasts like *Wallemia ichthyophaga*, instead cope with the osmotic stress by exporting salt, and import or synthesize compatible solutes such as glycerol. However, virtually nothing is known about bacterivorous halophilic protists that thrive in hypersaline habitats all over the world. We conducted transcriptomic investigations to unravel the molecular adaptations of two unrelated bacterivorous protists that are obligate halophiles, *Halocafeteria seosinensis* and *Pharyngomonas kirbyi.* Their predicted cytoplasmic proteomes show increased hydrophilicity compared to protists inhabiting marine habitats. Furthermore, analysis of reconstructed ancestral sequences suggests that, relative to mesophiles, proteins in halophilic protists have undergone fewer substitutions from hydrophilic to hydrophobic residues since divergence from their closest relatives considered in this study. Absence of a detectable canonical acidic signature, commonly observed in proteins of 'salt-in' microbes, suggests that *H. seosinensis* and *P. kirbyi* utilize organic osmolytes to maintain osmotic equilibrium. This is in agreement with the transcription of genes encoding enzymes involved in osmolyte synthesis and genes encoding osmolyte transporters differentially expressed at optimal compared to maximal salt concentration for growth in *H. seosinensis.*

SOIL PROTIST DIVERSITY AND COMMUNITY STRUCTURE ALONG A GRADIENT OF FOREST PRODUCTIVITY IN THE TEMPERATE RAINFOREST OF BRITISH COLUMBIA (CANADA)

Thierry Heger (**University of British Columbia**), **Colleen Kellogg** (**University of British Columbia**), **Julia Gustavsen** (**University of British Columbia**), **Ian Giesbrecht** (**Hakai Institute, Heriot Bay, British Columbia**), **Javier Del Campo** (**University of British Columbia**), **Kira Hoffman** (**University of Victoria**), **William Mohn** (**University of British Columbia**), **Ken Lertzman** (**Simon Fraser University**), **Patrick Keeling** (**University of British Columbia**).

Although abundant and functionally important, unicellular eukaryotes are still poorly characterized, particularly in soils from natural ecosystems. In this study, we examined protist communities along a gradient of forest primary productivity ranging from blanket bogs to zonal forests in the temperate rainforest of British Columbia (Canada). Our main objectives were to assess how species richness and composition of protist communities varied across the gradient and what are the primary environmental factors that structure protist community composition in the temperate rainforest. Illumina MiSeq was used to generate protist sequences of the 18S V4 region from the soil surface (moss and litter). With more than 3500 distinct operational taxonomic units (OTUs, 97%) identified, our study revealed a high diversity of protists from these soils. Our data show clear protist community differences along the gradient with an increase in protist richness and diversity from blanket bogs to zonal forests, similar to diversity patterns in trees. Protist community changes along the gradient were strongly correlated with soil pH, moisture content, substrate-type, calcium and nitrogen. Furthermore, we used microscopy to compare morphospecies data with high-throughput environmental sequencing (HTES) data of testate amoebae, one of the most important and abundant groups of protists in soil. For some testate amoeba groups, we found strong correlations between the two types of data, while for other groups, weak correlations were reported. Potential causes which might explain these discrepancies will be discussed during the talk.

Altogether, our study provides unprecedented insight into the diversity and community structure of diversity across distinct terrestrial ecosystems of the temperate rainforest and highlights the advantages and limitations of HTP sequencing to assess protist diversity in soils.

INVESTIGATION OF A NOVEL OPISTHOKONT WITH A PREDATORY LIFESTYLE IN THE CONTEXT OF THE EVOLUTION OF MULTICELLULARITY

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The origin of the multicellular metazoans from their unicellular ancestors has received a large amount of attention in recent years, with the continuous emergence of new genomic information from unicellular relatives being of essential importance for the understanding of this process.

In this context we are investigating a new unicellular opisthokont species, leading a predatory lifestyle by feeding on other eukaryotes. A first phylogenomic analysis revealed this opisthokont to be most closely related to another unicellular opisthokont group, the animal parasites/commensals ichthyosporea. However, data available at that time did not allow reliable positioning of the novel opisthokont + Ichthyosporea clade in the holozoa, which comprise metazoans and their unicellular relatives. To improve data quality we were seeking to generate a clean transcriptome for the novel opisthokont by sorting the opisthokont from its prey.

To date we were able to create RNASeq data from a sorted culture of the novel opisthokont that allows a more thorough phylogenomic analysis of this organism. Those data will be used, in the context of a stable phylogenomic framework, to investigate the domain and orthologous protein content of this predatory opisthokont in comparison with other lineages leading to the metazoans and the metazoans themselves.

APICOMPLEXAN PARASITES: INTRACELLULAR LIFE STYLE SPECIALISTS AND THEIR ASTONISHING ADAPTIVE POTENTIAL TO ANTI-PROLIFERATIVE DRUGS

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Neospora caninum, Toxoplasma gondii and *Besnoitia besnoiti* are closely related apicomplexan parasites, seriously affecting the health and productivity of large and small ruminants, and represent important economical factors. All three have an obligatory intracellular lifestyle, and can infect a variety of different cell types and tissues. Tachyzoites represent the proliferative and disease-causing stage, and bradyzoites form tissue cysts, surrounded by a thick cyst wall, and can persist for several years during the chronic stage of the disease. More recently, several studies have investigated drug treatment as an option to limit the effects of the diseases caused by these parasites. Compounds such as dicationic pentamidine derivatives, naphtoquinones and ruthenium-based anti-proliferative drugs interfere in the proliferation of tachyzoites, but these organisms have developed an astonishing ability to overcome the drug-mediated effects and adaptation to higher drug concentrations is achieved within a short time frame in vitro. On the other hand, adaptation to drugs that interfere in host cell invasion is less easily achieved. Bumped kinase inhibitors (BKIs) which are inhibitors of calcium dependent protein kinase 1 (NcCDPK 1) that is crucially involved in host cell invasion of *N. caninum* and other apicomplexans, have emerged as highly interesting drug candidates. In vitro studies showed that the BKI1294 inhibits host cell invasion, but also egress, but does not inhibit intracellular DNA replication, which leads to the formation of large multinucleated complexes that remain viable for extended periods of time in vitro. Nevertheless, BKI1294 has been shown to exhibit outstanding safety and efficacy in non-pregnant and pregnant mouse models for *Neospora* infection, rendering this candidate a prime candidate for future studies in large animal models. In addition, BKI1294 also exhibits promising in vitro activities against the related apicomplexans *Toxoplasma gondii* and *Besnoitia besnoiti.*

MORPHOLOGY BASED CLADISTIC ANALYSIS OF SELECTED DINOPHYSOID DINOFLAGELLATE SPECIES

Karina Esqueda-Lara (Centro del Cambio Global y la Sustentabilidad en el Sureste), David Hernández-Becerril (Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Ciudad Universitaria, D.F., Mexico) and Mona Hoppenrath (Senckenberg am Meer, German Centre for Marine Biodiversty Research, Wilhelmshaven, Germany).

There has been an increasing interest for studying species within the dinoflagellates order Dinophysales. Unfortunately, the microscopic documentations of these species are few, especially concerning rare species and studies by scanning electron microscopy. The main goal of this contribution is to cladistically assess the phylogenetic relationships among dinophysoid species based on morphological characters. Phytoplankton net-samples collected from coasts of the tropical Mexican Pacific (including the Gulf of California) and Gulf of Mexico were analyzed. The morphology of twenty planktonic species: *Amphisolenia globifera, Dinophysis acuta, D. argus, D. fortii, D. caudata, D. hastata, D. phalacromoides, D. pusilla, D. schuettii, Histioneis remora, Methaphalacroma skogsbergi, Ornithocercus magnificus, Oxyphysis oxytoxoides, Phalacroma rotundatum, P. porodictyum, P. doryphorum, P. cuneus, P. rapa, P. turbineum* and *Pseudophalacroma nasutum* was studied in certain detail by light microscopy and scanning electron microscopy, and the information of four benthic *Sinophysis* species: *S. grandis, S. stenosoma, S. canaliculata* and *S. microcephala* was taken from the literature. Additionally, *Prorocentrum micans* was used as outgroup. Two strict consensus trees are presented: weighted strict consensus tree and reweighted consensus tree. Both trees showed *Prorocentrum micans* and *Amphisolenia globifera* in basal position. The ingroup is divided into two clades: the first clade of the weighted strict consensus tree is formed by *Metaphalacroma skogsbergii, Sinophysis* spp and *Pseudophalacroma nasutum,* but in the reweighted strict consensus tree *Pseudophalacroma nasutum* is not in this clade. The second clade includes in turn two clades: in the case of weighted strict consensus tree *Phalacroma* spp. and *Oxyphysis oxytoxoides* are related in a subclade, whereas in reweighted strict consensus tree the clade has *Phalacroma* spp., *Oxyphysis oxytoxoides* and *Pseudophalacroma nasutum*. The last clade in both trees includes *Ornithocercus* spp., *Histioneis* spp. and *Dinophysis* spp.

Keywords: Dinophysales; systematic phylogeny; tropical Mexican Pacific.

HUNTING FOR AGILE PREY: TWO NOVEL LEPTOPHRYID AMOEBAE (VAMPYRELLIDA, CERCOZOA) DEVOURING PLANKTONIC FRESHWATER ALGAE

Sebastian Hess (**Biocenter, University of Cologne**).

Vampyrellid amoebae (Vampyrellida, Cercozoa) are well known for their fascinating ability to perforate algal cell walls and to feed on protoplast material, as seen in several species of the genus *Vampyrella*. Besides of these protoplast feeders there are vampyrellids in freshwater and soil ecosystems, that engulf whole prey organisms such as desmids, diatoms or even micrometazoa. Some of these voracious forms have been placed in the Family Leptophryidae, which is well separated from the protoplast-feeding Vampyrellidae by molecular analyses. The leptophryid representatives studied so far (*Leptophrys, Theratromyxa, Platyreta*) display an expanded, surface-attached morphotype and were thought to have a benthic and terrestrial life style, respectively.

Two novel vampyrellid amoebae feeding on motile, planktonic algae (*Euglena* and *Eudorina*) have been isolated from freshwater ponds. According to molecular analyses of the SSU rDNA gene the new isolates are members of the Leptophryidae, which agrees well with their voracious feeding behaviour and the digestive cyst morphology. A microcontroller-based setup for time-lapse photography and a special preparation technique were used to investigate the elusive food capture events and feeding processes. A feeding experiment involving zygnematophycean, volvocalean and euglenophycean algae revealed the food range specificity of the new isolates and enabled an autecological comparison with *Leptophrys vorax*.

HOW TO BUILD AN INVASION MACHINE

Ke Hu (**Indiana University**), **Jun Liu** (**Indiana University**), **Phoebe He** (**Indiana University**), **Jacqueline Leung** (**Indiana University**), **Ying Zhang** (**Indiana University**), **Laurence Florens** (**Indiana University**).

Toxoplasma gondii is one of the most successful parasites on earth. This unicellular organism can infect all warm-blooded animals and is carried by 20% of the global human population. Besides being an important human parasite itself, *T. gondii* is a model for its ~6000 relatives in the Phylum Apicomplexa, including the malaria parasites, which are much less accessible experimentally. To infect an animal, the parasite has to generate considerable force to move through multiple layers of tissues and penetrate host cells. It also needs a robust cortex that is strong enough to withstand the shocks of drastic changes in osmotic pressure, ionic strength and tissue stiffness as it invades different environments, yet is flexible enough to squeeze through narrow junctions. My group focuses on understanding the construction and function of the structural framework of this highly evolved invasion machine. In this presentation, I will discuss our recent efforts in understanding how the unique structures in *T. gondii* cytoskeleton are created, with the focus on the assembly of the cortical microtubules and the mitotic spindle, and how we dissect the functions of individual cytoskeletal components combining molecular genetics, in vivo measurements, and super-resolution imaging techniques. Our ultimate goal is to uncover fundamental principles for cellular structural inheritance and force transmission, taking advantage of the highly ordered cytoskeletal architecture and streamlined motility apparatus of *T. gondii,* which is far more tractable than most traditional model systems for cell biology.

UNEXPECTED DIVERSITY OF MARINE CILIATES FROM COASTAL WETLANDS OF SOUTH CHINA SEA

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Benthic ciliates are commonly thought to play crucial role in marine sediments; however, their biodiversity is comparatively far from understood in China. Therefore during the last four years a faunistic survey of these microorganisms has been carried out along coastal area, especially wetlands, of South China Sea. A total of 162 species have so far been identified and described based on modern taxonomic criteria, mainly including 23 karyorelictids, 23 cyrtophorids, 11 hymenostomatids, 28 scuticociliatids, 9 peritrichs, 10 oligotrichs, 40 hyptrichs s. l. Among them, one new family, 17 new genera and 82 new species as well 9 new combinations were established. Forty-five species were new records for China. Additionally, SSU rDNA sequence data of 86 species were available for the first time to analyze their phylogenetic position. All these revealed an unexpected diversity of marine, free-living ciliates in China. At the present stage of knowledge we are not sure whether these new taxa are endemic because of the lack of intensive investigation elsewhere. Comparative study suggests that low latitude marine environments tend to have higher ciliate diversity than high latitude ones.

NEW BIOREMEDIATION TECHNIQUE FOR RADIOACTIVE CESIUM-CONTAMINATED SOIL USING PARAMECIUM BURSARIA

MD Shafiqul Islam (Dept. Biol., Grad. Sch. Sci., Kobe Univ., Japan), Chisato Yoshimura (Ctr. Environ. Management, Kobe Univ., Japan) and Toshinobu Suzaki (Dept. Biol., Grad. Sch. Sci., Kobe Univ., Japan).

Physicochemical approaches for removal of radioactive cesium from contaminated soil proved to be cost-ineffective than biological methods, and many biological techniques have been proposed. However, none of the bioremediation techniques have been found suitable for fulfilling the purpose, especially for removing cesium contamination from the soil. *Paramecium bursaria* has a mechanism of dissociating metal elements that are strongly bound to the soil particles, and incorporating them into the cell. *P. bursaria* takes up soil particles of up to 10 μm in size. In the digestive vacuole, pH decreases from ~7 to 3 and dissociation of metal element from the soil particles is facilitated. This unique character encouraged us to use *Paramecium* for removal of radioactive cesium from the contaminated soil, and we found that *P. bursaria* has a strong ability of cesium accumulation. After treatment with 1 mM CsCl for 24 hours, the average concentration of cesium in *P. bursaria* cells (green *P. bursaria*) was increased to 8 mM, while aposymbiotic white *P. bursaria* (without symbiotic zoochlorellae) showed no remarkable accumulation. When cesium-adsorbed kaolin particles (a model soil) were mixed with green *P. bursaria* for 4 days, a pronounced accumulation of cesium was observed; the average concentration of cesium in green *P. bursaria* became ~300 times higher than that in the outer medium environment (0.1 mM). *Paramecium* cells were effectively recovered (~95%) by applying DC current to the soil suspension. This technique could be a promising cost-effective, eco-friendly and time-saving method for bioremediation that can be operated on-site at individual farms.

EFFECT OF HIGH LECANE INERMIS ROTIFERS ABUNDANCE ON ACTIVATED SLUDGE BIOCENOSIS

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Activated sludge is a consortium of different kinds of bacteria, flagellates, amoebas, ciliated protozoa and several kinds of small metazoans. The species composition and abundance vary in space and time and depend on many abiotic and biotic conditions. We recently found that rotifer *Lecane inermis* by feeding on filamentous bacteria can prevent the sludge from bulking. The rotifers control is effective, when the abundance of rotifers is higher than 500 ind/ml. We studied influence of high *L. inermis* density on protozoan community of activated sludge in 4 laboratory-scale sequencing batch bioreactors (SBRs). Two treatments and two controls, operated as sequence bioreactors with nutrient removal system in pattern similar to process in wastewater treatment plant. The experiment lasted 9 days in repeated 24 hours cycles including phases of agitation with feeding, aeration and agitation and sedimentation with decantation at the end of the cycle. *L. inermis* rotifers in final concentration of 500 ind/ml were inoculated into 2 bioreactors. Then, the taxonomic composition and abundance of activated sludge microfauna were checked on 1st, 2nd, 5th and 8th day. In total, 33 taxon's were found among which 26 were ciliated protozoa, 4 amoebae, 2 flagellates and one nematode. Mean density of ciliates on first day of experiment was 12 610 ind/ml and diminished to 4 868 ±432 ind/ml in control and 5 496 ±638 ind/ml in treatment bioreactor on the last day. The results show that even extremely high abundance of artificially introduced rotifers did not negatively affect protozoan community. On the contrary, protozoan biodiversity was even higher in treatment in comparison to control.

This work was financed by National Science Center allocated on the basis of a decision DEC-201.3/09/B/NZ8/03303.

PARASITE SECRETED MICROVESICLES: MEDIATORS OF HOST CELL COLONIZATION AND PROSTATE CANCER?

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Trichomonas vaginalis is a common sexually transmitted parasite that colonizes the human urogenital tract where it remains extracellular and adheres to epithelial cells. Infections range from asymptomatic to highly inflammatory, depending on the host and the parasite strain. Symptomatic women typically present with vaginitis, whereas infection in men is usually asymptomatic but can lead to untreated, chronic inflammation of the prostate. *T. vaginalis* infection is associated with increased incidence and severity of prostate cancer. Our research focuses on the role of *T. vaginalis* surface proteins and secreted vesicles in pathogenesis. We have found that T.vaginalis produces and secretes microvesicles with physical and biochemical properties similar to mammalian exosomes. Parasite-derived exosomes are characterized by the presence of core mammalian exosomal proteins as well as parasite-specific proteins. We have demonstrated that *T. vaginalis* exosomes fuse with and deliver their contents to host cells and modulate host cell immune responses. Moreover, exosomes from highly adherent parasite strains increase the adherence of poorly adherent parasites to vaginal and prostate epithelial cells. Studies on a *T. vaginalis* homologue of the human macrophage migration inhibitory factor (TvMIF) presence in parasite exosomes have shown that TvMIF can mimic human MIF by inducing cellular pathways linked to inflammation and increasing prostate cell proliferation and invasiveness: properties underlying the promotion and progression of prostate cancer. In summary, parasite exosomes modulate host:parasite interactions, promote colonization and may contribute to increased risk of prostate cancer in men infected by the parasite.

MORPHOLOGICAL VERSUS MOLECULAR PHYLOGENIES: A DEBATE

Komal Kamra (University of Delhi, India).

Often, there have been mismatches between description and therefore naming of taxa and proposed phylogenetic relationships among ciliate species based on morphology, morphometry, ultrastructure, morphogenesis and molecular data, the latter largely based on the sequence of 18S r DNA sequences. Some hypotrich ciliates described from the Indian subcontinent recently have added more fuel to this important issue. There needs to be a discussion on what is to be relied on more for ciliate description. Uptil now, this has been largely subjective. There are proponents for or against any of these characteristics being 'more important' than another. Two ciliate species matching in all respects looked different completely and the query still remains. The present paper puts up some case studies from Indian hypotrichs described as 'new', or 'new combinations', to assess new taxa and have an open house debate on this very important issue.

WATERBORNE PROTOZOAN INFECTIONS: EMERGING AND RE-EMERGING PATHOGENS IN THE GLOBALIZATION ERA

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The re-emergence of *Cryptosporidium* and other protozoan pathogens as life-threatening opportunistic pathogens, highlights an urgent need for effective control measures and research directions. The present discussion is based on historical facts, analytical reviews, evolution and update on development and evaluation of complete methods for the detection of water borne parasites in drinking and environmental waters. The number of reported waterborne parasitic outbreaks is increasing due to the better surveillance and reporting systems in several countries and continents. Quantity and intensity of the undiagnosed outbreaks stay uncovered. Data about those countries that are probably concerned most are lacking. Countries that established surveillance systems did not establish an international standardization of reporting system. An international agreement of reporting structure is still missing. As the scientific community faces emerging and re-emerging pathogens in the present era of globalization and climate change, conventional and molecular tools for effective detection methods in environmental samples, f. e. water sources, are discussed as well as hindering and confusing factors. Truly ideal methods that support the complete removal of protozoan oocysts during sampling and analysis are available but methods that permit removal, detection of infectious (oo) cysts that would allow to include all of the important biological parameters are missing. In this content, latest findings on cultivation of *Cryptosporidium in vitro* axenic culture based on TEM studies, genetic diversity and taxonomy are presented and discussed. Even though *Cryptosporidium* has been classified as a coccidia, it was always considered to be different and was atypical in terms of its epi-cellular localization, auto-infectivity of oocysts, feeder organelle, myzocytosis-like feeding and high phylogenetic affinity to the gregarine parasites. The ability of *Cryptosporidium* to replicate in host cell-free systems such as aquatic bio-films will be discussed. Understanding the likely occurrence of environmental-borne pathogens like *Cryptosporidium, Giardia* and others in surface water has been a subject obscured by information that has been inconsistent and prone to misinterpretation. Factors to permit successful sampling and analysis in the lab as the basis for reliable interpretation of the data are presented, including the knowledge of the pathogens' life-cycles.

OPISTHOSPORIDIA, A NEW DEEP LINEAGE OF OPISTHOKONTS AT THE BORDER OF HOLOMYCOTA AND HOLOZOA

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The supergroup Opisthokonta includes multicellular animals, fungi and a variety of unicellular organisms which, over the past decade, molecular phylogenetic analysis has assigned to each of the two following major clades: i) the Holozoa, including Metazoa, Choanoflagellata, and Mesomycetozoea,, and ii) the Holomycota, including nucleariid amoebae, fungi, rozellids (Cryptomycota), aphelids and microsporidia (Lui et al., 2009; Lara et al., 2010; Jones et al., 2011; Torruella et al., 2012 Karpov et al., 2013; 2014; Letcher et al., 2013; Paps et al., 2013). The phyla Microsporidia and Cryptomycota (Rozellida), and the class Aphelidea have recently been shown to be the deepest branches of the Holomycota lineage forming the monophyletic ARM-clade (Aphelidea-Rozellida-Microsporidia), which is sister to the classical fungi (Karpov et al., 2013) including Dikarya (Ascomycota and Basidiomycota), paraphyletic Zygomycota, and Chytridiomycota sensu lato (Voigt et al., 2013). Consequently, the taxonomy of ARM clade has been reorganized, and a new superphylum Opisthosporidia with three phyla: Aphelida, Cryptomycota and Microsporidia, has been proposed (Karpov et al., 2014). Opisthosporidia are not true fungi: not only their phylogenetic position place them as sister to true fungi, but also several of their biological peculiarities do not conform the classical definition of fungi. The most remarkable of these is the fact that the trophonts of Aphelida and Cryptomycota (but not of Microsporidia, which are extremely specialized and derived parasites) engulf the host cytoplasm by phagocytosis, like amoebae (Gromov, 2000). Among the Opisthosporidia the Aphelida is sister to both Microsporidia and Cryptomycota (Karpov et al., 2014; Corsaro et al., 2014a,b). The aphelids retained the amoeboid nature in all three genera not only at trophic stage like Rozella, but also in propagules, which agrees with the basal phylogenetic position of the Opisthosporidia nearest to the Holomycota ancestor in the molecular phylogenetic trees. New data on the aphelid and cryptomycota diversity will be presented and the necessity of opisthokont division on Holozoa and Holomycota will be discussed.

GENOME ASSEMBLY AND ANNOTATION OF BALAMUTHIA MANDRILLARIS

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The free-living, ubiquitous amoeba *Balamuthia mandrillaris* is an opportunistic causative agent of amoebic encephalitis, a rare but lethal infectious disease of humans and other mammals. Molecular data plays a critical role in unraveling factors governing the metabolic and pathogenic potential of a pathogenic agent and in developing rational and specific therapeutic strategies. The gateway for this concept becomes accessible through the pathogens genome and transcriptome.

With the help of high-throughput sequencing data, we assembled and annotated the first de novo draft genome and transcriptome of *B. mandrillaris.* The integrated data from Illumina, Roche 454, and PacBio sequencing platforms revealed a genome of 56 Mbp in length and predicted 21,623 genes. Representation of 458 core eukaryotic genes was at 94% identifiable as complete ORFs and at 97% when considering also evidence of partial ORFs. Analysis of the k-mer frequency spectrum of Illumina sequencing reads indicated a polyploid genome with a high level of heterozygosity. The mitochondrial genome had a length of 39,892 bp and encoded 48 genes. Comparison of *B. mandrillaris* nuclear genes and mitochondrial structure to that of *Acanthamoeba castellanii* suggested a more distant relationship between these organisms than conventionally assumed.

As a first proof of said concept, we applied the genomic and transcriptomic data to the investigation of genes involved in sterol synthesis to identify fundamental metabolic disparities between this amoeba and its mammalian host with potential for therapeutic intervention.

FUNCTIONAL DIVERSITY OF PEATLAND TESTATE AMOEBAE: FINDING RELEVANT TRAITS TO ASSESS THE RESPONSE OF COMMUNITIES TO ECOLOGICAL STRESS

Isabelle Koenig (**University of Neuchâtel**), **Matthieu Mulot** (**University of Neuchâtel**), **Edward A. D. Mitchell** (**University of Neuchâtel**).

Stresses and disturbances are major drivers of community assembly and ecosystem processes. Both vary in intensity, frequency, regularity and predictability. Accordingly, community responses range from slow turnover in community composition to total die-out and recolonisation. Stresses result of long-term changes or perturbations that affect directly or indirectly organisms. They affect diversity and community structure and increase spatial heterogeneity in ecosystems, thus contributing to enhancing biodiversity.

Peatlands play a key role in the global carbon cycle and are threatened by on-going climate change. Soil microorganisms are the drivers of changes in the carbon balance of these ecosystems and testate amoebae were shown to be a key functional group in microbial trophic networks of peatlands. However knowledge on the response of peatland microorganisms to climate change is still limited and especially the link between changes in soil communities and ecosystem function remain poorly known.

To estimate the ability of an ecosystem to perform its functions effectively, synthetic metrics and indices are needed. These should be based on key "ecosystem service providers" and in Sphagnum-dominated peatlands testate amoebae are therefore good candidates.

The study of the diversity of functional traits can be used to estimate how environmental changes are affecting ecosystem functioning. This approach is complementary to more classical taxonomy-based approaches. The underlying rationale for this approach is that if the functional diversity is preserved and if redundancy between all actors of the food web and connections at larger scales (time and space) exist, the ecosystem will be more resilient towards stresses.

We present here a study on the effect of drought on *Sphagnum* peatlands testate amoeba community in mesocosms. We analysed responses of testate amoebae using two approaches: 1) taxonomic (classical community ecology) 2) functional traits and in both cases comparing univariate diversity metrics and multivariate (RDA) approaches. Traits were chosen based on current knowledge on relationship between testate amoebae and niche constraints and measured directly on samples. Our results show that changes in communities are revealed sooner using functional traits than community structure. In the future, indexes based on these traits could be made allowing a more practical monitoring of ecosystem under pressure.

SINGLE CELL TRANSCRIPTOMICS OF OXYMONADS

Martin Kolisko (University of British Columbia), Vera Tai (University of British Columbia), Patrick J Keeling (University of British Columbia).

Oxymonads are a small group of anaerobic flagellates and most of the known representatives are obligatory symbionts of wood-eating insects. Majority of oxymonads are unculturable and they are in many ways one of the least understood groups of protists. The most common approach to obtain data from unculturable organisms is environmental sequencing and metagenomics, which provide information about the microbial diversity and ecology of the environment itself, but very little about the biology of the actual organisms. Very recently, a method for single cell transcriptome sequencing has been developed that is applicable to single cell eukaryotes. In the presented study we used an existing single cell transcriptome protocol to isolate single cells of several oxymonads: *Pyrsonymha, Streblomastix* and two species of *Saccinobaculus.* We amplified their mRNA, and sequenced it with MiSeq Illumina technology. Subsequently, we used the obtained data for phylogenomic analyses to resolve phylogenetic relationships of oxymonads and analyze basic metabolic processes of these protists, including searching for genes of putative mitochondrial origin.

CURRENT AND FUTURE PERSPECTIVES ON THE SYSTEMATICS, TAXONOMY AND NOMENCLATURE OF TESTATE AMOEBA

Anush Kosakyan (University of Sao Paulo), Enrique Lara (University of Neuchatel), Daniel Lahr (University of Sao Paulo).

Testate amoebae are a polyphyletic assemblage of at least three major, unrelated taxonomic groups of unicellular amoeboid eukaryotes exhibiting a test (shell). The use of testate amoebae in scientific research has greatly increased in the past 20 years: from an average of about 5 papers a year in the mid-1990's to the current rate of more than 50 papers published yearly. The application range of these organisms is rapidly expansing as well: from the traditional fields of environmental monitoring and paleoecology, to forensic sciences and ecotoxicology studies. All these areas are dependent on the proper use of taxonomy and nomenclature. However, scientometric data reveals that despite an ever increasing necessity for the use of names (the product of taxonmy), taxonomic training and molecular systematic surveys have not increased comparably. Subsequently, insufficient taxonomic training inhibits the growth of related disciplines. These and related problems are discussed in this study, highlighting the outcome of poor taxonomic expertise in understanding of accurate classification and phylogeny of testate amoebae, and the consequences derived from it. Additionally, this study is aimed to discuss the current stage of testate amoebae classification, and to present all nomenclature and taxonomic changes in higher and lower taxonomic levels of testate amoebae, as a result of recent molecular reconstructions. Finally, we conclude with a list of the needs and suggestions toward a unified and modernized taxonomy of testate amoebae.

SINGLE CELL TRANSCRIPTOMICS OF TWO UNCULTIVATED RADIOLARIAN SPECIES

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Recent advances in high-throughput sequencing have resulted in a revolution within protistomics. However, this revolution has not yet spread to the unculturable protists; single celled eukaryotes cannot be held in cultures and grown to the cell numbers needed for NGS. The revolution will come to the unculturables soon, though. Recent methodological developments have made it possible to amplify genomes and transcriptomes from single cells, producing enough molecular material to subject to high-throughput sequencing.

Although these methods for the most part have been developed for clinical testing of humans, or for studying model organisms, we will show that they can be applied to free-living protists as well. We have selected two radiolarian species to test the power of single cell transcriptomics for phylogenomic analyses, and functional analyses of single cells and their symbionts.

In this talk, I will discuss the applied method for single cell transcriptomics and present the first multi-gene phylogeny of Radiolaria with representatives from all the major Radiolarian groups, as well as *Sticholonche zanclea,* the only species in Taxopodida. I will also present analyses of some of the functional genes that seem to have played an important role in the evolution of the Rhizaria-supergroup.

IMPACT OF THE CONVERSION OF TROPICAL LOWLAND RAINFORESTS ON SOIL TESTATE AMOEBAE COMMUNITY COMPOSITION

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We investigated effects of the conversion of tropical lowland rainforests into rubber agroforests ("jungle rubber"), intensive rubber and oil palm plantations on the species richness, density and community composition of litter and soil testate amoebae, and identified factors responsible for these changes, focusing on Sumatra, a hotspot of rainforest transformation. In total 190 morphospecies of testate amoebae were identified. Mean taxa number and density varied significantly between rainforest conversion systems. In litter, species number and density of live testate amoebae were similar in rainforest and jungle rubber but much lower in rubber and oil palm. In contrast, in soil species number and density were similar in rainforest, jungle rubber and oil palm but much lower in rubber. Live biomass in litter was significantly higher in rainforest (62%) compared to any of the transformation systems. In contrast, in soil live biomass was at minimum in rubber and higher in rainforest, jungle rubber and oil palm (73%). As indicated by NMDS and DFA testate amoebae community structure in litter of rainforest differed significantly from that of each of the agricultural land-use systems. In contrast, in soil community structure was similar in rainforest and jungle rubber but differed significantly from that of rubber and oil palm. Canonical correspondence analysis confirmed that environmental factors controlling testate amoebae community composition in litter differ from those controlling testate amoebae community composition in soil. Moreover, the impact of rainforest conversion on trophic groups of live testate amoebae (based on aperture size to shell size ratio) was investigated. Overall, the analyses confirmed our expectations that testate amoebae sensitively respond to environmental changes associated with rainforest conversion and these changes are likely to be linked with changes in ecosystem functioning of the converted land-use systems.

MORPHOLOGY VERSUS DNA – THE TAXONOMIC STATUS OF PARAMECIUM BUETSCHLII SP. NOV. AND ITS NOVEL CRYPTIC CONGENERS

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Paramecium is one of the most intensely studied ciliate genera that nearly everyone knows from school days. Here we present a novel species, *Paramecium buetschlii* sp. nov., discovered in a freshwater pool in Norway. This is the first description of a new valid *Paramecium* morphospecies in Europe since the early 20th century. Interestingly, it features unusual combinations of morphological characters and a high genetic diversity relative to other congeners. Three further investigated *Paramecium* spp. from Germany, Hungary, and Brazil are difficult to discriminate from other members of the genus when relying on morphological criteria only. These species, however, can be clearly separated by DNA-based taxonomic markers (18S-rDNA and COI) indicating that we still can expect and prove a higher biodiversity in genus *Paramecium* then current knowledge would suggest. This supports the opinion that DNA barcoding can complement morphological identification and aid the discovery of cryptic species that show only minor or even no visible morphological differences to valid species, but are often genetically quite different and consequently may represent distinct biological entities. Integrative approaches to species delineation incorporating several adequate characteristics including DNA sequence data are therefore the best method at present, but do not require morphology as the sole element. They rather have to detect the most reliable and efficient character set for proper species delineation. Nevertheless, as long as DNA sequence characters are not conventionally accepted to distinguish candidate species from a valid morphospecies, cryptic speciation will continue to be important in taxonomic as well as ecological and biodiversity studies. In our opinion, we therefore need a common practice on how to formally name these species and to indicate their current cryptic status in order to distinguish them from valid biological species. We therefore propose and would like to canvass the provisional status 'Eucandidatus' as a component of the taxonomic name when describing new but cryptic eukaryotes. This term is intended to make a distinction between valid species and the provisional cryptic species status of novel eukaryote candidate species. Describing a candidate species, however, will not shelve a proper species description including thorough morphological analyses.

DISCORDANT MORPHOLOGICAL AND MOLECULAR EVOLUTION IN TESTATE AMOEBAE

Daniel Lahr (**University of São Paulo**), **Angela Oliverio** (**Smith College**), **Jessica Grant** (**Smith College**), **Laura Katz** (**Smith College**).

Microscopy has revealed tremendous diversity of bacterial and eukaryotic forms. Recent analyses show discordance in estimates of biodiversity between morphological and molecular analyses. Moreover, phylogenetic study of the diversity of microbial forms reveal evidence of convergence at scales as deep as interdomain: morphologies shared between bacteria and eukaryotes. Here, we highlight a few examples of discordance, focusing on testate amoebae. We reveal extensive phenotypic convergence and non-monophyly of genera and morphospecies of testate amoebae, using two independent markers: small subunit ribosomal DNA (SSU-rDNA) and mitochondrial COI. We argue that hypotheses about discordance can be tested using the concept of neutral morphologies, or more broadly neutral phenotypes, as a null hypothesis. Given that testate amoebae are used as bioindicators in both paleoecological and contemporary studies, understanding the discordance between morphology and genetics in testate amoebae is essential for development of indicator species.

CRISPR/CAS9 GENOME EDITING IN TRYPANOSOMA CRUZI REVEALS THE ROLE OF PARAFLAGELLAR ROD PROTEINS IN FLAGELLAR ATTACHMENT AND MOTILITY

Noelia Lander (Center for Tropical and Emerging Global Diseases, The University of Georgia, Athens, GA, USA and State University of Campinas, Campinas, SP, Brazil), Zhu-Hong Li (Center for Tropical and Emerging Global Diseases, The University of Georgia, Athens, GA, USA), Sayantanee Niyogi (Center for Tropical and Emerging Global Diseases, The University of Georgia, Athens, GA, USA), Roberto Docampo (Center for Tropical and Emerging Global Diseases, The University of Georgia, Athens, GA, USA and State University of Campinas, Campinas, SP, Brazil)

Trypanosoma cruzi is the etiologic agent of Chagas disease and current methods for its genetic manipulation have been highly inefficient. We report here the use of the CRISPR/Cas9 system for disrupting genes in this parasite by three different strategies. The system consists of the prokaryotic endonuclease Cas9 and an engineered RNA chimera or single guide RNA (sgRNA) conforming a ribonucleoprotein complex able to recognize a target sequence and produce double strand breaks, that in trypanosomes can be repaired by homologous recombination using donor DNA or by microhomology-mediated end joining (MMEJ), that always results in deletions. We used either vectors containing single guide RNA (sgRNA) and Cas9, separately or together, or one vector containing sgRNA and Cas9 plus a DNA donor for homologous recombination to rapidly generate mutant cell lines in which genes encoding paraflagellar rod proteins 1 (PFR1) and 2 (PFR2) have been disrupted. We demonstrate that genome editing of these endogenous genes in *T. cruzi* is successful without detectable toxicity of Cas9. Our results indicate that PFR1 and PFR2 contribute to flagellar attachment to the cell body and motility of the parasites. Therefore, CRISPR/Cas9 allows efficient gene disruption in an almost genetically intractable parasite and suggests that this method will improve the functional analyses of its genome. Work funded by NIH (AI107663) and FAPESP (2013/50624-0 and 2014/08995-4).

FRESHWATER PLANKTON COMMUNITY ECOLOGY UNDER HIGH THROUGHPUT SEQUENCING PERSPECTIVE

Enrique Lara (**University of Neuchâtel, Switzerland**), **Gabriela Mataloni** (**University of San Martin, Argentina**), **Christophe V. M. Seppey** (**University of Neuchâtel, Switzerland**), **David Singer** (**University of Neuchâtel, Switzerland**), **M. Romina Schiaffino** (**Universidad de Buenos Aires, Argentina**), **Irina Izaguirre** (**Universidad de Buenos Aires, Argentina**).

Recent advances in sequencing technology are currently changing radically our vision of environmental diversity by providing immense numbers of phylotypes that cover most of the diversity present in a sample. However, precisely because of this enormous amount of data, the entirety of data cannot be analyzed precisely. This is rendered possible when only a subset of all data is analyzed, for instance a single clade or only indicator taxa. Here, we show two case studies where phylotypes obtained by sequencing the ribosomal RNA SSU V9 variable region of freshwater planktonic eukaryotes with Illumina HiSeq technology were identified to the finest taxonomical level possible.

We studied the composition of microbial eukaryotes encountered in minerotrophic and ombrotrophic water bodies located in Tierra del Fuego. A GUNIFRAC analysis of community composition reflected perfectly the classification of the sites based on environmental data. However, this separation disappeared when more weight was given to abundant phylotypes, suggesting that subordinate phylotypes were responsible for site discrimination. The 5% best indicators for, respectively, minerotrophic and ombrotrophic environments were searched using an IndVal analysis. Autotrophic taxa were more common in minerotrophic environments, whereas mixotrophic taxa represented best ombrotrophic water bodies.

We also compared the diversity of freshwater planktonic ciliate communities from eight water bodies located in Southern Patagonia and Tierra del Fuego with six lakes located in Hope Bay (Peninsular Antarctica). We inferred then taxonomic composition and functional diversity from the obtained phylotypes. Small algivorous taxa (*Rimostrombidium, Strombilidium, Halteria*) dominated Patagonian communities and were underrepresented in Antarctic lakes, probably because of the absence of light (and thus algal growth) during the Antarctic winter. In contrast, Antarctic communities hosted diverse communities of bacterivores (Scuticociliatea) and omnivores (Stichotrichida, Colpodea, Prostomatea), practically absent from South American communities. We suggest that the latter replaced algivores in the Antarctic foodwebs due to their higher versatility towards a food source that changes over time. Freshwater planktonic ciliates represent a counter-intuitive example where diversity appears higher in more constraining environments.

Illumina opens therefore new perspectives for eukaryotic community ecology, but depends strongly on reliable databases and careful taxonomic affiliation of phylotypes.

SINGLE-CELL MULTIGENE AND TRANSCRIPTOMICS-BASED CATALOGING OF PHAGOTROPHIC EUGLENIDS: TOWARDS MULTIGENE PHYLOGENETICS

Gordon Lax (**Dalhousie University**).

The taxon Euglenida contains organisms with a broad range of morphologies and various nutritional modes including phagotrophy, osmotrophy, phototrophy and mixotrophy. Phagotrophic euglenids are the ancestral form from which other euglenids descended, and represent most of the phylogenetic diversity in the group. Molecular phylogenetic and biodiversity analyses of phagotrophic euglenids suffer from low taxon sampling since few of these species have been cultured, leading to the whole assemblage being understudied. In addition, the SSU rRNA gene which is the most widely used phylogenetic marker by far, can be highly divergent in euglenids, and likely contains insufficient reliable signal to robustly reconstruct their phylogeny alone. To tackle these issues we have isolated ~80 single phagotrophic euglenid cells (to date), and combined identification using high-quality light microscopy, with multiple displacement amplification (MDA) of genomic DNA. This product is used for PCR-amplification of both SSU rRNA genes and multiple other gene sequences. We have also conducted single-cell transcriptome sequencing on selected phagotrophic euglenids, and address outstanding issues with taxon sampling.

AN ANCESTRAL BACTERIAL DIVISION SYSTEM IS WIDESPREAD IN EUKARYOTIC MITOCHONDRIA

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Bacterial division initiates at the site of a contractile Z-ring composed of polymerized FtsZ. The location of the Z-ring in the cell is controlled by a system of three mutually antagonistic proteins, MinC, MinD, and MinE. Plastid division is also known to be dependent on homologs of these proteins, derived from the ancestral cyanobacterial endosymbiont that gave rise to plastids. In contrast, the mitochondria of model systems such as *Saccharomyces cerevisiae*, mammals, and *Arabidopsis thaliana* seem to have replaced the ancestral α-proteobacterial Min-based division machinery with host-derived dynamin-related proteins that form outer contractile rings. Here, we show that the mitochondrial division system of these model organisms is the exception, rather than the rule, for eukaryotes. We describe endosymbiont-derived, bacterial-like division systems comprising FtsZ and Min proteins in diverse less-studied eukaryote protistan lineages, including jakobid and heterolobosean excavates, a malawimonad, stramenopiles, amoebozoans, a breviate, and an apusomonad. For two of these taxa, the amoebozoan *Dictyostelium purpureum* and the jakobid *Andalucia incarcerata*, we confirm a mitochondrial localization of these proteins by their heterologous expression in *Saccharomyces cerevisiae*. The discovery of a proteobacterial-like division system in mitochondria of diverse eukaryotic lineages suggests that it was the ancestral feature of all eukaryotic mitochondria and has been supplanted by a host-derived system multiple times in distinct eukaryote lineages.

DEVELOPMENT OF NEW MOLECULAR TOOLS FOR THE GENETIC ENGINEERING OF EUKARIOTYC MICROALGAE

Rosa León (University of Huelva, Spain).

Microalgae are a heterogeneous group of photosynthetic microorganisms with high ecological importance and enormous biotechnological potential. In the last years, there has been an increasing interest on genetic engineering of microalgae, as a potential tool to get the economically feasible production of bulk materials and to enhance the productivity of high-added compounds. But routine genetic manipulation has been until recently limited to a few species (eg. the classical model microalgae *Chlamydomonas reinhardtii, Volvox cateri* and *Phaeodactylum tricornutum*). The lack of strong promoters and other regulatory sequences are, besides low efficiency and instability of transgenes expression, the main difficulties found to aim the genetic transformation of new microalgal strains. Here we suggest new approaches to overcome these difficulties. On one hand, we propose a new method to express transgenes in microalgae: cotransformation with two naked promoter-less genes, a selectable antibiotic-resistant gene and a gene of our interest, which are randomly inserted into the nuclear genome where their transcription relies on their adequate insertion in a region adjacent to an endogenous genomic promoter or in frame with a native gene. On the other hand, we suggest the translational fusion of the gene of interest with a selectable antibiotic-resistant gene and the subsequent selection of those transformants able to grow at increasing antibiotic concentrations, as a strategy to select vigorous transformants with high levels of transgene expression. Examples of successful application of both approaches are presented.

ORGANELLE EVOLUTION IN ANAEROBIC CILIATES

William Lewis (Newcastle University), Genoveva Esteban (Bournemouth University), Martin Embley (Newcastle University).

Anaerobic ciliates have evolved from aerobic relatives repeatedly and convergently in different lineages. Hydrogenosomes, anaerobically-functioning mitochondrial-related organelles, are the most common adaptation as they enable redox balance and perhaps the production of energy. Only one species of ciliate, *Nyctotherus ovalis*, has been shown so far to have retained a hydrogenosome genome. This is a reduced form of the mitochondrial genome of aerobic ciliate species and it has retained some components of the electron transport chain. An outstanding question concerns the origin(s) of the nuclear encoded, cytosolically synthesised enzymes, such as hydrogenase, that have a functional role within hydrogenosomes. In our present study we have established anaerobic cultures of free-living ciliate species, from the genera *Cyclidium, Metopus, Trimyema* and *Plagiopyla* isolated from their natural aquatic environments. Using RNA-sequencing and comparative transcriptomic techniques, we aim to assemble transcriptomes for these species and identify genes that have important functional roles within their hydrogenosomes. These include pyruvate metabolising enzymes, [FeFe]-hydrogenases and [FeFe]-hydrogenase maturation enzymes. The gene sequences encoding these proteins will be used for phylogenetic inference, in order to reconstruct their evolution. Since these proteins appear to be absent in the closest prokaryotic mitochondrial relatives, the alpha-proteobacteria, and are not consistently mono-phyletic within eukaryotes, evidence would suggest they have been acquired from prokaryotes as a result of lateral gene transfer. Here we aim to establish whether these genes were present in the root of the ciliate tree and therefore common to all ciliates, both aerobic and anaerobic, or whether they have been acquired independently and repeatedly via lateral gene transfer.

GENOMICS OF THE METHANOGENIC ENDOSYMBIONTS OF ANAEROBIC CILIATES

Anders Lind (**Uppsala University**), **William Lewis** (**Newcastle University**), **Lionel Guy** (**Uppsala University**), **Martin Embley** (**Newcastle University**), **Thijs Ettema** (**Uppsala University**).

Endosymbiosis is a wide spread phenomena, and many varying host-symbiont systems can be found all over the world. The common denominator between endosymbiont-host interactions seems to be a bacterial symbiont living inside a eukaryotic host. While examples that deviate from this formula can be found, they are rare. One such deviating system is the methanogenic endosymbiont living inside anaerobic, hydrogenosome-containing, ciliates. The methanogenic archaea living inside these cells have been known for over 20 years, but up until know there have been few clues as to how they have adopted to an intracellular lifestyle. One reason behind this is that the endosymbiotic lifestyle prohibits culturing of these organisms. This is of course a major obstacle when it comes to obtaining adequate amounts of genomic material for genome sequencing, which could provide insights into the adaptations of these cells. Here we show two methods in which we are able to circumvent the need for pure cultures, single-cell genomics and metagenomics. By obtaining high quality draft genome sequences from endosymbiotic methanogens from *Nyctotherus ovalis* and *Metopus contortus,* we have been able to do a genome-wide comparative study of the genomic content of the organisms, together with their free-living relatives, and found the first insights in the genomic basis for endosymbiosis that involves an archaeon.

SEX IN FORAMINIFERA

Jere Lipps (Univ. California Berkeley).

Like so many other microbial eukaryotes, foraminifera were regarded as simple by Carpenter, Darwin and other early workers, hence incapable of complex behaviors like sex. But unlike other microbial eukaryotes, life cycles of foraminifera were partially described by the mid-1800s and sexuality was inferred in 1895 based on dimorphic test morphology. Nevertheless life cycles of only a few of the very many living foraminifera are well known.

Many foraminifera asexually produce tests having a large proloculus (megalospheric) while sexually-produced tests have a smaller proloculus (microspheric). This dimorphism has been detected in fossil forms as old as the early Paleozoic. Other aspects of test morphology are also associated with sex. Thus foraminifera have long engaged in an alternation of asexual and sexual reproduction that is commonly tuned to environmental factors such as nutrient supply, primary productivity, water turbulence, or temperature. Sexual strategies are related chiefly to the mode of gametogenesis—gametogamy, autogamy, and plastogamy—and the locomotion of the gametes. Foraminifera do not have the capacity generally to produce abundant gametes so that gametogenetic strategies are selected for the preservation of gametes and successful gamete fusion. These include the protection of gametes until fusion, the placing of gametes in particular habitats to enhance encounters, and limiting the locomotion of gametes to prevent dispersal. Asexual strategies (schizogamy, budding) may build populations rapidly while genetic exchange through gametogenesis provides several evolutionary advantages.

Foraminiferal gamete and sexual strategies are varied across many phylogenetic lineages, and have a deep origin perhaps in the Proterozoic long before foraminifera evolved skeletons. Sex itself may have been established in eukaryotes in general in the ancestors of foraminifera. The particular gamete and sexual strategies used by foraminifera have evolved and diversified themselves during the evolution of the group.

EXPLORING THE MARINE PICOEUKARYOTIC DARK MATTER USING SINGLE-CELL GENOMICS

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Pico-sized eukaryotes play key roles in the functioning of marine ecosystems. Yet, our knowledge on their diversity, evolution, metabolisms and ecology is still limited. One of the reasons for this situation is that most species are still unculturable. Single-Cell Genomics (SCG) bypass this limitation by allowing accessing the genomes of single microbial cells. SCG is being applied to samples from the global circumnavigation campaign Tara Oceans, and to date about 900 picoeukaryotic Single Amplified Genomes (SAGs) have been generated. Here we present results from the analysis of 45 of them affiliating to MAST-4, MAST-7, Chrysophytes, *Micromonas* and *Bathycoccus.* Overall, we have observed that genome recovery following single-SAG assembly was variable between taxa (e.g. mean=20.6% for MAST-4A and mean=2.3% for *Micromonas*). In any case, we did not observe a genome recovery >44% in any of the SAGs, and genome recovery was not dependent on sequencing depth. Furthermore, within a single species, each SAG seems to recover different areas of the genome. In order to improve genome recovery, we have co-assembled together different SAGs belonging to the same species. Genome recovery improved significantly, obtaining 89.1% of genome completeness in MAST-4A after co-assembling 14 SAGs and 68.5% genome completeness in MAST-4E after co-assembling 9 SAGs. Mapping reads and contigs from single-SAGs back to co-assemblies showed several SNPs and indels, indicating that co-assemblies represent consensus sequences. This was expected, as individual SAGs from the same species were not identical. Gene prediction and annotation was carried out with the co-assemblies. A total of 27,443 genes were predicted for MAST-4A, a number that seems high compared to other taxa (e.g. 23K genes for humans). MAST-4A could actually have a relatively high number of genes, yet these numbers could also be explained by a pangenome as well as by the presence of genes from other taxa. Gene annotation worked better when using a marine reference gene-catalog than when using other general reference databases. Several genes were assigned to ecologically relevant metabolic pathways/functions, such as endocytosis, lysosome hydrolases and ABC transporters. Overall, SCG shows a great potential to continue opening the black-box of marine picoeukaryotes.

HEXAAZATRINAPHTHYLENES WITH APOPTOSIS-LIKE ACTIVITY AGAINST LEISHMANIA DONOVANI

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The available treatment against leishmaniosis include pentavalent antimonials, amphotericin B, miltefosine, paramomycin and pentamidine. These treatments normally present high toxicity to the patient even at low doses. Furthermore, most of these treatments require several days of hospitalization because of its intravenous or parenteral way of administration. Nevertheless, the appearance of resistant strains to these active compounds presents a major problem in the current therapeutic measures against these parasites. Moreover, since no immediate prospect of vaccines is expected, there is a great need to develop novel leishmanicidal agents with an acceptable efficacy and safety profile.

The two molecules used in this study, DGV-B (1, 2, 4, 5-benzenetetraamine tetrahydrochloride) and DGV-C were recently reported to present leishmanicidal activity inducing apoptosis-like processes in both *Leishmania amazonensis* and *Leishmania donovani* species, demonstrated by phosphatidylserine exposure, DNA fragmentation, chromatin condensation, loss of mitochondrial membrane potential among other evidences. In addition it has been reported that DGV-B, which is precursor of hexaazatrinaphtylene synthesis, has been recently used as an anti-cancerigenous drug, decreasing some tumour as breast cancer, even in vivo.

In order to elucidate possible targets related to apoptosis, two different arrays were performed to determine the proteome profile of the parasite: one focused in the determination of the programmed cell death mechanism by detecting the relative expression of 35 human apoptosis-related proteins, and the following array distinguished between 9 different human kinases phosphorylated or not in specific sites. The results for the apoptosis-related proteins array showed that the two molecules activated the programmed cell death by different pathways on the promastigote stage of the parasite.

Funded by grants: RICET/ RD12/0018/0012, Project PI13/00490. ALA and MRB were funded by Becas de Investigación Obra Social La Caixa-Fundación Cajacanarias para Postgraduados 2014". JLM was supported by the Ramón y Cajal Subprogramme RYC-2011-08863. Se agradece la financiación concedida a la ULL por la Agencia Canaria de Investigación, Innovación y Sociedad de la Información, cofinanciada en un 85% por el Fondo Social Europeo.

EXPLORING THE DIVERSITY OF DIVERGENT PROTIST LINEAGES IN FRESHWATER ECOSYSTEMS

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Culture-independent molecular approaches based on 18S rRNA gene amplification and sequencing have largely extended our knowledge on microbial eukaryotic diversity and revealed the occurrence of novel lineages in various ecosystems, and most particularly in the more thoroughly studied marine plankton. While some of these lineages are truly novel, others were known from classic studies but their diversity was largely underestimated on the account of morphological observations only and their phylogenetic novelty unsuspected in the absence of molecular phylogenetic markers. Despite so, an unknown fraction of protist lineages exhibiting phylogenetic novelty and with potential ecological significance is difficult to unveil by classical 18S rDNA amplicon sequencing owing to low abundance, seasonality or divergence at the general eukaryotic primers currently used. A variety of approaches can help to disclose such lineages, including deep amplicon sequencing across temporal series, cultivation and single cell-based approaches. We will present a few such examples on the generally less-explored freshwater ecosystems, focusing most particularly on the opisthokont super-group and the early-branching apusozoans. We will also show that several protist lineages easily transgress marine-freshwater barriers.

ACANTHAMOEBA KERATITIS: UPDATE ON CURRENT TREATMENT OPTIONS

Jacob Lorenzo-Morales (University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna).

Free living amoebae of *Acanthamoeba* genus are causal agents of a severe sight-threating infection of the cornea known as *Acanthamoeba* keratitis (AK). Moreover, the number of reported cases worldwide is increasing year after year, mostly in contact lens wearers. *Acanthamoeba* keratitis has remained significant, despite our advances in antimicrobial chemotherapy and supportive care. In part, this is due to an incomplete understanding of pathogenesis and pathophysiology of the disease, diagnostic delays and problems associated with chemotherapeutic interventions. In view of the devastating nature of this disease, in this workshop we aimed to present the current understanding of *Acanthamoeba* keratitis and molecular mechanisms associated with the disease, as well as virulence traits of *Acanthamoeba* that may be potential targets for improved diagnosis, therapeutic interventions and/or for the development of preventative measures.

UNLOCKING THE TOXOPLASMA GONDII GENOME THROUGH CRISPR/CAS9

Sebastian Lourido (Whitehead Institute), Saima M. Sidik (Whitehead Institute), Diego Huet (Whitehead Institute).

Apicomplexans encode vast numbers of completely uncharacterized genes. Their distance from other eukaryotic lineages makes homology to model organisms difficult and often misleading. Despite the wealth of molecular tools available to manipulate apicomplexan genomes, the lack of RNAi in these organisms has precluded high-throughput functional genetic screens. We recently established the use of CRISPR/Cas9 as a flexible and efficient tool to edit the *Toxoplasma gondii* genome. In this system, the Cas9 nuclease is targeted by a small guide RNA (sgRNA) with 20 bp of homology to the gene of interest. The resulting double stranded DNA break is typically repaired by non-homologous end-joining in *T. gondii,* frequently disrupting the targeted gene. In mammalian cells, pooled screens have been performed using libraries of sgRNAs targeting each gene in the genome. Depletion of certain mutants can be tracked by using the sgRNAs as barcodes. We have built a library that targets each of the ~8200 genes of *T. gondii* with 10 different sgRNAs per gene. By introducing this library into Cas9-expressing parasites and monitoring the population over time, we determine the contribution of each gene to *T. gondii* replication in human fibroblasts. We also demonstrate the power of this approach in a positive selection screen using FUDR, which accurately identifies loss of UPRT as a mechanism of drug resistance. Our work reveals the utility of this technology in investigating the function of parasite genes and presents the first comprehensive functional analysis of any apicomplexan genome.

BEYOND EVERYTHING IS EVERYWHERE – THE BURGEONING FIELD OF LANDSCAPE GENETICS AND ITS APPLICATION FOR UNDERSTANDING PROTIST BIOGEOGRAPHY

Chris Lowe (University of Exeter).

Biogeography remains fundamental to understanding evolution as the distribution of organisms in the environment, and the degree of connectivity between populations, are critical in driving/constraining speciation and extinction. Historically, our understanding of the distributions and diversity of free-living unicellular eukaryotes (aka the protists) has been limited by over-generalisations, and only relatively recently has research moved toward characterising how protist populations are shaped by the land/seascape in which they are embedded. A diverse set of resources including high-resolution environmental datasets, increasingly accessible DNA sequencing technology, and the development of a range of multivariate statistical approaches are providing an unprecedented opportunity to examine the roles of historical and contemporary environmental factors in shaping protist populations.

Here I will provide a brief overview of some current developments in microbial biogeography and landscape genetics and provide an example from our own work examining the distribution and diversity of the common and broadly dispersed marine flagellate *Oxyrrhis marina*. Using a combination of phylogenetic, ecophysiological and environmental data, we show that physical environmental parameters play an important role in shaping genetic structure independent of space/distance. In addition, *O. marina* shows strong patterns of local adaption in response to temperature and salinity further re-enforcing the key role played by physical environmental variation in shaping population structure. These data provide further support for an emerging picture in which broadly distributed free-living protists, despite high dispersal potential, are strongly structured by adaptation to the contemporary physical environment.

ANTI-ACANTHAMOEBA ACTIVITY OF DIFFERENT ARTIFICIAL TEARS USED IN THE TREATMENT OF AMOEBA KERATITIS

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Acanthamoeba is a free living amoeba that can cause keratitis, a disease associated to contact lens (CL) wearers. This pathology is associated with the appearance of dry –eye which makes necessary the treatment with artificial tears. The present work studies the amebicidal capacity of some artificial tears with *Acanthamoeba* trophozoites

Optava FusionTM, Artelac Splash® multidosis, Oculotec®, Hyluprotect® and Systane® Ultra were selected according to their formulation. *Acanthamoeba* T4 trophozoites were used in the study. The viability of amoeba trophozoites was determined with Trypan Blue stain and by flow cytometry with Bacstain- CTC Rapid Staining Kit, using *Acanthamoeba* maintained in NEFF saline as a viability control. The effect of the artificial tears was studied at different hours.

The results showed a decrease in viability in the case of OptavaTM and Artelac® during the first 4 hours. After then, the amoebae started to divide reaching concentration levels similar to those of the viability control. With Systane ® Ultra a high percentage of amoeba death was also observed (78%) while, in the case of Oculotec® a complete cell death was observed after 2 hours of incubation.

Optava® includes in its formula Purite® that is a microbicide that can be killing the trophozoites at the beginning of the incubation but loses its strength after 4 hours. On the other hand Artelac® doesn't have any preservatives in its formula but also produces the decrease in the viability probably due to an osmolarity change of the medium, nevertheless after 4 hours this effect is lost. It's important to highlight that both tears have sodium hyaluronate in their composition, a salt proven as a stimulator of proliferation of the corneal epithelium therefore, can be also stimulating amoebae growth.

In the case of Oculotec®, the preservative/biocide used is Benzalkonium chloride and in Systane® Ultra is Poliquad®. Both preservative have shown an amebicide effect that suggests that some artificial tears might be use in the prophylaxis of AK.

Finantial Support: PI12/02725 from FIS, FEDER, and USPCEU-PC07/2013 and USP-PC07/2014 (Fundación Universitaria San Pablo-CEU). Thiago Gomes by EADS CASA-Brasil and by CAPES Foundation grant (DF 70040-020) FPU grant AP2009-0415.

ACCESSING THE ECOLOGY OF UNCULTURED PICOEUKARYOTES THROUGH A HIGH-THROUGHPUT AUTOMATIC CELL ENUMERATION APPROACH

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Small planktonic protists (i.e. picoeukaryotes, cells of 0.8-3 µm) have fundamental roles in the functioning of marine ecosystems, both as primary producers and as microbial grazers, and are likely the most abundant eukaryotes on Earth. Over the last fifteen years, technological progresses in molecular ecology and environmental sequencing have substantially boosted our understanding of these marine microbes, unveiling an unexpected diversity and notably the existence of new uncultured clades. These methodological improvements have notably offered the possibility to analyse simultaneously a substantial number of samples, allowing a more accurate description of their diversity in time and space. Faced with this growing amount of data available on microbial diversity, it is now challenging to design parallel molecular tools combined with microscopy to acccess their quantititive importance in environmental systems. However, this quantitative approach is relatively expensive, time-consuming and observant-dependant. Furthermore, their tiny size, their lack of specific morphological traits and the changing number of 18S rDNA copies between taxa among the picosize protistan community do not allow the use of classical microscopy and SSU-based molecular approaches (qPCR) to access their abundance in a large numbers of samples. Within this study we tested a newly developed automatic image acquisition and subsequently cell enumeration system of picoeukaryotic organisms. Two uncultured groups of picoeukaryotes differing by their cell size, MAST-4 (2 µm) and MAST-1C (5 µm), have been considered here to optimize this high-throughput quantitative approach for picoeukarytic cells. After targeting these groups by TSA-FISH using specific oligonucleotide probes, microscopic images were acquired fully automatically and cells enumerated using the program ACMEtool 2.0. In general, direct microscopic cell counts were in agreement with our automatic cell counting approach. The automated method can process a larger number of fields of view (FOVs) and consequently analyzes more cells, so it provides an estimate that is closer to true cell abundance. We were further able to test this automatic microscope and cell enumeration system and also its application on a spatial survey with a wide geographical coverage, about 100 stations from a circumglobal expedition, by depicting the spatial distribution of the two above-mentioned MAST groups.

UREA VS. NITRATE: CONCURRENT UPTAKE OF NUTRIENTS BY DINOFLAGELLATES PROROCENTRUM MINIMUM AT A POPULATION AND SINGLE-CELL LEVELS

Olga Matantseva (**Institute of Cytology RAS, Russia**), **Angela Vogts** (**Institute for Baltic Sea Research Warnemuende, Germany**), **Natalia Filatova** (**Institute of Cytology RAS, Russia**), **Maren Voss** (**Institute for Baltic Sea Research Warnemuende, Germany**), **Sergei Skarlato** (**Institute of Cytology RAS, Russia**).

Many phototrophic dinoflagellates are capable of utilizing both inorganic and organic nutrients, and this ability is often linked to initiation and proliferation of harmful algal blooms. Urea is one of the most common organic compounds in eutrophied coastal waters. Its concentration may be variable and strongly depends on anthropogenic pollution events. Therefore, it is important to understand how bloom-forming dinoflagellates respond to urea inputs in the presence of other N sources. Using stable isotope tracers (15N and 13C) and isotope ratio mass spectrometry, we investigated the effect of urea addition on nitrogen and carbon uptake by nitrate-acclimated cultures of bloom-forming dinoflagellates *Prorocentrum minimum*. Our experiments showed that dinoflagellates growing on nitrate were able to consume urea shortly after its addition to the medium. Such a quick response to a new nutrient may be explained by very fast or permanent expression of molecular machinery required for urea utilization. Although dinoflagellates consumed both available substrates, the rate of urea uptake was 2-4 times higher than that of nitrate when both substrates were present at nearly equal N concentrations. Moreover, urea addition led to 30-40% inhibition of the nitrate uptake as compared to control experiments. Remarkably, single-cell analysis by nanoscale secondary ion mass spectrometry (NanoSIMS) showed that the degree of the nitrate uptake inhibition by every single cell within a population was not uniform. We registered rather high variation of the nitrate and urea uptake rates among distinct *P. minimum* cells. Thus, single-cell data deepen our knowledge on functioning of dinoflagellate populations. Using 3H-labeled uridine, we demonstrated a pronounced increase in RNA synthesis following the addition of urea, that can be related to increased transcription of genes involved in urea assimilation. Little is known about nitrogen metabolism genes of dinoflagellates, since the majority of their genomes has not been sequenced. We identified genes responsible for urea and nitrate metabolism (urea and nitrate transporters, urease, assimilatory nitrate reductase, etc.) in the transcriptomes of *P. minimum* from MMETSP database. This information is essential to further studies on the regulation of nitrogen metabolism in dinoflagellates. This work was supported by the RFBR grants 14-04-32146-mol_a and 13-04-00703-a.

NEW HIGH-THROUGHPUT METHODOLOGIES IN EPIDEMIOLOGY AND DIAGNOSIS OF PNEUMOCYSTIS JIROVECII PNEUMONIA

Olga Matos (Global Health and Tropical Medicine, Unidade de Parasitologia Médica, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Portugal).

Pneumocystis jirovecii is known for causing specific infections in the respiratory tract of humans, mostly in immunocompromised patients, leading to a severe, and often fatal, pneumonia known as *Pneumocystis* pneumonia (PcP). In the absence of a well-established culture system to isolate and maintain live organisms, prior efforts to understand the patterns of transmission, so as to develop methods of intervention and management for PcP, have relied on PCR-based approaches. The characterization of the genetic diversity of *P. jirovecii* has shown that some specific single nucleotide polymorphisms (SNP) have been recognized as the molecular markers of choice to study the geographical distribution, modes of transmission, drug susceptibility/resistance, virulence factors, and population genetics of specific genetic subtypes. Recently, the de novo assembly of the *P. jirovecii* genome was published. However, the novel whole-genome based approaches can be onerous, cumbersome, and time consuming. Also recently, the multiplex amplification of genomic *P. jirovecii* DNA associated with single base extension (SBE) and DNA pooling were reported to be a reliable alternative high-throughput DNA sequencing technique, allowing the calculation of the SNP allele frequencies on a large scale, and giving the opportunity to detect possible associations between SNP and multilocus genotypes with demographic and clinical data of infection by *P. jirovecii*. This powerful methodology can provide useful information to understanding the patterns, causes, and control of *P. jirovecii* infection, enhancing the research of this important pathogen.

OPPORTUNISTIC PROTISTS: WHAT WE KNOW AND WHAT WE HAVE TO KNOW

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Cryptosporidium, Free-living amoebae, *Toxoplasma, Microsporidia* and *Pneumocystis* are protists pathogenic for humans and other animals, and some are potential bioterrorist weapons. These organisms cause opportunistic infections in HIV/AIDS patients, in children, organ transplant recipients, and the elderly.

Cases and outbreaks of these organisms are recognized as diagnostic markers of system breakdowns and of major changes in the environment. As water-borne infections, some of these protists figure significantly in the agricultural and veterinary fields as well as their obvious danger to associated human populations. *P. jirovecii,* has increasingly been implicated as a potential co-morbidity factor in humans with underlying disease. *Microsporidia* are pathogens of immunodeficient humans, but can also cause major problems in the rabbit, fish, aviary, and honeybee industries. Some of these organisms (*Cryptosporidium*, some genera of *Microsporidia* and *Pneumocystis*) are not currently cultivable in high densities or in axenic cultures, thus considered as difficult experimental systems causing research performed on these protists to be costly and labor-intensive, and rely heavily on PCR-based approaches.

These protists not only attract those who work in human health, but also those concerned with infections caused by them in non-human hosts and biodiversity.

MALARIA PARASITE RESISTANCE TO THE COMMON DRUG ATOVAQUONE IS UNABLE TO TRANSMIT VIA MOSQUITOES

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Drug resistance severely erodes our ability to control malaria. Mutant parasites resistant to chloroquine, pyrimethamine, and artemisinin have emerged and then spread geographically costing millions of lives and engendering a constant search for replacement drugs. Resistance control strategies include drug rotation, drug combinations, and pursuing targets refractory to resistance. A less explored strategy is to identify drug targets where resistance mutations are unable to spread from patient to patient, which must occur via mosquitoes. We reasoned that some resistance mutations conferring selective advantage under drug pressure in human stages might suffer a fitness deficit in the mosquito stages thereby reducing transmission, and we focused on mitochondrial electron transport drug targets. Malaria parasite requirement for mitochondrial electron transport in the mammalian blood phase is minimal but is highly up regulated in the sexual mosquito stages, creating starkly different selection regimes across the life cycle. We tested the ability of malaria parasites with mutations in the mitochondrial DNA encoded cytochrome B gene (cytB), which are resistant to the widely used electron transport inhibitor atovaquone, to transfer resistance to new hosts via mosquitoes. Five different rodent malaria (*Plasmodium berghei*) cytB mutants tested were unable to successfully infect mosquitoes and hence unable to transmit resistance mutations to naïve mice. Two tested atovaquone resistant cytB mutants of the human cerebral malaria parasite (*P. falciparum*) were also unable to successfully infect mosquitoes. None of seven mutants tested generated sporozoites, the infectious form of the parasite injected into new mammalian hosts by mosquitoes, and were thus unable to transmit the resistance mutation. Resistance, which must be maternally inherited as cytB is mitochondrially encoded, was not able to transmit by outcrossing to atovaquone sensitive (wild type) parasites either. Our results suggest that the commonly occurring atovaquone resistance mutations are unable to increase in frequency in the population and cannot spread geographically, which radically improves the long-term prospects for this antimalarial.

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CILIATES AS MODEL SYSTEMS TO STUDY MOLECULAR ADAPTATION AND ENVIRONMENTAL RESPONSES

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Ciliates provide optimal model systems to study genome evolution and environmental adaptation. Addressing evolution and adaptation requires access to large sample sizes of genome or transcriptome sequencing. In this context, we analysed the genome and transcriptome of *Euplotes focardii*, a strictly psychrophilic ciliate isolated from Antarctic seawater samples. Comparative genome analysis of *E. focardii* and the mesophilic congeneric species *E. crassus* revealed rapid evolution and unusual plasticity of the programmed +1 ribosomal frameshifting, a standard feature of the genetic code that affects decoding over 3,000 genes in these genomes. Furthermore, approximately 5.5% of the obtained *E. focardii* genomic contigs were potentially of symbiotic bacterial origin suggesting that different organisms cooperate for environmental adaptation. The sequencing of the transcriptome revealed that the majority of the transcripts correspond to proteins involved in oxidoreductase activity, as reported for Antarctic fishes and krill. These results confirm that a major problem of Antarctic marine organisms is to cope with increased O2 solubility at low temperatures, and suggest that an increased defence against oxidative stress likely constituted an important evolutionary aspect that allowed the adaptation of Antarctic organisms in their oxygen-rich environment.

While *E. focardii* provides a new system for genomic and transcriptomic studies, *Tetrahymena thermophila* has been largely investigated and functional genomic databases are available. Therefore, *T. thermophila* provides an optimal model system for studying molecular bases of environmental responses, since molecular data obtained in different environmental conditions can be easily compared. We recently used *T. thermophila* to elucidate the environmental effects of silver nanoparticles by analyzing *T. thermophila*'s gene expression profile after exposure to Collargol (protein-stabilized silver nanoparticles) and comparing with the effect of the soluble silver salt, AgNO3. Currently, silver nanoparticles are increasingly used as biocides and they can affect non-target organisms in the environment. Therefore, understanding the toxicity mechanisms is crucial. We found that genes involved in mRNA splicing and oxidation reduction appear down and up regulated, respectively, only in Collargol treated samples. This research provides evidence that silver nanoparticles might be toxic due to combined effects of soluble silver ions released from the particles and the particles themselves.

INTRODUCTION TO THE WORKSHOP ON SOIL PROTISTS AND FIRST QUESTION: CAN WE TRUST DATA ON PROTIST DISTRIBUTION AND ECOLOGY?

Edward Mitchell (**University of Neuchâtel, Switzerland**)

Soils are home to a high abundance and diversity of protists, which remain ignored by many soil biologists. In response to this, we have initiated the "Soil Protist Inititative" which is linked to the Global Soil Biodiversity Initiative. In this session we will present and discuss several key aspects of soil protistology, starting with the simple question: "Can we trust data on protist distribution and ecology?"

Since the microscope was invented in the 17th century many studies on soil protists were published, starting with taxonomic descriptions soon followed by studies of protist biogeography and ecology. However the validity of most work on protist biogeography and ecology cannot be assessed for lack of illustration.

While obtaining taxonomically informative illustrations remains a challenge for many groups of protists, this is quite easy for some, such as testate amoebae. Yet even for this group the situation is far from optimal. Apparently surprising records may reflect our imperfect knowledge on the ecology and distribution of organisms (i.e. systematic bias in ecosystems and regions studied), and/or simple errors (i.e. mis-identifications, taxonomic over-fitting, possibly revealing new species).

While standard (albeit imperfect) repository for molecular data (e.g. GenBank) exist, no such system is systematically used for protist morphology (or other organisms for that matter). As most scientific journals now offer the possibility to publish online supplements there is no reason not to request authors to provide illustrations for the organisms they mention in their publications. An even better alternative would be a single address repository such as Wikimedia. Even sub-standard illustrations would be better than the current almost total lack of iconography. Making such data accessible would allow checking at any time the identity of listed taxa, or re-assigning names if the taxonomy of a group has been revised (e.g. splitting species complexes). It would also allow reviewers to better evaluate the quality of submitted papers.

With the increase in meta-analyses and global diversity studies we urgently need a better standard for data on protist biodiversity, biogeography and ecology. We therefore suggest that journals should request illustrations of all taxa mentioned in studies.

HARVESTING PHYLOGENETIC INFORMATION TO UNVEIL THE GLOBAL MARINE DIVERSITY AND BIOGEOGRAPHY OF THE MAMIELLOPHYCEAE LINEAGE OF EUKARYOTIC PHYTOPLANKTON

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Environmental cataloguing of marine protists, through 18S SSU rDNA gene sequencing, has revealed the widespread distribution of the Mamiellophyceae, a class of single-celled green algae, which have been shown to occupy a range of environments, from polar to tropical waters. The ecological success of these phytoplankton, which often dominate the pico-sized planktonic biomass (<2 µm), is further demonstrated by their fast growth rates and contributions to primary production. Moreover, select Mamiellophyceae species have increased in abundance in the Arctic Ocean while larger phytoplankton have decreased in association with climate-influenced changes. Based on molecular analyses of the Arctic Ocean, we further show that minor disturbances in nutrient and light regimes lead to large-scale changes in Mamiellophyceae abundances. Because of their influence on oceanic ecosystems, and sensitivity to environmental disturbances, it is crucial to monitor and model potential changes in Mamiellophyceae biogeography, and how these shifts may alter higher marine trophic levels and carbon export to the deep sea. To this aim, the present distribution and diversity of these fundamental phytoplankton must be assessed on a planetary scale. While 18S surveys of local communities recovered valuable baseline information about local and regional distributions of Mamiellophyceae species, these studies provided a narrow picture of their global diversity. A global dataset of Mamiellophyceae molecular diversity built from standardized sampling and sequencing procedures is lacking. Moreover, virtually all surveys of Mamiellophyceae have focused on the pico-size fraction, dominated by well-studied key members of the Mamiellales order —*Bathycoccus, Micromonas* and *Ostreococcus*. Little is known about distributions of larger, nano-sized Mamiellophyceae cells (<5 µm) such as members of the overlooked Dolichomastigales order, even though Mediterranean Sea surveys hint at high level of diversity. Here, we will present results from phylogenetic analyses of the 18S data from the 'piconano' size fraction (0.8-5 µm) generated by Tara Oceans, a four year circumnavigation that sampled plankton across the world's oceans. We harvested phylogenetic information from Mamiellophyceae sequences to unveil the global diversity and biogeographic distribution of this important phytoplankton group. Our results demonstrate that the diversity of the Dolichomastigales has been hitherto hugely underestimated, and that Mamiellophyceae abundances and community compositions were influenced by geographical origins rather than depth.

EVALUATING PREDATOR-PREY DYNAMICS: MAKING THE MOST OUT OF FUNCTIONAL AND NUMERICAL RESPONSE DATA

David Montagnes (University of Liverpool, UK).

Parameters obtained from measuring functional and numerical responses (the change in ingestion and growth rates with increasing prey abundance, respectively) aid in defining a consumer's competitive ability. Consequently, over the last four decades, as protists have been recognised as key components of food webs, researchers have made substantial efforts to obtain data for these two responses, primarily using them to describe mechanistic behaviours. Functional and numerical responses can, however, be employed much more extensively, to evaluate predator-prey dynamics through numeric modelling. Surprisingly though, modellers who simulate predator-prey (and for that matter food web dynamics) typically ignore numerical response data. Instead, following practices established ~100 years ago by Lotka and Volterra, they indirectly obtain growth rates from ingestion rates, by assuming a fixed conversion efficiency and fixed mortality rate. I will first briefly indicate why neither conversion efficiency nor mortality rate is invariant with prey abundance and then outline the difficulties associated with embedding these variable responses into the traditional Lotka-Volterra structure. I will then provide a solution: a parsimonious model-framework that uses empirically obtained functional and numerical responses. Finally, through examples, I will illustrate the robustness of this new approach to evaluating a range of issues associated with the complexity of protist dynamics (e.g. mixotrophy, temperature responses, strain differences, and if time permits nutritional history). In doing so, I aim to encourage protistologists to not only collect and evaluate functional and numerical response data but to use these responses in models that evaluate population dynamics.

SEX IN DIATOMS: INSIGHTS AND INSPIRING QUESTIONS

Marina Montresor (Stazione Zoologica Anton Dohrn, Villa Comunale, Napoli, Italy).

Diatoms are unicellular microalgae that have a key-role in the biogeochemical cycles of the oceans, where they are responsible for about 20% of the global primary production. The hallmark of diatoms is the presence of a rigid siliceous wall, the frustule, constituted by two slightly unequal thecae, fitting together as a box and its lid. This feature and the unique cell division modality imply a progressive cell size reduction as cell divisions proceed. The restitution of the large cell size is accomplished within the sexual phase, where a cell of maximum size is produced in the specialized zygote called 'auxospore'. It follows that sex has an additional fundamental role in the life cycle of these microalgae.

I will focus on marine planktonic diatoms, and will provide an overview of the general architecture of the sexual phase in these microalgae. Evidence for sex is largely based on 'visual observations' from studies carried out with cultures in the laboratory, but reports became available also in the natural environment. Cell size dictates the window for sex, but it is not the sole control. Experimental studies are exploring endogenous and exogenous cues for sexualization and the increasing availability of genomic resources allow unveiling the molecular mechanisms that regulate different aspects of the sexual phase. The sex determination system, the capability to perceive cells of the opposite mating type, the mechanisms regulating gametogenesis and gamete conjugation, are key points for understanding the sexual behavior of diatoms.

THE CYANOBACTERIAL ANCESTOR OF EUKARYOTIC CHLOROPLASTS PINPOINTED

David Moreira (**CNRS France**), **Rafael Ponce** (**CNRS France**), **Purificacion Lopez-Garcia** (**CNRS France**), **Philippe Deschamps** (**CNRS France**).

It is widely accepted that eukaryotes acquired photosynthesis by the endosymbiosis of a cyanobacterium within a heterotrophic eukaryotic host. This occurred in a lineage that diversified into three contemporary groups of primary photosynthetic eukaryotes: Viridiplantae (green algae and land plants), Rhodophyta and Glaucophyta, grouped collectively within the eukaryotic superphylum called Archaeplastida or Plantae. Subsequently, red and green algae participated in a number of secondary endosymbioses that gave rise to a vast diversity of algal groups (diatoms, dinoflagellates, chlorarachniophytes, etc.). An important question that remains unclear concerns the identity of the cyanobacterium involved in the initial primary endosymbiosis. Attempts to identify the cyanobacterial lineage most closely related to chloroplasts have been done using phylogenetic analysis of different markers, which led to contradictory results. Most phylogenetic trees, either based on chloroplast genes or on nuclear genes of chloroplast origin, placed chloroplast as a very early-diverging branch in the cyanobacterial tree, not closely related to any contemporary cyanobacterial lineage. However, some recent analyses retrieved chloroplasts as a late-diverging branch related to nitrogen-fixing filamentous cyanobacteria. Such contradictory results can be explained either by the lack of phylogenetic signal in the sequence datasets or by the lack of sequences for the actual cyanobacterial sister group of chloroplasts. We have tackled this question using a double approach. First, we increased the cyanobacterial taxonomic sampling by sequencing the genomes of two early-diverging species recently isolated: *Gloeomargarita lithophora* and *Synechococcus calcipolaris,* both coccoid unicellular species. Second, we analyzed three complementary sequence datasets: i) chloroplast 16S+23S rRNA genes, ii) 102 chloroplast-encoded protein-coding genes, and iii) 154 nucleus-encoded protein-coding genes, including in all cases the corresponding cyanobacterial homologues. Maximum likelihood and Bayesian inference phylogenetic analyses of these three datasets retrieved identical results concerning the position of chloroplasts: in all cases, chloroplasts emerged as sister group of *G. lithophora* with maximal statistical support. Thus, chloroplasts appear to derive from a lineage that diverged early during cyanobacterial evolution. That lineage contains freshwater non-filamentous nitrogen-fixing species, very often found in high-temperature environments. This discovery has important implications for the mechanism and the environmental conditions regarding the primary chloroplast endosymbiosis.

HIGH PREVALENCE OF THE 'BOVINE GENOTYPE' T. FOETUS IN DOMESTIC PIG FAECAL SAMPLES AND TRANSCRIPTOMIC COMPARISONS OF THE PORCINE, BOVINE AND FELINE T. FOETUS ISOLATES

Victoria Morin-Adeline (University of Sydney), Ana Conesa (Prince Felipe Research Centre and University of Florida), Jan Šlapeta (University of Sydney).

Bovine reproductive tract disease caused by the flagellate *Tritrichomonas foetus* is a notifiable disease in Australia. Recently, *T. foetus* has also been implicated as a cause of chronic large bowel diarrhea in domestic cats worldwide. While *T. foetus* is pathogenic in both cattle and cats, it has long been established that the same *T. foetus* also colonizes the stomach, caecum and nasal cavity of pigs without apparent clinical significance. Previous multi-locus genotyping has grouped the non-pathogenic porcine *T. foetus* with the pathogenic 'bovine genotype', rather than with the 'feline genotype' *T. foetus*. Indeed, it was shown that the porcine *T. foetus* is experimentally capable of causing disease in cattle. While bovine trichomonosis is now uncommon due to wide-spread use of artificial insemination, whether *T. foetus* remains prevalent in pigs where bovine trichomonosis has been eradicated remains unknown. We report the first firm evidence of *T. foetus* 'bovine genotype' present in Australian domestic pigs farmed in close proximity to *T. foetus*-negative cattle and establish a reference strain of porcine *T. foetus* designated as PIG30/1. Multi-locus genotyping at 10 loci match PIG30/1 identically to the 'bovine genotype' *T. foetus*. We sequence the transcriptome of PIG30/1 and show that it demonstrates, at a cell wide level, a closer relationship with the 'bovine genotype' *T. foetus* than the 'feline genotype' *T. foetus*. Furthermore, we compare virulence factors between the porcine, bovine and feline isolates and identify candidates that may determine parasitic potential of *T. foetus*.

THE EVOLUTIONARY ORIGIN OF MITOCHONDRIAL CRISTAE FROM ALPHA-PROTEOBACTERIA

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Mitochondria are organelles of endosymbiotic origin that produce most of the cell's energy in the presence of oxygen. This essential bioenergetic function tightly depends on carefully regulated membrane invaginations of the inner mitochondrial membrane that create cristae. In recent years, the development of cristae has been shown to be caused by a protein complex called MICOS (MItochondrial Contact Site and Organizing System). We studied the evolutionary history of MICOS to shed light into the origin and evolution of mitochondrial structure. We report that MICOS is widespread among eukaryotes, suggesting its ancient nature. We also show that the phylogenetic distribution of MICOS matches that of acristate anaerobic mitochondria, indicating that MICOS loss is a critical event in the structural simplification of mitochondria in anoxic environments. Furthermore, we found that among prokaryotes, only alpha-proteobacteria (the mitochondrial progenitors) have a homologue of MICOS' core protein Mic60, which suggests that MICOS has a pre-endosymbiotic origin. Interestingly, intracytoplasmic membranes (ICMs), some of which resemble mitochondrial cristae, are phylogenetically widespread among alpha-proteobacteria. We therefore propose that mitochondrial cristae evolved from bioenergetic ICMs in 'purple bacteria'. This study exposes a new and unexpected deep evolutionary connection between mitochondria and their bacterial progenitors, and contributes to the understanding of the evolution of prokaryotic organelles. Undergoing work attempts to experimentally prove the homology hypothesis between mitochondrial cristae and alpha-proteobacterial ICMs.

PHYLOGENETIC POSITION OF METCHNIKOVELLIDS (MICROSPORIDIA: METCHNIKOVELLIDAE)

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Metchnikovellids is a group of hyperparasites of gregarines inhabiting alimentary tract of polychaetes and some other invertebrates. Despite long history of discovery (first described at the end of 19th century), metchnikovellids remain poorly studied. It is difficult to find and isolate these hyperparasites; publically available molecular data are absent. The most of evolutionary hypotheses, based on morphology, consider metchnikovellids as a deviated group of microsporidia. Recent studies of organisms branching close to animal-to-fungi boundary resulted in recognition of ARM clade (Aphelida + Rozellida + Microsporidia), the members of which were united in the superphylum Opisthosporidia. The position of metchnikovellids may be one of the decisive points to recover the relationships of lineages within ARM clade.

We isolated and re-described *Metchnikovella incurvata* Caullery and Mesnil 1914 from the samples collected at Kandalaksha Gulf of the White Sea, Russia. This metchnikovellid infects gregarines *Polyrhabdina* sp. from the intestines of polychaetes *Pygospio elegans* (Sokolova et al. Parasitology (2013) 140: 855-867). Using micromanipulations and single-cell PCR technique we amplified and cloned SSU rDNA and beta-tubulin genes from trace amount of genomic DNA extracted from the individually isolated cysts of *M. incurvata*. Molecular cloning of amplicons revealed unusual length polymorphism in variable regions of SSU rDNA gene due to the presence of numerous tandem repeats. Two-gene phylogenetic inference and analysis of molecular signatures in SSU rDNA gene suggested that metchnikovellids are the earliest branching group of microsporidia. Isolation of other representatives of metchnikovellids and including more genes in the analysis is of a key importance for further understanding of origin and evolutionary history of microsporidia and related lineages.

Supported by RFBR grant 15-04-08870 and SPSU projects (1.38.251.2014 and 1.41.555.2015).

AIRBORNE SPREAD OF PNEUMOCYSTIS JIROVECII AND NOSOCOMIAL INFECTIONS

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Airborne transmission of *Pneumocystis* sp. from host-to-host has been demonstrated in rodent models and occurrence of *Pneumocystis* pneumonia (PCP) case clusters in hospital suggests that interindividual transmission occurs in humans. Moreover, it is accepted that the *Pneumocystis* organisms infecting each mammalian species are host specific and that the hypothesis of an animal reservoir for *Pneumocystis jirovecii* (*P. jirovecii*), the human-specific *Pneumocystis* species, can be excluded. Since no exosaprophytic form of the fungus has been identified yet, these data point toward the potential for the specific host to serve as its own reservoir and for *Pneumocystis* infection in humans to be an anthroponosis with humans as a reservoir for *P. jirovecii.* The present communication highlights the main data on host-to-host transmission of *Pneumocystis* sp. by the airborne route in rodent models and in humans through the occurrence of PCP case clusters in hospitals. Particularly, we describe an outbreak of *P. jirovecii* infections that occurred at Brest University Hospital (Brest, Brittany, France). Eighteen renal transplant recipients (RTRs) developed *P. jirovecii* infections at the renal transplantation unit from May 2008 through April 2010, whereas no cases of *P. jirovecii* infection had been diagnosed in this unit since 2002. This outbreak was investigated by identifying *P. jirovecii* types and analyzing patient encounters. The results provided additional data on the role of PCP patients and, to our knowledge, the first data on the role of colonized patients as potential sources of *P. jirovecii* in a context of nosocomial acquisition of the fungus. In 2010, quantitative data on spread of *P. jirovecii* in exhaled air from PCP patients have been reported. Likewise, in 2015, quantitative data on spread of *P. jirovecii* in exhaled air from colonized patients were reported. These results emphasize the risk of nosocomial transmission of *P. jirovecii* via the airborne route from patients harboring the fungus, whatever the presentation of their *P. jirovecii* infection (pulmonary colonization or PCP). The CDC recommend applying standard precautions and avoiding placement of a patient developing PCP in the same room with an immunocompromised patient. There are now sufficient data to extend this recommendation to colonized patients but also to replace standard precautions with droplet precautions.

EVOLUTIONARY HISTORY OF THE ARGININE DEIMINASE PATHWAY AMONG EUKARYOTES

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Multiple prokaryotic lineages use the arginine deiminase (ADI) pathway for anaerobic energy production by arginine degradation. Eukaryotes have been thought to be generally devoid of this pathway, with two notable exceptions: trichomonads and diplomonads, closely related groups of protists living in low-oxygen niches. Our survey of newly available genomic and transcriptomic data shows that the complete ADI pathway is present also in representatives of Preaxostyla, Heterolobosea, Breviatea, Amoebozoa, and Chlorophyta, while individual enzymes constituting the pathway are distributed among almost all other major eukaryotic groups in a patchy pattern. The subsequent phylogenetic analyses suggest a complicated evolutionary history of the ADI pathway enzymes within the eukaryotic domain driven by multiple losses of the individual enzymes, duplications, and lateral gene transfers. Two points arise as the most important from these analyses: 1) the ADI pathway is likely an ancestral feature of Metamonada and 2) the results are consistent with presence of the ADI pathway in LECA as a result of vertical inheritance from the archaeal ancestors of eukaryotes.

CHOLESTERYLSULFATE SYNTHESIZED VIA THE MITOSOME-COMPARTMENTALIZED SULFATE ACTIVATION PATHWAY IS REQUIRED FOR ENCYSTATION OF ENTAMOEBA

Tomo Nozaki (National Institute of Infectious Diseases).

Hydrogenosomes and mitosomes are mitochondrion-related organelles (MROs) in anaerobic/microaerophilic eukaryotes with highly reduced and divergent functions. *Entamoeba* possesses a highly divergent MRO known as the mitosome. The biological functions and their origin of *Entamoeba* mitosomes have been a longstanding enigma in the evolution of mitochondria. We previously demonstrated that sulfate activation, which is not generally compartmentalized to mitochondria, is a major function of *E. histolytica* mitosomes. However, as final metabolites of sulfate activation remain unknown, the overall scheme of this metabolism and the role of mitosomes in *Entamoeba* have not been elucidated. We recently purified and identified cholesteryl sulfate (CS) as a final sulfate activation metabolite. We futher identified the gene encoding the cholesteryl sulfotransferase responsible for synthesizing CS. Addition of CS to culture media increased the number of cysts, while, conversely, chlorate, a selective inhibitor of the first enzyme in the sulfate activation pathway, inhibited cyst formation. These results indicate that CS plays an important role in differentiation, an essential process for transmission of *Entamoeba* between hosts. Furthermore, *Mastigamoeba balamuthi*, an anaerobic, free-living amoebozoan species, also has the sulfate activation pathway in MROs, but does not possess the capacity for CS production. Hence, we proposed that a unique function of MROs in *Entamoeba* contributes to adaptation of its parasitic life cycle.

Understanding of metabolite trafficking across the two mitosomal membranes is important to understand metabolic functions of mitosomes. We recently discovered a novel mitosomal β-barrel outer membrane protein of 30 kDa (MBOMP30) by in silico prediction. We experimentally confirmed that MBOMP30 is indeed a β-barrel protein by circular dichroism analysis. Localization and integration of MBMP30 to mitosomes was verified by Percoll-gradient fractionation, carbonate fractionation, proteinase K digestion, immunofluorescence assay, and immunoelectron microscopy. MBOMP30 represents only the seventh subclass of eukaryotic BOMPs discovered to date and lacks detectable homologs outside *Entamoeba*, suggesting that it may be unique to *Entamoeba* mitosomes.

MOLECULES AND MOLECULAR COORDINATION DURING PHAGOCYTOSIS OF ENTAMOEBA HISTOLYTICA

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Phagocytosis of *Entamoeba histolytica* trophozoites is a fascinating example of molecular coordination during this cellular event, since the target recognition until the cargo digestion or recycling. Our aim is to identify molecules and intermolecular interactions involved in this phenomenon. Earlier, we disclosed the EhCPADH complex, an important player in phagocytosis of trophozoites, formed by EhCP112, a cystein protease, and EhADH, an ALIX family member with a Bro1 and an adherence domain. By its adherence domain, EhADH acts as a receptor for erythrocytes. Here, we showed that in addition to EhCP112, EhADH binds to EhVps32 (a member of the ESCRT machinery), after ingestion of target cell, and to LBPA and cholesterol mainly in the late phases of erythrophagocytosis. After erythrocytes ingestion, EhVps32 forms a cap around the phagosomes to generate the multivesicular bodies. Ehvps32-overexpressing trophozoites increased twice their rate of erythrophagocytosis and EhVps32-silenced trophozoites ingested 80% less erythrocytes than the wild type strain, pointing out the EhVps32 importance in phagocytosis. On the other hand, cholesterol sequestering in vesicles, diminished the rate of erythrophagocytosis and the ability of trophozoites to damage epithelial cell monolayers. Thus, our group and others have elucidated key molecules involved in different steps of phagocytosis, to be used as markers in the study of this important virulence-related event and to development new pharmacology strategies against amoebiasis.

COMBINED CULTURE-BASED AND CULTURE-INDEPENDENT APPROACHES PROVIDE INSIGHTS INTO DIVERSITY OF JAKOBIDS, EXTREMELY PLESIOMORPHIC EUKARYOTIC LINEAGE

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We use culture-based and culture-independent approaches to discover diversity and ecology of anaerobic jakobids (Excavata: Jakobida), an overlooked, deep-branching lineage of free-living nanoflagellates related to Euglenozoa. It belongs among a few lineages of nanoflagellates frequently detected in anoxic habitats by PCR-based studies, however only two strains of a single jakobid species have been isolated from those habitats. We recovered 712 environmental sequences and cultured 21 new isolates of anaerobic jakobids that represent at least 10 different species. Two species have never been detected by environmental, PCR-based methods, and at least 4 still remain uncultured. Our phylogenetic analyses based on SSU rDNA and six protein-coding genes showed that anaerobic jakobids constitute a clade of morphologically similar, but genetically and ecologically diverse protists – Stygiellidae fam. nov. Our investigation combines culture-based and environmental molecular-based approaches to capture a wider extent of species diversity and shows Stygiellidae as a group that ordinarily inhabits anoxic, sulfide- and ammonium-rich marine habitats worldwide.

PROTOZOAN DIVERSITY IN ACTIVATED SLUDGE FROM MBR SYSTEMS

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Membrane Bioreactor (MBR) systems and Advanced Wastewater Treatment Plants (AWWTP) showed different communities of protists than those observed in Conventional Activated Sludge Process (CASP), with different bioindicator organisms than those used in the Sludge Biotic Index (SBI) in CASP. Because of this, the goal of this research was to analyze the role of certain groups and protist species as well as the use of SBI in activate sludge from urban wastewater through a denitrification-nitrification MBR systems during two years. Sludge Retention Time (SRT) and Hydraulic Retention Time (HRT) were modified and acted as variables, together with temperature and variation in organic loading. With SRT values of 20 to 35 days, HRT of 32 to 40 h, four ranges of temperature (< 15, 16-20, 21-25 and > 26 º C), organic loading 0.4 to 1.1 Kg DQO/d m3. Under these operational conditions suspended solid in mixed liquor (MLSS) vary between 1-16 g/L. These operational conditions, different from any CASP, cause a specific development of the microfauna, so the assigned role as indicator of different species and protist groups are not the same in MBR systems and AWWTP. Our results show that indexes used to evaluate sludge quality, such as SBI, do not seem applicable. The group of crawling ciliates (positive group in SBI) lost prominence in MBR systems and AWWTP. *Chilodonella* spp. showed high Pearson´s correlations coefficients with effluent quality in parameters of DBO5 (0.161**) and COD (0.122**), indicating low effluent quality in MBR systems. Small flagellates (negative group in SBI) and naked amoeba (not included in SBI) showed positive association with the effluent quality in MBR systems and associated to good nitrification in MBR and AWWTP, the opposite role is assigned in CASP associated with low effluent quality and poor performance of the systems. Swimming and carnivorous ciliates showed low abundance and are occasionally observed in activate sludge from MBR systems and AWWTP compared with CASP.

MECHANISMS OF DISCRIMINATION AND KIN RECOGNITION: FROM UNICELLULAR TO MULTICELLULAR EUKARYOTES

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Because unicellular eukaryotes are the evolutionary precursors of multicellular life, we infer that their mechanisms of taxa-, clone-, and possible kin-discrimination gave origin to the complex diversification and sophistication of traits associated with species and kin recognition in plants, fungi, invertebrates and vertebrates. We will discuss exemplar cases of taxa-, clone-, and possible kin-discrimination in five major lineages: Mycetozoa, Dikarya, Ciliophora, Apicomplexa and Archamoebae. We will summarize the proposed genetic mechanisms involved in discrimination-mediated aggregation (self versus different) and the Proliferation Activation Factors (PAFs), which facilitate clustering in some protistan taxa. We shall caution about the experimental challenges intrinsic to studying recognition in protists, and highlight the opportunities for exploring the ecology and evolution of complex forms of cell-cell communication, including social behavior, in a polyphyletic, still superficially understood group of organisms.

INORGANIC PYROPHOSPHATE METABOLISM IN PROTISTS: METABOLIC AND EVOLUTIONARY IMPLICATIONS

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Inorganic pyrophosphate (PPi) is a metabolite generated from ATP in many anabolic reactions. Most of these reactions are reversible, therefore, an accumulation of PPi would produce a collapse in the synthesis of biomolecules. Consequently, PPi removal is important in order to pull anabolic reactions in the direction of biosynthesis. In organisms such as animals and fungi, this removal is accomplished by enzymes that hydrolyse PPi releasing heat, the so-called soluble inorganic pyrophosphatases; however, in other eukaryotic organisms like plants and many protists other PPi-utilising enzymes have been identified and characterised. Amongst these are the PPi-dependent phosphofructokinase and the membrane-bound Na+/H+ inorganic pyrophosphatases. Recently, PPi has been reported to be a critical element of cellular metabolism both as an energy donor and as an allosteric regulator of several metabolic pathways in some protists. The occurrence of a PPi-dependent metabolism in parallel to the "classical" ATP-dependent routes provides these organisms with a remarkable metabolic flexibility. A broad view on the metabolism of PPi in protists and its evolutionary implications will be given in this communication.

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CILIATES AS NATURAL RESERVOIR OF POTENTIALLY PATHOGENIC BACTERIA: STATE OF THE ART AFTER A FOUR YEAR NETWORKING PROJECT

Giulio Petroni (Department of Biology, University of Pisa).

Most protists can harbour microorganisms in a symbiotic relationship. Normally benign, in some cases these microbes could prove to be pathogenic to humans and animals like in the well-known case of *Legionella* and *Acanthamoeba*. The recently ended CINAR PATHOBACTER project was aimed at evaluating the possibility that also ciliates could act as natural reservoir of potentially pathogenic bacteria. The project involved ten laboratories from European and non-European countries including Italy, Germany, France, Canada, USA, Japan, Russia, Brazil and India. During the four-year project, the consortium characterised many novel symbiotic bacteria living in ciliates. Several of these symbionts showed phylogenetic affinities to pathogenic bacteria especially to the Order Rickettsiales (Alphaproteobacteria, more than 13 new genera and 18 new species discovered) and to the Family Francisellaceae (Gammaproteobacteria). Additionally, several new bacterial symbionts phylogenetically associated to non-pathogenic bacteria have been described. Ecological, functional and evolutionary genomic investigation on some selected symbionts has been also performed and are ongoing. The joint international collaboration has considerably enhanced research capacity and knowledge exchange on ciliates and their bacterial symbionts among involved laboratories and will hopefully boost future related research collaborations on an international scale. An overview of achieved results will be presented with a special emphasis on ongoing molecular and ultrastructural characterization on newly retrieved Rickettsiales.

A STILL NOT WELL-KNOWN PARASITE: BALANTIDIUM SP., EPIDEMIOLOGY AND DIAGNOSTIC TOOLS

Francisco Ponce-Gordo (Complutense University, Spain).

The genus *Balantidium* includes ciliated protozoa inhabiting the intestine of a broad spectrum of vertebrate hosts. Despite there have been several taxonomic controversies, it seems that there is only one species infecting mammals, *Balantidium coli* (recently renamed *Balantioides coli*). It is transmitted from one host to another by a fecal-oral route; the cysts (the infective stage) are shed in feces and can be easily identified. *Balantidium* (or *Balantioides*) coli is commonly found in pigs and other suids, and it can also infect humans being usually asymptomatic. The data available from epidemiological studies indicate that human infections by this parasite (most commonly detected as secondary findings in coprological analysis) are related to close contact with pigs and to poor condition of the water sources. Its pathogenicity is controversial and it is possible that this ciliate could be a commensal species that becomes, in some situations, an opportunistic, parasitic species that may lead to disease and even to the death of the infected host. It is possible that pathogenicity depends on a combination of host (individual susceptibility, diet, other infections) and parasite (genetic variants) characteristics; however the results from the studies in human hosts are controversial and there are very few data about the biochemistry of the parasite (in part due to the lack of adequate, successful culture protocols). The genetic characterization of *B. coli* is limited to the analysis of ribosomal genes and these have been done in a small number of isolates (including, to date, only one human isolate). These genetic analyses have shown unusual results (two different genetic variants within each single cell) but they have also shown that there is a great uniformity in the sequences of the highly variable regions of these genes, this apparently confirming that there is only one single species infecting mammals (and birds). In conclusion, despite *B. coli* is the largest protozoa inhabiting the human intestine and it can be easily identified, in fact little is known about it and further genetic analysis of other genes and of more isolates are encouraged.

PHYLOGENETIC POSITION OF NEPHRIDIOPHAGIDAE AT THE FUNGAL ROOT AND DESCRIPTION OF A NEW SPECIES OF NEPHRIDIOPHAGA

Renate Radek (**Free University of Berlin**), **Kerstin Voigt** (**Friedrich Schiller University Jena**), **Christian Wurzbacher** (**Berlin Center for Genomics in Biodiversity Research, Berlin**), **Sebastian Gisder** (**Institute for Bee Research, Hohen Neuendorf**), **Anja Owerfeldt** (**Free University of Berlin**).

Nephridiophagids are unicellular, spore-forming parasites infecting the Malpighian tubules of insects. Their life cycle includes merogony with multinucleate plasmodia and sporogony leading to small, uninucleate spores. The systematic position of the nephridiophagids has been thoroughly discussed. Morphological features did not allow a clear affiliation with one of the known protist groups. A first molecular study of one species suggested a relation to the fungus taxon Zygomycota. We now included three species in our phylogenetic approach, including one yet undescribed *Nephridiophaga* species from the Madeira cockroach *Leucophaea maderae*. Besides its specific host, the new species slightly differs from known ones by the size of its spores and by the number of spores within the sporogenic plasmodium. The constructed trees on the basis of 18S-rRNA sequences show that nephridiophagids belong to the fungal base. In order to prevent a biased view by long branch attractions we calculated bayesian trees on a conservative species selection and found a polytomy of nephridiophagids with flagellated fungi. Thus they may represent a novel basal fungal phylum.

NEW INTRACELLULAR SYMBIONTS OF PARAMECIA

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Paramecium is one of the most studied Ciliate's genera and there are many prokaryotic symbionts described for different *Paramecium* species. Bacteria occupy specific species and specific cell compartments: cytoplasm, both nuclei, perinuclear space.

We revealed many never-before-seen symbiotic bacteria in Paramecia during our expeditions for collecting living Paramecia. Here´s short description of our findings.

New *Holospora* species infecting *P. putrinum* macronucleus were found in Yakutia Republic. Sergey Fokin previously found probably the same bacteria in Italy, but their identification is questionable without sequencing 16S rDNA.

We found new two strains of P. putrinum with non-*Holospora* symbiotic bacteria inhabiting both macronucleus and micronucleus. One strain originate from Buryat Republic, the second one from Kamchatka peninsula. It is noteworthy that these two stands are separated by three thousand kilometers.

We isolated new macronuclear symbiont of P. bursaria. These bacteria have the ability to infect aposymbiotic cells, but morphologically differ from well-known *H. curviuscula*.

Other *P. bursaria* strain are hosts for very small cytoplasmic bacteria that are localized in vacuoles, from few to dozens in each vacuole. None of previously described cytoplasmic bacteria of *P.bursaria* had surrounding cell membrane.

We also found new isolates of cytoplasmic symbionts in species of *P. aurelia* complex, *P. caudatum, P. multimicronucleatum, P. nephridiatum* and new isolates of so-called "*Nonospora*-like" bacteria from *P. caudatum* macronucleus.

We sequenced 16S rDNA to clarify systematic position of these uncultured bacteria. Distribution of *Paramecium* symbionts among main bacterial groups will be presented.

MOLECULAR AND MORPHOLOGY METHODS FOR THE ASSESSMENT OF MARINE DINOFLAGELLATES DIVERSITY: DO THEY AGREE?

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High-throughput sequencing (HTS) is a powerful tool to study the community composition and diversity of microorganisms. It is the only way to explore the diversity of protist communities of small size (20 µm observed by microscopy and 454 sequencing was compared. Some morphospecies observed by microscopy were not present in HTS dataset and vice versa, highlighting the limitations of both methods. The lack of resolution of the sequenced region became apparent in some cases. Consequently, the relative abundance obtained by both methods for a given species agreed in some cases, but strongly differed in others. In order to conciliate the microscopy and molecular methods, the results obtained using different clustering thresholds and methods were explored for some genera in particular. Our results highlight useful insights but also important biases in the molecular assessment of dinoflagellate diversity.

DETECTION OF ACANTHAMOEBA STRAINS IN THE OCULAR SURFACE OF CONTACT LENS WEARERS USING THE SCHIRMER STRIP TEST

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Pathogenic strains of the opportunistic parasite *Acanthamoeba* are causative agents of a sight-threatening infection of the cornea known as *Acanthamoeba* keratitis (AK). This disease is often associated with an improper handling of contact lenses, such as poor hygiene or extended use over time. In a previous study, our laboratory demonstrated the presence of *Acanthamoeba* in the ocular surface of healthy individuals. Therefore and in order to move to the next level, it was decided to study if *Acanthamoeba* are present on the ocular surface of contact lens wearers since they are the potential host of these pathogens. For this reason, the aim of this study was to determine the presence of *Acanthamoeba* in the ocular surface of healthy patients using contact lenses sterile Schirmer test strips. All samples included in the study, were collected from a group of individuals attending a local ophthalmology consultation. In some cases, contact lenses and their cases were also investigated for the presence of amoebae.

The samples were collected in sterile conditions and were quickly seeded in 2% Non-Nutrient Agar (NNA) plates. The positive samples were then transferred to axenic conditions for further molecular analysis. All the isolated strains were classified as *Acanthamoeba* genotype T4 and osmotolerance and thermotolerance assays revealed that all strains were potentially pathogenic as they were able to grow at temperatures higher than 37 ºC and under osmotic pressure. To conclude, the Schirmer strip test is proposed as an effective tool for the detection of *Acanthamoeba* in ocular surface and also as a tool to isolate *Acanthamoeba* strains from healthy patients, who are contact lens wearers, a population at risk of suffering AK.

Funded by grants: RICET/ RD12/0018/0012, Project PI13/00490 , Project ref. AGUA3 ALA and MRB were funded by Becas de Investigación Obra Social La Caixa-Fundación Cajacanarias para Postgraduados 2014". JLM was supported by the Ramón y Cajal Subprogramme RYC-2011-08863.

THE DIVERSITY AND ORIGINS OF ANAEROBIC METABOLISM IN MITOCHONDRIA AND RELATED ORGANELLES

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Protists that experience hypoxia often possess metabolically distinct mitochondria called mitochondrion-related organelles (MROs). Although there are common metabolic features shared between MROs of distantly related lineages, these organelles have evolved independently multiple times in eukaryote evolution. Until recently our knowledge of the metabolic potential of such organelles was skewed towards those of parasitic protists. Over the last decade deep-sequencing studies of free-living facultatively or premanently anaerobic protists have revealed novel configuations of metabolic pathways that allow these organisms to thrive in low oxygen conditions. Here I review the predicted functions of MROs from free-living protists and their parasitic relatives. Using the various observed 'types' of MROs across eukaryotic diversity, I discuss scenarios for how the various metabolic features of these organelles have evolved.

A CHLAMYDOMONAS GENE CO-EXPRESSION NETWORK REVEALS GLOBAL PROPERTIES OF ITS TRANSCRIPTOME AND THE EARLY ESTABLISHMENT OF KEY CO-EXPRESSION PATTERNS IN THE GREEN LINEAGE

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The unicellular green alga *Chlamydomonas reinhardtii* has been used to study the establishment, conservation and divergence of key biological processes in photosynthetic organisms [1–3]. *Chlamydomonas* has also attracted interest for biotechnological applications in biofuel and hydrogen production [4–6]. Recently, massive amounts of RNAseq transcriptomic data have been produced from *Chlamydomonas* grown in diverse physiological conditions. Gene co-expression networks integrate transcriptomic data from different conditions and ecotypes in order to characterize patterns of coordinated gene expression at the whole transcriptome level. Nodes represent genes that are connected by an edge if the corresponding genes are significantly co-expressed [7] .We constructed a gene co-expression network and developed a web-based tool, ChlamyNET, for the exploration of the *Chlamydomonas* transcriptome. The topological properties of ChlamyNET suggest that the *Chlamydomonas* transcriptome possesses important characteristics related to error tolerance, vulnerability and information propagation. Clustering techniques identified nine gene clusters that can explain *Chlamydomonas* transcriptome structure under the analyzed conditions. ChlamyNET center constitutes the core of the transcriptome where most authoritative hub genes are located, interconnecting key biological processes such as light response with carbon/nitrogen metabolism. A great number of transcription factors and regulators in the *Chlamydomonas* genome were identified, demonstrating that key elements in the regulation of metabolism, light response and cell cycle, identified in higher plants, were already established in *Chlamydomonas*. These conserved elements are not only limited to transcription factors, regulators and their targets, but also include the cis-regulatory elements recognized by them. Finally, we used an independent data set to cross-validate the predictive power of ChlamyNET.

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PARAMECIUM AND ITS MOTILE ENDOSYMBIONTS: FOR BETTER AND FOR WORSE

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Paramecium species are known to harbor diverse endosymbiotic bacteria. Most often, endosymbionts demonstrate specific localization in the host cell, being either nuclear or cytoplasmic. However, recently described *"Ca. Trichorickettsia mobilis"* occurs both in the macronucleus of *P. multimicronucleatum* and in the cytoplasm of *P. calkinsi*. The endosymbiont is extremely motile, especially the nuclear one. Antibiotic treatment of several cell lines of *P. multimicronucleatum* bearing *Trichorickettsia* in the macronucleus revealed strong correlation between the presence of bacteria in the host cell and its survival, suggesting that the specific host strains might be dependent on its prokaryotic partner. This is particularly intriguing, taking into consideration the changes in the macronuclear architecture of the host shown by TEM.

In antibiotic treated cells, oval non-motile forms of bacteria were observed both in the nucleus and in vesicles in the cytoplasm. These oval shaped bacteria might be presumably considered L-forms of *Trichorickettsia*. L-forms are known to account for latent infection in many pathogens.

The endosymbiont of *P. calkinsi,* though always showing cytoplasmic localization, belongs to the same species, as confirmed by 16S rDNA sequencing and FISH using a species specific oligonucleotide probe. Flagella of the cytoplasmic *Trichorickettsia* differ in their appearance from those of other known endosymbionts of ciliates. High density of bacteria in the cytoplasm seems to affect host cell division, leading to micronuclear loss, formation of monsters and strain extinction. Surviving hosts often demonstrate peculiar wriggling movement and distorted cell shape.

A great part of this work was performed at the Core Facility Centers for Microscopy and Microanalysis and for Molecular and Cell Technologies of St.-Petersburg State University. Some ciliate strains were provided by the Core Facility Center for Cultivation of Microorganisms, St.-Petersburg State University. Financial support was provided by the European Commission FP7-PEOPLE-2009-IRSES project CINAR PATHOBACTER (247658) and the RFFI grant no. 15-04-06410 to E. Sabaneyeva.

NATURE AND SPECIFICITY OF DIATOM-BACTERIA INTERACTIONS IN MARINE INTERTIDAL SEDIMENTS

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It is becoming increasingly clear that many protists are engaged in intense and often highly specific interactions with bacteria. In the present study, we investigated the nature and specificity of diatom-bacteria interactions in marine intertidal biofilms. In addition, we performed experiments to assess whether these interactions can be modulated by bacterial density and the composition of the background microbial community. 16S rDNA amplicon sequencing was used to assess variation in the associated bacterial communities of about 80 strains (isolated from various estuarine and marine tidal flats in The Netherlands, Belgium and France) of the common and widespread benthic diatom *Cylindrotheca closterium*. The observed variation patterns were then related to host phylogeny, environment and geographic location. A first set of co-culture experiments (43 diatom-associated bacterial isolates, one *C. closterium* strain) revealed strong negative but also positive effects of the bacteria on diatom growth. A second set of co-culture experiments showed that *Cylindrotheca* strains were differentially impacted by a selection of bacteria (selected on the basis of a clear positive or negative impact in the first set of co-culture experiments). Finally, we found that the effect was density–dependent, and that the antagonistic effect of a *Marinobacter* sp. could be neutralized with a mixed bacterial inoculum from the natural habitat of the diatom (marine tidal flat) but not with a 'foreign' bacterial inoculum (forest soil). Our study thus clearly shows that a wide range of diatom-bacteria interactions exists, ranging from antagonistic to synergistic, and that these interactions are to a certain degree species- and strain-specific. In addition, these interactions can be modified by the density of the bacterial inoculum, and the presence of other bacteria in the cultures. Apart from effects of the bacteria on diatom fitness, the bacteria also caused remarkable behavioural changes in growth form and aggregation patterns of the diatom strains.

PERCEPTIONS OF BIOGEOGRAPHY: CORRESPONDENCE BETWEEN MOLECULES AND MORPHOLOGIES IN TINTINNID CILIATES

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Tintinnid ciliates display well-known spatial and temporal distributions in marine plankton. Bathymetric, latitudinal and seasonal fluctuations in tintinnid distribution have been documented using mostly the morphology of the lorica attached to the cell. Although the lorica has been a valuable means for taxonomic and ecological studies for more than two centuries, limitations due to its plasticity are largely acknowledged, thus raising the question if examining the morphology alone has distorted the known distribution patterns. We are studying the correlation between morphological and molecular information at two levels, species and assemblages, using single cell sequencing and high-throughput sequencing (HTS). At the level of species delimitation, there is a general agreement between morphology and DNA sequences. Nevertheless, the relationship between both criteria is complicated by cases of polymorphism (different lorica morphologies corresponding to identical sequences), crypticity and pseudo-crypticity (identical or almost identical morphologies corresponding to divergent sequences), or low molecular resolution (different morphospecies that are identical in one molecular marker but different in others). At the level of assemblages, HTS has revealed rare or potentially novel taxa not observed in the microscope during environmental surveys in NW Atlantic waters. However, molecular operational taxonomic units have been less efficient than morphospecies to capture the relationship between distribution patterns and environmental factors. Despite the differences, both approaches have detected distinct inshore and offshore assemblages, hence supporting the known coastal versus oceanic biogeographical patterns. Assemblage fluctuations in terms of taxonomic composition and lorica sizes are related to proxies for prey quantity and quality, thus suggesting niche partitioning as a structuring mechanism in the waters examined. The combination of molecular and structural data, either by parallel morphological examination or via barcoded morphospecies included in public repositories, is key to understand tintinnid biogeographical patterns and underlying evolutionary and ecological processes.

THREE-DIMENSIONAL STRUCTURE OF RIVER BIOFILMS IN ITS IMPORTANCE FOR PROTOZOANS IN BIOFILMS

Anja Scherwass (University of Cologne), Annette Schluessel (), Heidrun Budde (), Hartmut Arndt (University of Cologne).

Biofilms serve as important habitats for protozoans. In the study presented in the talk we investigated the three-dimensional structure of a river-borne biofilm over a period of six months by staining of the exopolymeric matrix with fluorochromes and further analysis at the Laser-Scanning-Microscope. Additionally, the occurrence of the bacterial community within the microbial biofilms was observed. We investigated short-term grown biofilms (3 weeks old) and long-term grown biofilms (aged up to six months). Results concerning the biofilm structure revealed a variety of three-dimensional structures and indicatedthe maturing process for many features of the biofilm (e.g. biofilm volume, surface enlargement etc.).

Furthermore, in a parallel set of laboratory experiments, the sensitivity of surface-associated protozoans to increasing flow velocity in experiments in a flow chamber by the use of particle image velocimetry and conventional video particle tracking was investigated. Here, we focussed especially on the importance of sheltered biofilm regions for protozoans confronted with flow velocity. The experiments in the flow chamber revealed that even low flow velocities close to the substrate (0.001 m s-1) can cause the detachment of e.g. nanoflagellates and pointed out that sheltered regions within the biofilm are of importance for the maintenance of protists confronted with flow velocity.

KITCHEN GARDEN OR RECYCLING CENTER? COMPARATIVE GENOMICS OF THE KENTROPHOROS CILIATE-BACTERIA SYMBIOSIS

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The Karyorelictea are a group of ciliates found almost exclusively in marine sediments, where they graze on microbes living between the sediment grains. The exception to this lifestyle is the genus *Kentrophoros*, which lacks a defined oral apparatus and carries a dense layer of bacterial ectosymbionts on one surface of its body. The symbionts have been called a "kitchen garden" because they are thought to feed their host by fixing carbon dioxide, using reduced sulfur as an energy source. The host harvests bacteria from this "garden" by phagocytosis. To understand the metabolism of this symbiosis, we sequenced metagenomes from six morphospecies of *Kentrophoros* from Elba, Italy. Metatranscriptomes were also sequenced from two morphospecies.

Contrary to previous expectations, evidence for autotrophic fixation of carbon dioxide was lacking. Sequences encoding ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the key enzyme of the Calvin cycle for inorganic carbon fixation, could not be found in the symbiont genomes. Alternative pathways for inorganic carbon fixation also appear to be absent. However, both genomic and transcriptomic data indicate that heterotrophy, i.e. the assimilation of organic carbon, plays an important role: a complete oxidative tricarboxylic acid (Krebs) cycle, pathways for assimilation of small organic molecules such as acetate and propionate, and transporters for organic substrates. The symbionts appear to gain energy for organic carbon assimilation through sulfur oxidation, using the hybrid rDsr-Sox pathway.

We propose that the *Kentrophoros* symbionts are not an autotrophic "kitchen garden", but instead a heterotrophic "recycling center", using energy from reduced sulfur to assimilate fermentation waste products from the environment or the host for biosynthesis. Many symbiotic systems that are known to fix inorganic carbon have now been shown to also have the capacity for heterotrophy. The *Kentrophoros* symbiosis may show that symbionts can dispense with autotrophy entirely but still make a living and feed their hosts.

A HOUSE FOR TWO – DOUBLE BACTERIAL INFECTION IN EUPLOTES WOODRUFFI SQ1 (CILIOPHORA, EUPLOTIA) SAMPLED IN SOUTHEASTERN BRAZIL

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Several ciliated protists form symbiotic associations with a diversity of microorganisms, leading to drastic impact on their ecology and evolution. In this work two *Euplotes* spp. sampled in Rio de Janeiro – Brazil were identified based on morphological and molecular features as *Euplotes woodruffi* strain Sq1 and *E. encysticus* strain Sq2, and investigated for the presence of endosymbionts. While *E. woodruffi* Sq1 stably hosts two bacterial populations, namely *Polynucleobacter necessarius* (Betaproteobacteria) and a new member of the family "*Candidatus Midichloriaceae*" (Alphaproteobacteria, Rickettsiales), here described as "*Candidatus Bandiella woodruffii*", branching with a broad host range bacterial group found in association with cnidarians, sponges, euglenoids and some arthropods; in *E. encysticus* Sq2 no symbiotic bacterium could be detected. The dispersion ability of this novel bacterium was tested by co-incubating *E. woodruffi* Sq1 with three other ciliates. Among the tested strains "*Ca. B. woodruffii*" could only be detected in association with *E. encysticus* Sq2 with a prevalence of 20% after 1 week and 40% after 2 weeks, maintaining this level for up to 6 months. Nevertheless, this apparently in vitro association was abolished when *E. woodruffi* Sq1 donor were removed from the microcosm suggesting this bacterium has the capacity for at least a short term survival outside its natural host and the aptitude to ephemerally interact with other organisms. Together these findings strongly suggest the need for more detailed investigations to evaluate the host range for "*Ca. B. woodruffii*" and any possible pathogenic effect of this bacterium on other organisms including humans.

ACIDIFICATION-DEPENDENT DEFECTS IN MEMBRANE TRAFFIC AND AUTOPHAGY IN YEAST STEROL MUTANTS

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We have found that Δ8-unsaturated sterols (e.g. fecosterol), like those produced by morpholine fungicides, inhibit V-ATPases in *Saccharomyces cerevisiae*. This inhibition is not due to ergosterol depletion and is specific of this and a few other types of abnormal sterols. This is in agreement with some sterol (erg) mutants showing phenotypes similar to those of V-ATPase mutants. Photosynthetic eukaryotes are much less sensitive to the accumulation of abnormal sterols than fungi and, remarkably, they show two different primary proton pumps in endomembranes: V-ATPases and H+-pumping pyrophosphatases. Consequently, introduction of a second H+-pump (a vacuole-targeted quimaera of *Arabidopsis thaliana* AVP1) alleviates growth defects such as Zn and alkaline pH sensitivity in erg2Δ yeast mutants. Abnormal (Δ8-unsaturated) sterols accumulating mutants show also a set of other phenotypes such as endocytosis and exocytosis defects. We have analysed the endocytosis in erg2Δ mutants using Lucifer Yellow and found it to be reverted to near wild-type levels if AVP1 was heterologously expressed. We also found mistargeting of Pma1p to the vacuole in erg2Δ mutants, an exocytosis defect first observed in V-ATPase yeast mutants. Similar to endocytosis, this defect was also alleviated in erg2Δ cells when expressing the plant H+-pyrophosphatase. Autophagy is a evolution conserved process that helps recycling proteins and organelles in all eukaryotes. This process is based on the transport of vesicular structures named autophagosomes. These structures need to fuse with late endosomes or, directly, with the vacuole in order to deliver their cargoes to this last organelle for degradation by hydrolytic enzymes. It is well stablished that defects in the acidification of intracellular organelles impair the autophagic flux in yeast [1] and mammals [2]. Accordingly, we observed that erg2Δ yeast cells present impaired autophagy. Autophagy dysfunction in erg2Δ cells translates into the accumulation of PAS (Pre-Autophagosomal Structures) under normal growth conditions and AB (Autophagic Bodies) under nitrogen starvation conditions. Remarkably, accumulation of AB is dependent on organelle acidification while accumulation of PAS is exclusive of defects in sterol synthesis.

Supported by BFU2010-15622 grant (partially founded by EU-FEDER program).
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CHEMOTAXIS RESPONSE OF PHYTOPLANKTON TO THE EXUDATES OF CILIATES

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Marine environment at the micro scale level is heterogeneous in terms of nutrient distribution and many microbial species can actively exploit these nutrient patches. Exudates of microbes act as nutrient hotspots and also provide chemical cues to their prey or predators. In the present study we examined the chemotactic response of starved and/or non-starved *Dunaliella salina* to the exudates of ciliate grazers (*Euplotes vannus, Euplotes* sp., and *Diophrys oligothrix*) by using two approaches; 1. Co-culturing of two species to evaluate the growth, ingestion and behavioural response of the phytoplankton and/or ciliates and 2. Using microfluidics and image analyses to evaluate the response of *D. salina* to the exudates of ciliates and related nutrient media. *D. salina* showed active swimming and an 'attack-like' behaviour towards ciliates, despite the high cliate grazing rates on them. When exposed to the patches of ciliate exudates, f/2 growth medium, yeast extraction and ammonium solution, both starved and non-starved *D. salina* showed chemotactic accumulation on/around the exudate and nutrient patches. In both cases positive chemotaxis indices were found towards ciliate exudates suggesting that *D. salina* could actively uptake nutrients released by its ciliate grazers. This specific behavioural response however, could be costly to *D. salina*. We suggest that this behaviour could also serve as a defence mechanism against the grazers thereby increasing the benefits for *D. salina*.

NATURAL PRODUCTS AS A SOURCE OF POTENTIAL THERAPEUTICS AGAINST ACANTHAMOEBA INFECTIONS

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Acanthamoeba keratitis cases are increasing worldwide due to the higher number of contact lens wearers and a general lack of hygiene in the handling of lenses and their cases. The existence of a cyst stage in *Acanthamoeba* genus is the main concern in the development of effective therapeutic agents. Currently, the first line treatments against *Acanthamoeba* keratitis are not fully effective and highly toxic for the patients. Moreover, the treatment schemes are often lengthy making the patients recovery rate very low. We have been working on finding novel active compounds against these pathogens using natural products as a source. In this study, we present the obtained results so far from Olive Leaf Extracts and other natural products of Tunisian origin. The identified molecules highlight the potential of these sources as a good base for the development of novel anti-*Acanthamoeba* agents in the near future.

Acknowlegments:
This work was supported by the grants RICET (project no. RD12/0018/0012 of the programme of Redes Temáticas de Investigación Cooperativa, FIS), Spanish Ministry of Health, Madrid, Spain and and PI13/00490 "Protozoosis Emergentes por Amebas de Vida Libre: Aislamiento, Caracterización, Nuevas Aproximaciones Terapéuticas y Traslación Clínica de los Resultados" from the Instituto de Salud Carlos III. ALA was funded by a grant "Ayudas del Programa de Formación de Personal Investigador, para la realización de Tesis Doctorales" from the Agencia Canaria de Investigación, Innovación y Sociedad de la Información from the Canary Islands Government. CMMN was supported by Canary Islands CIE: Tricontinental Atlantic Campus. MRB and ALA were funded by Becas de Investigación Obra Social La Caixa-Fundación CajaCanarias para Posgraduados de la Universidad de La Laguna. Convocatoria 2014. JLM was supported by the Ramón y Cajal Subprogramme from the Spanish Ministry of Science and Innovation RYC-2011-08863. IS was supported by a grant from the Laboratoire Matériaux- Molécules et Applications, IPEST, and Ayudas para estancias estudiantes de posgrado e investigadores del continente americano y africano 2015 from the ULL.

IN VITRO EFFECT OF SEVERAL OLIVE LEAF EXTRACT MOLECULES ON ACANTHAMOEBA CASTELLANII NEFF

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Olive leaves has been used from the past in traditional medicine to cure many infections such as malaria and ulcers. Considering their richness in bioactive molecules olive products have been used in pharmaceutical and food industries. Phytochemical investigations of olive leaves led to the isolation of various polyphenols and triterpenes, some of which were found to possess several pharmacological properties. Free living amoebae are ubiquitous organisms widely distributed in the environment. *Acanthamoeba* genus is one of the four genera able to cause different pathologies in humans such as Granulomatous Amoebic Encephalitis (GAE) and Amoebic Keratitis (AK). At present, Therapy against *Acanthamoeba* infections remains as an issue to be solved due to the existence of a cyst stage. Recently, the activity of Olive Leaf Extracts (OLE) was demonstrated against *Acanthamoeba* species. However, the molecules involved in this activity were not identified and/or evaluated. During the present work, the anti-*Acanthamoeba* activity of 24 molecules usually present in OLE was studied. Among the tested molecules, apigenin present the highest activity with an IC50 of 6.59 µg/ml. The action mode of the bioactive molecules was evaluated by the detection of changes in the phosphatidylserine (PS) exposure, the permeability of the plasma membrane and the mitochondrial membrane potential. Results showed that the bioactive compounds could induce apoptosis in the treated parasite via the mitochondrial membrane potential collapse. Even though, those bioactive molecules could be considered as a future therapeutic alternative against *Acanthamoeba* spp. Further studies are needed in order to establish their action mode.

ENVIRONMENTAL DIVERSITY OF CRYPTIC SPECIES FROM THE NEBELA COLLARIS COMPLEX IS STRONGLY CORRELATED WITH ENVIRONMENTAL FILTERS.

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The phylogenetic niche conservatism theory predicts that closely related species should occupy similar niches and therefore colonize similar environments. Closely related species are therefore not expected to co-exist as they should compete for the same resources. In protists, however, strong top-down regulation has been shown to mitigate competition, at least in plankton and there is also evidence of low competition in the soil environment. Here, we studied the distribution of members of the *Nebela collaris* species complex, a group of at least eight morphologically resembling species of arcellinid testate amoebae in the different micro-habitats of peatlands. We studied community composition in Sphagnum mosses collected from hummocks, lawns, pine forests, poor fens and peatland margin in two peatlands in the Swiss Jura Mountains by environmental DNA sequencing. We applied a protocol for specific amplification of the COI gene of *N. collaris* s.l. to *Sphagnum* DNA extractions and cloned the PCR products. Sequence analysis revealed six of the eight previously barcoded species, plus three new genetically defined lineages whose morphology is still unknown. The distribution patterns among the studied habitats show that, in agreement with our hypothesis, species do not coexist randomly. Instead, we observed a strong correlation between community composition and both nitrogen content and water table depth. Members of the *Nebela collaris* s.l. exhibit a reduced niche overlap, as suggested by calculating overall, and between pairs Pianka indices. We found no evidence for competitive exclusion, based on C-score and NTI/NRI calculations. Furthermore, plotting NTI values versus nitrogen content suggested strong adaptive pressure for low N values on a specific clade. Our study demonstrates that cryptic species play different roles in the environment, and for this reason should be studied in detail. Furthermore, we confirm that extreme lack of nitrogen in peatlands is a major driver of diversity.

HIGH-THROUGHPUT SEQUENCING REVEALS STRONGLY SEASON-DEPENDENT DIVERSITY AND DYNAMICS OF HAPTOPHYTES IN NORTH ATLANTIC COASTAL WATERS

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Microalgae in the division Haptophyta play key roles in the marine ecosystem and in global biogeochemical processes. Despite their ecological importance, knowledge on seasonal dynamics, community composition and abundance at the species level is limited due to their small cell size and few morphological features visible under the light microscope. Here we present unique data on haptophyte seasonal diversity and dynamics from two annual cycles, with the taxonomic resolution and sampling depth obtained with high-throughput sequencing. From outer Oslofjorden, S Norway, nano- and picoplanktonic samples were collected monthly for two years, and the haptophytes targeted by amplification of RNA/cDNA with Haptophyta-specific 18S rDNA V4 primers. We obtained 156 operational taxonomic units (OTUs) from c. 400.000 454 pyrosequencing reads, after rigorous bioinformatic filtering and clustering at 99.5% similarity. Most OTUs represented uncultured and/or not yet 18S rDNA-sequenced species. Haptophyte OTU richness and community composition exhibited high temporal variation and significant yearly periodicity. Richness was highest in September-October (autumn) and lowest in April-May (spring). Some taxa were detected all year, such as *Chrysochromulina simplex, Emiliania huxleyi* and *Phaeocystis cordata*, whereas most calcifying coccolithophores only appeared from summer to early winter. We also revealed the seasonal dynamics of OTUs representing putative novel classes (clades HAP-2 - 5) or orders (Clade D, E, F). Season, light and temperature accounted for 29% of the variation in OTU composition. Residual variation may be related to biotic factors, such as competition and viral infection. This study provides new, in-depth knowledge on seasonal diversity and dynamics of haptophytes in North Atlantic coastal waters.

ENGINEERED NANOPARTICLES AND OXIDATIVE STRESS IN AQUATIC PROTISTS

Vera Slaveykova (University of Geneva).

Engineered nanomaterial (ENM) use in various applications is rapidly growing and their concomitant environmental release is inevitable and therefore bears important societal and environmental implications. ENM can be considered as emerging stressors of anthropogenic origin with a potential to persist and exercise toxic action in the environment. Although the increasing efforts to assess and clasify the toxicity potential of ENM in the environment, it is still unclear what are the major drivers and underlaying mechanisms. The present work focusses on the interactions between ENMs and two representatives of the aquatic protists: photosynthetic plant-like protist *Chlamydomonas reinhardtii*, and protozoa injestive animal-like protist *Tetrahymena thermophyla*. More specifically the applicability of the oxidative stress paradigm to assess the toxicity potential of metal containing nanomaterials in environmental settings is explored. The results demonstrated that short-term exposure to CuO-NPs, TiO2-NPs or quantum dots (QDs) suspensions induces oxidative stress and damage, in medium and time dependent manner. However no ENPs uptake by *C. reinhardtii* was observed and no relationship between ENP concentration and oxidative stress response was found. By contrast all the studied nanoparticles, Ag, Au, CuO-NPs, TiO2-NPs and QDs, accumulated in *T. thermophyla* by different uptake pathways. The ENPs aggregates are found in food vacuoles and cytoplasm, but the accumulation varied according to the concentration and exposure time. The induced oxidative stress correlated to ENPs uptake rate however some deviation from good correlations for the more inert NPs compared to more dissolvable and bioreactive NPs was observed. Overall, the oxidative stress and damage in aquatic protists induced by metal-based NPs can be triggered directly, promoted by particle properties at the nanoscale, or indirectly by dissolved, toxic metal ions from NPs. Therefore both the oxidative potential and ENPs dissolution have to be taken into a consideration in evaluation toxicity potential of ENMs.

THE GENETIC STRUCTURE OF AMOEBAE MORPHOSPECIES – PATTERN IN SPACE AND TIME

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The morphospecies concept, routinely applied for amoebae, was almost satisfactory when most of studies were limited to ecological, faunistic and taxonomic purposes. Molecular biological tools, applied in order to increase the resolution of species distinction, show that genetic structure of amoebae morphospecies is rather complex. To challenge the problem we performed wide-scale studies of several widely distributed amoebae species belonging to the genera *Vannella, Korotnevella, Flamella* and *Cochliopodium*. Cox I gene and portions of 18S r DNA genes were used as a DNA barcode; amoebae were sampled in many regions around the world and sampling sites were located at the distances from several meters to several thousand kilometers from each other. Results were congruent for all studied species and genes and indicated that: (1) properly defined amoebae morphospecies represents genetically distinct unit (2) an amoeba morphospecies consists of limited set of genetic lineages (phylotypes) forming monophyletic clade in phylogenetic trees; (3) within the single morphospecies the same phylotype may be found in distant locations, while some phylotypes appear to be endemic; (4) in local population morphospecies of amoeba usually comprises limited number of phylotypes, of them some are unique for this habitat while others are shared with other locations.

Observed pattern may be explained with the following model: cysts and trophozoites of amoebae are dispersed with the global and local-scale mass flows. This process seeds local habitats with numerous amoebae lineages; among them there are phylotypes belonging to the same morphospecies. Local selection, random events and on-site competition leave few of them alive and active. Within every amoeba species this process forms set of phylotypes showing worldwide distribution. If amoebae population is stable, local evolution of phylotypes results in appearance of unique phylotypes, which for some time seem to be endemic for a habitat. Further these phylotypes start to spread around becoming more widely distributed. However, high dispersion rate probably prevents formation of too many endemic phylotypes. The most of amoebae habitats are ephemeral. Dispersion may be the only way to maintain species is space and time; entire amoeba species thus exists as metapopulation. Support: Russian Science Foundation grant 14-14-00474.

THE TWO APICOMPLEXA BESNOITIA BESNOITI AND TOXOPLASMA GONDII DIFFERENTIALLY ALTER INTRINSIC HOST CELL POLARITY BY MANIPULATING CENTROSOME AND GOLGI APPARATUS

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Obligate intracellular parasites have co-evolved with hosts to be able to invade their cells and flourish. To be successful they need to establish specific molecular parasite-host cell interactions, and then manipulate the host cell structures, mechanisms and pathways in order to replicate and grow. In a previous work we described that upon interaction with the host cell, the apicomplexa *Besnoitia besnoiti* undergoes dramatic modifications of shape and surface, as revealed by atomic force microscopy, accompanied by a distinct tubulin labeling on the posterior region. In the host cell, the microtubule cytoskeleton shows a re-arrangement around the parasitophorous vacuole (PV). This phenomenon was also observed in the closely related parasite *Toxoplasma gondii*. During this event our data suggest that this parasite modulates the levels of tubulin polyglutamylation by controlling the factors that regulate the levels and pattern of tubulin post-translation modifications namely TTLLs, CCPs and severing microtubule enzymes such as spastin, katanin and fidgitin. Also, we have observed that *T. gondii* recruits the host cell centrosome towards the PV, whereas *B. besnoiti* does not. Notably, both parasites recruit the host Golgi apparatus to the PV but its organization is differentially affected. Moreover, *T. gondii* replication rate decreases in cells over-expressing TBCCD1 but not in TBCCD1 depleted cells, while for *B. besnoiti* no differences were found. However, *B. besnoiti* promotes a reorganization of the Golgi ribbon previously fragmented by TBCCD1 depletion. In fact, as described by us, TBCCD1 is involved in centrosome positioning and Golgi apparatus integrity Furthermore, the *T. gondii* tubulin cofactor B (TBCB) gene, a member of tubulin folding pathway that also controls microtubule dynamics through the recycling/degradation of the native tubulin heterodimers is involved in the invasion process of *T. gondii*. Taken together our results strongly support the importance that a successful establishment of the PVs in the host cell requires a cross-talk between the parasite and the host cytoskeleton through the regulation of the factors that control cytoskeleton specific functions and dynamics. Lastly, the differences found in how *T. gondii* and *B. besnoiti* interact with their host cells may indicate different evolutionary paths.

HOW MANY CELL POLARITY RELATED GENES ARE CONSERVED FROM TETRAHYMENA TO METAZOA?

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Cell polarity can be seen as an asymmetric distribution and spatial arrangement of biomolecules, cellular components (e.g., membrane domains and organelles such as the Golgi apparatus, mitochondria, cilia and others) and cytoskeleton such that, their specific positioning in the cell, in close relationship with their functions, generates a structural/functional asymmetry that can be conserved and transmitted to new cells during cell division.

In fact, cell polarity controls the morphology from single cells to whole tissues. Cellular organizational/functional asymmetry is required for a variety of cell functions in both unicellular and multicellular organisms such as correct symmetric and asymmetric cell division, differentiation, motility and cell migration. Moreover, in mammalian cells, polarity can be challenged by environmental cues, and cells are able to remodel their intrinsic polarity.

The ciliate *Tetrahymena thermophila* is a highly differentiated cell organism that possesses a permanent anterior–posterior axis and left–right asymmetry. *Tetrahymena* cells are also characterized by a complex cortex where basal bodies are longitudinally arranged in close association with cytoskeleton appendages and networks originating a complex pattern. The molecular mechanisms that control the formation and regeneration of this complex cortical patterning in each daughter cell after cytokinesis are still not well understood.

We have shown that the *Tetrahymena* Mob1 protein is essential for maintenance and regeneration of cell polarity, proper cell proportions, correct division plane placement and finally to cytokinesis completion. At the time, Mob1 was already described as a member of the mitotic exit network, a signaling cascade that controls mitosis to interphase transition. In metazoans Mob1 is a member of the Hippo signaling pathway, a major conserved mechanism governing cell contact inhibition and organ size control. Due to its cell features *Tetrahymena* emerges as a good model to address the regulatory mechanisms underlying cell polarity/morphogenesis/ and cell division. To test this idea we went throughout the *Tetrahymena* genome looking for genes already described to be involved in cell polarity in other unicellular and multicellular model organisms. Interestingly, some of these core genes involved in cell polarity appear to be conserved in this ciliate. In this presentation we will discuss our findings.

STRESS RESPONSES AND PHOTOPROTECTIVE STRATEGIES OF CILIATES EXPOSED TO ULTRAVIOLET RADIATION

Bettina Sonntag (Mondsee, Austria).

Solar ultraviolet radiation (UVR, 280 – 400 nm) is an environmental stress factor directly and indirectly affecting freshwater organisms at the molecular and the ecological level. The effects of UVR exposure on ciliates and how they manage their survival are elucidated in this talk. Particularly UV-B (280 – 315 nm) causes severe direct damages on nucleic acids and proteins followed by lower cell division rates or reduced speed. Indirectly, UV-B can also accelerate oxidative stress by the formation of reactive oxygen species. On an ecological level, the structure of microbial food webs is affected when, for example, a predator declines leading to a positive feedback on prey abundance. Longer wavelengths in the range of UV-A (315 – 400 nm) and visible light (400 -700 nm), however, can also be beneficial for an organism and induce photo-repair mechanisms. Overall, a variety of strategies how freshwater ciliates minimize stress and damage caused by the impact of the solar UVR have been identified so far: i) avoidance of lake areas irradiated by high incident solar radiation as is the case for the UV-sensitive *Balanion planctonicum*, ii) the acquisition of sunscreen compounds (mycosporine-like amino acids, MAAs) through food or by the synthesis of MAAs by algal symbionts, iii) physical shading of the nuclei by dense layers of symbiotic algae as observed in *Paramecium bursaria* or iv) DNA repair mechanisms as seen in experiments with *Pelagodileptus trachelioides*. Taken together, the effects as well as the strategies to minimize stress under UVR exposure are species-specific and in the case of planktonic ciliates they are dependent on the UVR transparency of the habitat.

ARE AMOEBOZOA ANCESTRALLY AMOEBOID?

Frederick Spiegel (Department of Biological Sciences, University of Arkansas), Seungho Kang (Department of Biological Sciences, Mississippi State University), Alexander Tice (Department of Biological Sciences, Mississippi State University), Daniel Lahr (Department of Zoology, University of Sao Paulo), Matthew Brown (Department of Biological Sciences, Mississippi State University).

The last common ancestor of Amoebozoa was a flagellated, sexual organism. We hypothesize that it also had amoeboid states in its life cycle. That is, at least one part of its life cycle was a cell that moved using pseudopodia and probably ingested food from anywhere on its surface. However, there is a wide range of morphologies that fit the definition of being an amoeba. We propose a specific hypothesis: that the last common ancestor of amoebozoans had a polyphasic life history that included an amoeboid state with a morphology that was tubular in cross section and distinct cytoplasmic streaming and also a state with a flatter profile and less organized cytoplasmic flow. Therefore, the ancestor had an "amoebal toolkit" that allowed it to assume aspects of morphology that are found scattered throughout all the major lineages of extant amoebozoans. Thus, simpler extant amoebozoans are best interpreted as having reduced their repertoire of possible morphologies rather than invoking the concept that simpler morphologies are primitive, and more complex forms are derived. We will demonstrate that this hypothesis is congruent with several possible phylogenies and show how comparative phylogenomics can be used to test it.

EVOLUTION AND CELLULAR LOCALIZATION OF RHODOQUINONE BIOSYNTHESIS IN PYGSUIA BIFORMA AND OTHER ANAEROBIC EUKARYOTES

Courtney Stairs (Dalhousie University), Laura Eme (Dalhousie University), Andrew Roger (Dalhousie University).

Complex II (CII) of the respiratory chain typically catalyzes the conversion of succinate to fumarate with the concomitant reduction of ubiquinone (UQ). In some anaerobic bacteria and eukaryotes CII functions as a fumarate reductase to convert fumarate to succinate with the oxidation of a different quinone species (rhodoquinone, RQ). RQ is structurally similar to UQ, but has a lower electron potential favouring CII-catalyzed fumarate reduction over succinate oxidation. Recently, a putative methyltransferase homolog was discovered in *Rhodospirillum rubrum* (named RQUA) that was shown to be involved in RQ biosynthesis (Lonjers et al. 2012). We have previously shown that RQUA is rare in prokaryotes, and is encoded by only 50 distinct bacterial genomes from the α-, β-, and γ-proteobacterial divisions (Stairs et al. 2014). Interestingly, in nine of these lineages, the rqua gene appears to be encoded in an operon with CII subunits suggesting that the expression of these complimentary proteins might be linked. Here, we identified a number of novel RQUA in a variety of anaerobic or facultatively anaerobic microbial eukaryotic genomes including genomes of two subtypes of *Blastocystis* sp., *Mastigamoeba, Pygsuia, Euglena, Neoparamoeba,* and *Monosiga*. All of the full-length RQUA sequences from these protists were found to contain a predicted mitochondrial targeting sequence, suggesting they function within mitochondria or related organelles. Using immunofluorescence microscopy, homologous antibodies directed against RQUA localized to *Pygsuia biforma* mitochondrion-related organelles. We are using mass spectrometry to confirm the presence of RQ in protist cell extracts. Finally, phylogenetic analysis revealed a patchy distribution and relationships of the eukaryote and bacterial RQUA sequences suggesting that the gene has been laterally transferred multiple times between Domains of Life. This suggests that the transfer of the gene between protists is probably selectively advantageous by allowing the mitochondrial electron transport chain to function in hypoxia via an RQ-utilizing CII.

RECOGNITION GENES, POPULATION DENSITY, SORTING, AND CHEATING IN THE SOCIAL AMOEBA DICTYOSTELIUM DISCOIDEUM

Joan Strassmann (Washinton Unversity in St. Louis).

Kin recognition is only likely to be evolutionarily favored when it increases the inclusive fitness of the bearer of the trait. Fitness can be increased by avoiding potential exploiters or by directing cooperation towards relatives. In the social amoeba *Dictyostelium discoideum* recognition genes tgrB and tgrC are highly variable and appear to function in sorting after amoebae aggregate upon starvation. These aggregates are made up of thousands of cells that ultimately form a fruiting body with a stalk of dead cells that lifts the living spores for dispersal. Recognition genes could keep amoebae from aggregating with non-clonemates, or they could facilitate cheating to avoid contributing to the dead stalk cells once the amoebae have aggregated. The importance of how recognition overall plays out depends also on population density. If the likelihood of encountering a non-clonemate is low, then recognition genes may not be crucial in many interactions. Understanding these complex interactions is an ongoing challenge that requires knowing not only how the gene products function, but also how *D. discoideum* clones interact in nature. This unusually well characterized system should tell us much about the early steps of recognition and its consequences in protists.

CYTOPLASMIC DOUBLE-INFECTION – PARAMECIUM BIAURELIA INFECTED BY TWO NOVEL RICKETTSIA-LIKE BACTERIA

Franziska Szokoli (Department of Biology, University of Pisa, Italy), Michele Castelli (Department of Biology, University of Pisa, Italy), Elena Sabaneyeva (Department of Cytology and Histology, St. Petersburg State University, Russia), Martina Schrallhammer (Institute of Biology II, University of Freiburg, Germany), Sascha Krenek (Institute of Hydrobiology, Dresden University of Technology, Germany), Tom Doak (Indiana University, IN, USA), Thomas U. Berendonk (Institute of Hydrobiology, Dresden University of Technology, Germany), Giulio Petroni (Department of Biology, University of Pisa, Italy).

Symbiosis is a widespread phenomenon in protists: over 50 bacterial endosymbionts have been detected in various species of the ciliate Paramecium, and the number is still rising. Occasionally double-infections of two different bacterial endosymbionts occur; especially the combination of *Holospora* species and other bacteria has been reported in *Paramecium*. In this work, we characterize two novel bacterial endosymbionts inhabiting a *Paramecium biaurelia* isolate collected near Bloomington, Indiana (USA). For characterization of these bacterial endosymbionts, prokaryotic SSU rRNA genes were amplified and sequenced, and used for taxonomic assignment and species-specific oligonucleotide probe design. Phylogenetic analyses revealed that both endosymbionts belong to the order Rickettsiales (Alphaproteobacteria), with *"Candidatus Bealeia paramacronuclearis"* clustering with the so-called "basal" Rickettsiales and the second species belonging to the *"Candidatus Midichloriaceae"* family. Both endosymbiotic species inhabited the host cell cytoplasm. The Gram-negative bacterium *"Candidatus Bealeia paramacronuclearis"* (1.8-2.4 x 0.4-0.5 µm in size) occurred in groups of up to eight generally parallel orientated cells and was frequently found associated with the macronucleus, sometimes forming rows in cytoplasmic invaginations; the second endosymbiont showed neither grouping nor a preferential location within the host cytoplasm. *"Candidatus Bealeia paramacronuclearum"* with its electron-dense cytoplasm and a distinct halo was easily distinguishable from the second smaller symbiont (1.1 x 0.35-0.5 µm in size), whose cytoplasm was electron-lucent and lacked any halo. Instead it was always surrounded by a symbiontophorous vacuole.

MASTIGAMOEBA BALAMUTHI AND ENTAMOEBA HISTOLYTICA: SO SIMILAR YET SO DIFFERENT

Vojtech Žárský (Charles University in Prague), Eva Nývltová (Charles University in Prague), Ivan Hrdý (Charles University in Prague), Jan Paces (IMG AVCR, Prague), Jan Tachezy (Charles University in Prague).

Archamoebae is an attractive clade of flagellates or amoebae of Amoebozoa group for tracing evolutionary history of cell adaptation to anaerobic niches and parasitic style of life. As the sister group of Archamoebae, Eumycetozoa are aerobes with regular aerobic mitochondria, it is likely that Archamoebae, converted their aerobic metabolism including an ancestral aerobic mitochondria to their anaerobic counterparts (hydrogenosomes, mitosomes). This transition included the loss of most canonical mitochondrial pathways, and the acquisition of anaerobic pathways by lateral gene transfer. Comparative analysis of 7 selected genome of Amoebozoa group members including *Mastigamoeba balamuthi* and *Entamoeba histolytica* together with 4 genome of related opisthokonts revealed 5299 orthologous groups of genes that are shared by Opisthokonta and Amoebozoa groups. Genome of archamoebal ancestor was reduced by about 1736 orthologous groups, while 173 orthologous groups were gained. A free living *M. balamuthi* lost additional 487 orthologous groups. Remarkable reduction of orthologous groups was found in parasitic lineages of *Entamoeba* species (2240).

Next we specifically focused on metabolism of *M. balamuthi* and *E. histolytica.* The common features for both organisms includes (i) acquisition of anaerobic energetic metabolism including PFO, hydrogenase, acetyl CoA synthetase (ii) iron-sulfur cluster assembly machinery of bacterial type (NifS, NifU), and (iii) sulfate activation pathway. However, while in *M. balamuthi,* the enzymes of energetic metabolism and NIF pathway have a dual localization in the cytosol and hydrogenosomes, these pathways are not present in mitochondria (mitosomes) of *E. histolytica*. Interestingly, both hydrogenosomes and mitosomes possess sulfate activation pathway, however, only in *E. histolytica* it plays important role for entamoeba encystation (Mi-Ichi et al., 2015). In addition, hydrogenosomes of *M. balamuthi* retain several canonical mitochondrial components including TCA enzyme malate dehydrogenase, respiratory complex II and the glycine cleavage system, and possess an unusual D-lactate dehydrogenase. We propose that the more complex hydrogenosome in free living *M. balamuthi* represents the intermediate step between the ancestral most likely aerobic organelle and highly reduced mitosomes in parasitic *E. histolytica*.

TETRAHYMENA THERMOPHILA SHOWS INCREASED EVOLVABILITY FOLLOWING SEXUAL REPRODUCTION

Jason Tarkington (University of Houston), Rebecca Zufall (University of Houston).

Understanding the mechanisms that generate genetic variation, and thus contribute to the process of adaptation, is a major goal of evolutionary biology. *Tetrahymena thermophila* is a ciliate with an unusual genetic feature, called phenotypic assortment, which may allow for an increase in the amount of genetic variation following sex, thereby increasing its evolvability. To test this hypothesis, I compared the rate of adaptation in *T. thermophila* populations that were allowed to undergo phenotypic assortment to those that were not. These populations were maintained at two different temperatures and fitness was measured every 25-50 generations for approximately 1000 generations. Under some environmental conditions, the populations that underwent phenotypic assortment adapted more quickly than those that did not. This suggests that the additional genetic variation generated by phenotypic assortment can increase the rate of adaptation under certain conditions.

GENETIC REGULATION OF SPOROCARP DEVELOPMENT IN A PROTOSTELOID AMOEBA, PROTOSTELIOPSIS FIMICOLA (VANELLIDAE, AMOEBOZOA)

Alexander Tice (Mississippi State University), Frederick Spiegel (University of Arkansas, Fayetteville), Matthew Brown (Mississippi State University).

Protosteloid amoebae are amoeboid protists, found only in the eukaryotic supergroup Amoebozoa, with the ability to form a simple spore dispersal structure known as a sporocarp. Sporocarps develop from a single amoeboid cell with a discrete developmental process leading to the formation a fungal-like fruiting body that consists of an extracellularly produced stalk with a cellular spore atop. Despite the developmental similarity of protosteloid amoebae, they are polyphyletic. In molecular phylogenies, they are found on both sides of the deepest bifurcation of Amoebozoa, occurring in seven distinct clades. This observation leads to the question of whether this developmental program has been converged upon many times independently, or was present in the last common ancestor of Amoebozoa. In order to begin to elucidate the genetic toolkit involved in regulating sporocarp formation, we isolated RNA from trophic cells, and two stages of sporocarp development in the protosteloid amoeba Protosteliopsis fimicola. Our data show clear patterns of differential expression of transcripts in each of the three developmental stages sampled. These patterns show a mass down regulation of the expression of trophozoite unique transcripts during the onset of sporocarp development. This is coupled with the up regulation of a small set of genes likely crucial to sporocarp formation. This data represents the first developmental transcriptomic data on protosteloid amoebae, which will be used to compare expression patterns and, through annotation of these transcripts, the genetic pathways used in the production of sporocarps across Amoebozoa.

VARIATIONS IN ANAEROBIC METABOLISM OF PROTISTS

Aloysius G.M. Tielens (Dept. Medical Microbiology and Infectious Diseases, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands).

Many protists inhabit environments where virtually no oxygen is available, such as anaerobic sediments or the mammalian gut. The spectrum of endproducts and also the type of their mitochondrion-related organelles (MROs) varies among the different anaerobic protists.

All eukaryotes contain MROs and all MROs are descendants of one symbiotic event. By differential loss and gain of metabolic functions the organellar evolution resulted in a mosaic of mitochondrial functions in the various eukaryotic lineages and unifying features of mitochondria are sparse. A classification of these diverse organelles into five classes was proposed earlier on the basis of their energy metabolism, as that was supposedly the original driving force for the endosymbiotic event [1]. In all classes that can be discriminated based on their energy metabolism, numerous variations of the organelles exist within each class. The variations in anaerobic metabolism among protists will be discussed in relation to this functional classification.

1. Müller, M., Mentel, M., van Hellemond, J.J., Henze, K., Woehle, C., Gould, S.B., Yu, R.Y., van der Giezen, M., Tielens, A.G.M., and Martin, W.F. (2012). Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. Microbiol. Mol. Biol. Rev. 76, 444–495.

NEW LINEAGES OF DEEP-BRANCHING PREDATORY FLAGELLATES AND THEIR EVOLUTIONARY SIGNIFICANCE

Denis Tikhonenkov (University of British Columbia), Jan Janouškovec (San Diego State University), Fabien Burki (University of British Columbia), Ryan Gawryluk (University of British Columbia), Alexander Mylnikov (Institute for Biology of Inland Waters, Russian Academy of Sciences), Patrick Keeling (University of British Columbia).

Predatory protists are understudied, but often represent important deep-branching evolutionary lineages and new eukaryotic supergroups with unique morphology and ultrastructure, slowly evolving nuclear proteins, large and slowly-evolving mitochondrial gene sets. Their studying may be important in addressing previously puzzling evolutional problems, such as the trajectory of plastid spread, evolution of mitochondrial genomes, origins of parasitism. Here we show phylogenomic and morphological data on several novel or rare free-living predatory flagellates.

1. Using phylogenomic analysis we have find that predatory colpodellids and photosynthetic chromerids represent a monophyletic clade, which is sistergroup to parasitic apicomplexans. The revealed relations indicate the complex scenario of acquisition/loss of plastids and transition to parasitism in alveolate evolution. Our observation suggests that the evolution of parasitism is not primarily linked to the acquisition of novel structures or components, but rather to loss and modification of those already present.

2. We introduce Twirling Disk (TD), a new deep-branching eukaryote isolated from coral reef samples. TD occupies a deep evolutionary position within the Corticates (eukaryotes other than Unikonts and Excavates) and probably represents a new eukaryotic phylum with a novel type of extrusive organelles for active hunting. The TD mitochondrial genome is the second largest after jakobids: it is circular and encodes a full set of tRNA genes and 47 mitochondrion-encoded proteins.

3. We discovered several predatory kinetoplastids, which is related to endosymbiotic *Ichtyobodo* and *Perkinsiella*-like species and occupies a deepest position at the base of kinetoplastids, including free-living (Bodonids) and parasitic species, like well-known *Trypanosoma*. These new deep-branching kinetoplastids are characterized by highly edited mitochondrial RNAs.

4. We have isolated new deep-branching predatory rhizarian. Phylogenomic analysis reveals that this organism goes very deep and doesn't belong to cercozoans, foraminifera's and radiolarians, and probably represents a basal branch of whole Rhizaria.

5. We report the establishment of multiple cultures of basal predatory stramenopiles, candidates for the ochrophyte ancestor.

SYMBIOSIS IN THE COLD: IDENTIFICATION AND CHARACTERIZATION OF A NEW FRANCISELLA ENDOSYMBIONT FROM THE POLAR CILIATE, EUPLOTES PETZI

Adriana Vallesi (**University of Camerino, Italy**), **Dezemona Petrelli** (**University of Camerino, Italy**), **Graziano Di Giuseppe** (**University of Pisa, Italy**), **Andreas Sjödin** (**Swedish Defence Research Agency and Umeå University, Sweden**), **Johanna Thelaus** (**Swedish Defence Research Agency, Umeå, Sweden**), **Elin Nilsson** (**Swedish Defence Research Agency, Umeå, Sweden**), **Caroline Öhrman** (**Swedish Defence Research Agency, Umeå, Sweden**), **Gabriel Gutierrez Pozo** (**Universidad de Sevilla**), **Eduardo Villalobo** (**Universidad de Sevilla**).

Ciliates of the genus *Euplotes* are commonly found in polar environments, and different species isolated from Arctic and Antarctic coastal seawaters are currently studied for their genome evolution and adaptation. In analyzing whole genome sequences of a wild-type *E. petzi* strain collected from Terra Nova Bay (Antarctica), it appeared that more than 3% of the assembled contigs had a bacterial origin and overlapped (one contig containing rDNA operon included) with DNA sequences of the gamma-proteobacterium *Francisella* (represented by extremely infectious species to a wide array of different organisms man included).

Given that an *Euplotes* species of temperate seawaters, *E. raikovi*, has already been found to host a *Francisella* species (namely *F. endociliophora*), we searched for and succeeded in isolating *Francisella*-like endosymbionts from *E. petzi* cells. Colonies of these endosymbionts (grown optimally at a temperature range from 4 to maximum 30 °C) have been analyzed for their genome and found to represent a new clade with a basal position in the *Francisella* phylogenetic tree. This clade is unequivocally distinct from *F. endociliophora* (living in *E. raikovi*) as well as from all the other well-recognised *Francisella* clades.

The finding that *Francisella* is adapted to live in the extreme environmental conditions of the polar regions implies that this bacterium is much more common and geographically widespread than previously known, and that free-living *Euplotes* species may represent a natural reservoir of *Francisella* in every aquatic environments.

MECKELIN GUIDES BASAL BODY LOCATION

Judith Van Houten (**University of Vermont, USA**) , **Ashik Nabi** (**University of Vermont, USA**), **Tyler Picariello** (**University of Massachusetts, USA**), **Megan Valentine** (**University of Vermont, USA**), **Junji Yano** (**University of Vermont, USA**).

Meckelin (MKS3) is protein of the primary cilia transition zone that functions in ciliogenesis and ciliary gating. MKS3 appears to have similar functions and location in *Paramecium tetraurelia* since FLAG-MKS3 is found associated slightly above each basal body and RNAi for MKS3 leads to loss of cilia. However, RNAi for MKS3 also leads to the disorganization of rows of basal bodies that normally run from anterior to posterior. In the areas of misalignments, the basal bodies with their post ciliary and transverse rootlets are found out of their expected rows. While it appeared that the rootlets were attached to the basal bodies at the expected angles relative to each other, we confirmed this with immunofluorescence for all 3 rootlets plus basal body centrin. That is, if there are markers on the basal body for the rootlet positions, these have been maintained.

We propose that MKS3 guides new basal bodies as they move toward the anterior of the cell along the striated rootlet (SR) of the parent basal body. The loss of MKS3 results in loss of interactions between the basal body and the SR. Without a guide to maintain orientation, the new basal bodies migrate off the expected line and, when they form their SRs, these too cannot project toward the anterior as expected.

To test for interactions of MKS3 with SR components, we identified 24 potential SR protein genes and expressed 13 them (considering paralogs as duplicates) with epitope tags. Nine with SF assemblin domains (similar to those in the *Chlamydomonas* rootlet proteins) were we found in the *Paramecium* SRs; conversely those without this domain were not in the rootlets, but were in cilia and cytoplasm. Using a selection of the SR tagged proteins, we found that other SR proteins co-IP, but MKS3 is not among these proteins. If there is an interaction between MKS3 and SR proteins, it likely is indirect or weak.

P20 GM103449; P20 RR016435; R01 GM59988.

REDOX-BASED SENSING OF ENVIRONMENTAL STRESS – FROM ORGANELLE SIGNALING TO CELL FATE DECISION

Assaf Vardi (Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel).

Diatoms are one of the most successful groups of photosynthetic protists in the modern oceans, responsible for about 20% of global primary productivity. The molecular basis for diatom's ecological success and the role of cell signalingare still poorly understood, although recent studies suggested that diatoms utilize sophisticated sensing mechanisms to respond to environmental stress conditions. During bloom succession algal cells are subjected to abiotic (nutrientsdeprivation, high light) and biotic stress (viruses, grazers and allelopathic interactions). Production of Reactive Oxygen Species (ROS) under stress conditions and consequently alterations in cell redox state have been shown to play a central role in regulation of cell fate signal transduction pathways in plants and animals. We therefore explored diatom mechanisms of perception of stress conditions by combining in vivo imaging of redox responsewith quantification of the whole redox proteome (redoxome) in the model diatom *Phaeodactylum tricornutum.* In vivo imagingof the redox state in various subcellular compartments, using the redox sensitive GFP (roGFP), revealed distinct compartmentalized signaling in response to light regime, nitrogen or iron availability as well as infochemicals that are derived from diatom biotic interactions. We further identified intriguing correlations between early oxidation patternsin the mitochondria and subsequent induction of cell death.Using a redox proteomics approach we were able to unravelthe redox-sensitive protein network which includeskey enzymes in diatom metabolic pathways. Comparative analysis of the diatom redoxome across 48 genomes revealed reactive cysteines that are evolutionarily conserved across kingdoms. We propose that redox regulation may provide diatoms with important machinery for rapid and reversible responses to multiple environmental cues therefore essential for their ecological success in the marine ecosystem.

ECO-PHYSIOLOGICAL CONTEXT FOR THE CONSERVED AUTOPHAGY PATHWAY DURING ALGAL BLOOM DYNAMICS

Daniella Schatz (Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel), Adva Shemi (Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel), Shifra Ben-Dor (Department of Biological Services, Weizmann Institute of Science, Rehovot, Israel), Assaf Vardi (Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel).

Phytoplankton contribute ca. 50% of the global photosynthesis and serve as the foundation of marine food webs. Although their eco-physiology is extensively studied, some basic aspects of the algal cell biology remain obscure. The recent wealth of algal genomic resources has opened new frontiers to decipher cellular pathways and their ecological function. Autophagy is a common eukaryotic pathway that recycles unwanted cytoplasmic content via specialized vesicles, serving as a key cellular mechanism against pathogens and nutrient starvation. We performed a genomic analysis of autophagy-related (ATG) proteins in green, red and chromalveolate algae. We elucidated that ATG proteins are conserved among green algae, but intriguingly missing from red algal genomes. This is the first demonstration of a eukaryotic genome that does not harbor the autophagy pathway, raising major evolutionary questions regarding the conservation and function of this cellular survival process. Among chromalveolates, *Emiliania huxleyi* (Haptophyta), a bloom-forming coccolithophore, possesses a complete set of ATG genes. *E. huxleyi*'s vast blooms in the oceans are usually terminated by a large double-stranded DNA viruses (EhV). In light of this context, the role of autophagy was investigated during the host-pathogen interactions. We showed that hallmarks of autophagy, such formation of double-membraned vesicles, vacuolar acidification and upregulation of a suite of autophagy related genes, are highly induced during the lytic phase of viral infection. Furthermore, we detected a host encoded autophagy related protein (Atg8) in EhV virions, demonstrating the pivotal role of an autophagy-like process in viral assembly and egress from the cells. We further showed that during phosphate limitation of *E. huxleyi* cultures, ATG gene expression was induced, together with formation, acidification and degradation of autophagic vesicles, coupled with profound remodeling of the membrane's phospholipid composition. We propose that autophagy plays an important cellular role and therefore, has a unique ecological significance in acclimation of marine protists to nutrient limitation and viral infection within algal blooms in the oceans.

DIVERSITY AND PHYLOGENETIC RELATIONSHIPS WITHIN THE GENERA PARAMOEBA AND NEOPARAMOEBA (AMOEBOZOA, DACTYLOPODIDA)

Ekaterina Volkova (**Saint-Petersburg State University**), **Alexander Kudryavtsev** (**Saint-Petersburg State University**).

Neoparamoeba is a group of free-living and amphizoic marine and aestuarine amoebae which possess an intracellular symbiont related to Kinetoplastida (*Perkinsela*-like organism, PLO). Because of amphizoic members living in tissues of fishes, sea urchins and other invertebrates, this group has a practical interest. During last several decades a lot of new strains belonging to this group have been isolated and included in the molecular analysis. Descriptions of several species (e.g. *N. perurans*, *N. branchiphilla*) were based on molecular data only, while morphological data were either collected, but not discussed, or not even obtained. By now, numerous SSU rRNA gene sequences of these amoebae form a poorly resolved phylogenetic tree with several incongruences between morphological and molecular data. In particular, *Neoparamoeba aestuarina* strains often branch within a clade comprising *Neoparamoeba pemaquidensis.* With this contribution, we present an overview of the biodiversity and phylogenetic relationships within *Paramoeba*/*Neoparamoeba* clade, and report an isolation and study of a number of new *Neoparamoeba* strains using morphological and molecular methods. One of these strains isolated from the deep-sea environment is obviously a new species. Other strains can be identified as *N. aestuarina* and *N. pemaquidensis.* The molecular analysis showed branching of *N. aestuarina* among strains previously identified as *N. pemaquidensis* making the latter species paraphyletic. However, two other new strains branch separately in the two parts of *N. pemaquidensis* divided by *Neoparamoeba aestuarina*. We suggest that one of these two parts of *N. pemaquidensis* strains not containing the type strain of this species should be described as a new species. The results of phylogenetic analysis of *Paramoeba*/*Neoparamoeba* clade obtained using SSU rRNA gene of amoebae are partly congruent with those obtained using the SSU rRNA gene of PLOs.

This study was partially supported by the grants 12-04-33229 and 15-29-02749 from the Russian Foundation for Basic Research and utilized the equipment of the core facility centers of Saint-Petersburg State University.

THE RESPONSE OF MYXOMYCETE COMMUNITIES TO 14 YEARS OF N, P, AND K ADDITION IN A LOWLAND TROPICAL RAIN FOREST

Laura M. Walker (University of Arkansas), Benjamin L. Turner (Smithsonian Tropical Research Institute), S. Joseph Wright (Smithsonian Tropical Research Institute), Franck Carbonero (University of Arkansas), Steven L. Stephenson (University of Arkansas).

Myxomycetes (also called plasmodial slime molds) are among the most abundant protozoans in many soils, where they feed on bacteria and other microorganisms. In doing so, these organisms help mediate the flow of nutrients to plants and higher trophic levels, playing a critically important role in the functioning of global ecosystems. Nutrient availability is a primary constraint on the productivity and distribution of organisms in tropical forests. As global temperatures rise and atmospheric CO_2 increases, nutrient availability will become increasingly important in lowland tropical forests. The extent to which nutrient limitation affects the myxomycete community is unknown. To increase our understanding of myxomycete ecology and possible nutrient limitations, this project takes advantage of a long-term nutrient fertilization experiment in the lowland forests of Panama to investigate the impacts of increased levels of nitrogen (N), phosphorus (P) and potassium (K) on myxomycete community structure. Samples of litter and soil were collected from all eight factorial NPK treatments including an untreated control with four replicates each, during the summer of 2013. All litter samples were placed in moist chamber culture and were very productive for myxomycetes. Because soil myxomycetes cannot be reliably cultured, total DNA was isolated from soil samples for high-throughput environmental sequencing of the small subunit (SSU) ribosomal DNA. Preliminary results are in line with our hypotheses and indicate an increased abundance and diversity on the P and NPK plots, relative to the N, K, and control plots. The significance of these and other findings will be discussed.

MILTEFOSINE AS AN ANTI-ACANTHAMOEBA DRUG

Julia Walochnik (Medical University of Vienna), Andreas Obwaller (Clinical Data Management and Statistics GmbH), Michael Duchêne (Medical University of Vienna).

Acanthamoebae are ubiquitously occurring protozoa that can be found in natural as well as in man-made habitats, including tap water world-wide. They generally do not need a host, but if they (accidently) enter the human body they can cause serious disease, being the causative agents of very different disease entities, the so-called *Acanthamoeba* keratitis associated with contact lens wear, on the one hand and several severe disseminating infections in the immunocompromised host, on the other hand. The treatment of *Acanthamoeba* infections is still problematic due to the lack of sufficiently effective and also easily manageable drugs.

Miltefosine, an alkylphosphocholine, has known activity against several protozoan parasites and has been approved for oral and topical treatment of visceral and cutaneous leishmaniosis, respectively. Several studies have demonstrated its high efficacy against *Acanthamoeba* spp. and other amphizoic amoebae in vitro. Moreover, we have shown in different ex vivo model systems that penetration of amoebae into human skin and cornea, respectively, is prevented by topical treatment with miltefosine, while miltefosine treatment was generally well tolerated. In vivo hamster and rat models proved miltefosine to be very effective in the treatment of *Acanthamoeba* keratitits, alone and particularly in the combination with polyhexamethylene biguanide. Due to the lack of an established medication, miltefosine has also been used as an investigational drug in several cases of human *Acanthamoeba* infections, including skin lesions, granulomatous encephalitis and keratitis, achieving very good results. The drawbacks of miltefosine are its relatively high costs, its general susceptibility to emerging resistance and its toxicity, which is however low compared to the standard treatment. In the current situation with no specific anti-*Acanthamoeba* treatment available, miltefosine appears to be a very good alternative treatment, particularly in complicated *Acanthamoeba* infections.

USER-FRIENDLY METHODS FOR THE IDENTIFICATION OF CILIATE SPECIES IN BIOLOGICAL AEROBIC WASTEWATER-TREATMENT PROCESSES

Alan Warren (Natural History Museum, London, UK).

Although it has long been known that ciliates can be used as reliable indicators of effluent quality in biological aerobic wastewater-treatment processes, they are rarely used on site because of the difficulty that non-specialists have in identifying them. In this talk I will discuss two approaches to resolving this problem. Firstly, I will describe an interactive guide to sewage ciliates that was developed several years ago by the NHM and University of Barcelona (http://ciliateguide.myspecies.info/ciliates-activated-sludge). This is a multi-entry, multimedia, user-friendly guide that can be used by ciliate specialists and non-specialists alike. Brief diagnoses and line diagrams of 175 species that have been reported in wastewater-treatment systems are provided. Video clips of cells in vivo are included for ~75 species that are most commonly encountered and/or are of greatest indicator value. A multi-entry key has been developed that is essentially pictorial and allows comparisons to be made with several taxa on the same screen. Other data provided for each species includes their indicator value (e.g. saprobic valency) and references to the literature. Future work will focus on updating the guide, incorporating an automated effluent predictor function, and making the guide available as a web-based facility. Secondly I will briefly discuss an ongoing project to develop a method for identifying spirotrichean ciliates using DNA barcoding. Spirotricheans are one of the most diverse and speciose ciliate groups and are often dominant in biological wastewater-treatment systems. Many are superficially very similar and therefore difficult to identify from in vivo observation. DNA barcoding is a method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species. There is, however, no currently recognised universal DNA barcode for the ciliates and it is possible that different gene markers may be more suitable for different ciliate groups. In collaboration with South China Normal University, work has commenced to investigate the relative merits of four candidate barcode gene markers in the spirotricheans: SSU rDNA, LSU rDNA, ITS, and Cox1. Preliminary findings will be presented based on the sequences of ~60 species and ~135 populations.

FUNCTIONAL ECOLOGY OF AQUATIC PROTISTS – KEY ISSUES AND OPEN QUESTIONS

Thomas Weisse (Mondsee, Austria).

The evaluation of numerical and functional responses (i.e. the change in growth rate and ingestion rate with changing prey abundance, respectively) is key to understanding survival of heterotrophic protists under various environmental conditions. For instance, the threshold food level, where specific growth equals mortality and the initial slope (α) of the numerical response define a species competitive ability at low food conditions. Likewise, the maximum growth rate (μmax) and the maximum ingestion rates (Imax) characterise a species performance under food replete conditions. Together with 'top down' forces such as loss rates due to grazing and parasitism and prey dependent responses (e.g. logistic growth), these parameters may be used to predict population dynamics of a given species; then the responses can be modified to evaluate outcomes when environmental/biotic conditions change. However, in most cases, only one population or one clone of a given protistan species has been studied, i.e. intraspecific genetic variance and phenotypic plasticity remained poorly assessed. In spite of their great theoretical and practical significance, key aspects of the numerical and functional responses are only now being carefully examined. For instance, interaction with ambient parameters such as food quality (stoichiometry) and type, climate change mediated shifts in temperature and pH, and biotic factors such as mixotrophy, shifts to cannibalism, and nutritional history have received little attention. This presentation will introduce the symposium Functional Ecology of Aquatic Protists, briefly illustrating key issues and open questions using examples from different taxonomic groups.

CILIATES IN THE OLIGOTROPHIC OCEAN - DO TRANSIENT DYNAMICS DETERMINE LONG TERM PATTERNS?

Stephen Wickham (University of Salzburg), Monika Claessens (University of Salzburg), Anton Post (University of Rhode Island).

Our work in the Gulf of Aqaba has shown that, despite extremely low productivity, both ciliate abundance and diversity is high. Chlorophyll ranges between 0.02 - 2 µg Chl a L-1 (summer stratification and spring bloom), but up to 55 ciliate species can be found at any one time, with abundances reaching 3.5 cells ml-1. Moreover, there is relatively little overlap in the species composition, either across seasons or depths. Highest diversity was measured after 5 months of stable stratification, where equilibrium conditions should allow dominance by the best competitor. Conversely, lowest diversity was at the onset of stratification, where rapidly changing nutrient dynamics should act as a disturbance and promote species diversity. To investigate the drivers of ciliate diversity, we ran both short-term (24 h) and long-term (96 h) experiments with size fractionation cross-classified with nutrient addition during summer stratification, winter mixing and the spring bloom. All experiments showed minimal top-down and strong bottom-up control, independent of season. Ciliates responded more strongly to nutrient addition than phototrophs, becoming markedly more abundant in treatments with added nutrients relative to those without, an effect lasting over the 96 h of the long-term experiments. Treatments with added nutrients were also considerably more dissimilar to one another than those without added nutrients: which species benefitted from added nutrients appeared random. This could explain the high ciliate species diversity in the Gulf of Aqaba, if small nutrient pulses are rapidly transferred to different random collections of ciliate species in the vicinity of the pulse.

INTERACTIONS BETWEEN THEILERIA AND THE HOST CELL CYTOSKELETON

Kerry Woods (Vetsuisse Faculty, University of Bern, Switzerland).

Theileria annulata, the causative agent of Tropical Theileriosis, manipulates its bovine host to an impressive extent. *Theileria* infection confers a cancer-like (transformed) phenotype upon the infected leukocyte, inducing anti-apoptotic signaling, uncontrolled proliferation and increased invasiveness. Transformation depends on the presence of the parasite within the host cytoplasm, and is linked to the modification of host cell signaling cascades. However the molecular mechanisms by which *Theileria* triggers these processes remain largely unknown. To ensure its persistence within the cytoplasm of the continually proliferating cell, *Theileria* interacts closely with the host cell mitotic machinery. Several host molecules, including some kinases and microtubule-associated proteins (MAPs), are found to bind to the parasite surface. We are using BioID technology to investigate protein interaction networks at the parasite surface. The principle of BioID involves the fusion of a promiscuous biotin ligase (BirA*) to a protein of interest, expression within cells, and the subsequent biotinylation and purification of interacting and proximal proteins. Because biotin is covalently bound to proteins, stringent conditions can be employed to solubilise protein complexes prior to purification – a huge advantage when dealing with membrane proteins. We used this powerful technique to identify the parasite binding partner of the microtubule stabilizing protein CLASP1. Excitingly, this approach also revealed the interaction of other host cell proteins, including signal transduction adaptor proteins and regulators of the actin cytoskeleton, with the parasite. We are currently investigating the significance of these interactions in terms of parasite division and host cell signaling.

STRAIN PAP020, A NOVEL ANAEROBIC MICROEUKARYOTE BRANCHING AT THE BASE OF FORNICATA

Euki Yazaki (Graduate School of Life and Environmental Sciences, University of Tsukuba), Takashi Shiratori (Graduate School of Life and Environmental Sciences, University of Tsukuba), Kietaro Kume (Graduate School of Life and Environmental Sciences, University of Tsukuba), Tetsuo Hashimoto (Graduate School of Life and Environmental Sciences, University of Tsukuba), Ken-ichiro Ishida (Graduate School of Life and Environmental Sciences, University of Tsukuba), Yuji Inagaki (Center for Computational Sciences Institute of Biological Sciences, University of Tsukuba).

A novel microeukaryote, strain PAP020, was isolated from mangrove sediments sampled in the Republic of Palau in November 5, 2011. The laboratory culture of strain PAP020 has been maintained under the anaerobic condition with prey bacteria. PAP020 is oval-shaped cell with two flagella. A preliminary electron microscopic observation identified no typical mitochondrion in the cell. As PAP020 shared no clear morphological characteristic with other previously described eukaryotes, we further explored the position of this microeukaryote using the maximum-likelihood (ML) phylogenetic analysis of small subunit ribosomal DNA (SSU rDNA) sequences. In the SSU rDNA tree, PAP020 showed no strong affinity to other eukaryotes. The position of this microeukaryote was difficult to be settled in the SSU rDNA phylogeny, as the PAP020 sequence appeared to be rapidly evolving. As neither microscopic observation nor SSU rDNA phylogeny provided any clues for the phylogenetic affiliation of PAP020, we suspected that this microeukaryote belongs to an as-yet-to be recognized lineage. To determine the precise phylogenetic position of PAP020, we ran a 'phyogenomic analysis' based on the transcriptomic data of PAP020, which was generated by an Illumina Hi-seq 2000 platform. We prepared an alignment comprising 147 proteins sampled from 79 of phylogenetically diverse eukaryotes (including PAP020). The ML tree inferred from the 147-protein alignment reconstructed a clade comprising PAP020, parabasalids and diplomonads with a BP of 100%. Within this clade, PAP020 showed a specific affinity to diplomonads with a BP of 79%. Due to lack of large transcriptomic/genomic data of CLOs in public databases, we could not include any CLOs in the phylogenomic analysis. Thus, the relationship among PAP020, CLOs, and diplomonads remains uncertain in this study. Nevertheless, we here propose that PAP020 branches at the base of the clade of CLOs and diplomonads (Fornicata), as the SSU rDNA phylogeny recovered Fornicata by excluding PAP020. If PAP020 is genuinely basal to Fornicata, this microeukaryote may hold keys to predict the ultrastructure and anaerobic metabolism of the common ancestor of Fornicata.

RECONSTRUCTION OF CHLOROPLAST PROTEOME OF THE EARLIEST BRANCHING PHOTOTROPHIC EUGLENID, RAPAZA VIRIDIS BASED ON TRANSCRIPTOMIC DATA

Naoji Yubuki (**Department of Parasitology, Charles University in Prague, Czech Republic**), **Vladimir Hampl** (**Department of Parasitology, Charles University in Prague, Czech Republic**), **Brian Leander** (**Departments of Botany and Zoology, University of British Columbia, Canada**), **Anna Karnkowska** (**Department of Parasitology, Charles University in Prague, Czech Republic**).

The chloroplasts of phototrophic euglenids originated through a secondary endosymbiotic relationship between a phagotrophic euglenid and a prasinophyte-like green alga. Molecular phylogenetic analyses revealed that the chloroplast of *Pyramimonas* is a close approximate of the most recent ancestor of all euglenid chloroplasts. Nonetheless, there is still substantial missing data that limit our ability to fully portray the origin and early evolution of euglenid plastids.

A mixotrophic (phototrophy plus phagotrophy) euglenid, *Rapaza viridis*, was described as a new species in 2012. This microalga possesses functional chloroplasts and consume a specific strain of a prasinophyte alga, *Tetraselmis* sp. Behavioral and ultrastructural data, and molecular phylogeny analyses of this euglenid flagellate demonstrated the intermediate features between phototrophic euglenids and phagotrophic lineages. In order to study the evolutionary history of secondary plastid endosymbiosis in euglenids, we sequenced transcriptome from *Rapaza viridis* and assembled it into 107,092 transcripts. 8,875 transcripts contained euglenid specific splice leader at the 5 end indicating their completeness and these were selected for further analyses. Based on automatic annotations and further manual analyses, we hitherto identified 78 sequences encoding putative plastid-targeted proteins. Most proteins are green algal origin but some proteins are originated from lateral gene transfer from red algae. Phylogenetic analyses of concatenated datasets will help to indentify algae that provided the plastid to *Rapaza* as well as infer the evolutionary history of plastid targeted proteins among phototrophic euglenids.

THERE AND BACK AGAIN: COVERINGS EVOLUTION IN CENTROHELID HELIOZOANS

Vasily Zlatogursky (Saint-Peterburg State University).

Centrohelid heliozoans are non-ciliated axopodial protists of uncertain affinities. Cell coverings (organic spicules, siliceous plate-scales and spine-scales) are the main criterion for species identification and higher-rank taxonomy in this group. Phylogenetic reconstructions of centrohelids evolution are mostly based on the sequences of 18S rRNA gene. Newly obtained 18S rRNA sequences, as well as light- and electron-microscopic data led to discoveries of some previously unknown evolutionary events in centrohelids. An interpretation of the cell coverings evolution as a mostly parallel process in two orders is proposed. According to this hypothesis cell coverings were ancestrally organic, then independently silicified and after that multiple cases of desilicification or even complete loss of coverings took place. The family Raphidiophryidae was shown to be polyphyletic and spread between two centrohelid orders. The organisms with organic spicules only (here referred to as *Heterophrys*-like organisms, HLO) revealed to be very broadly spread on the phylogenetic tree of centrohelids. One of such organisms, representing a new undescribed genus presumably occupy a basal position in order Pterocystida which is very similar to the situation in the order Acanthocystida where the most basal position is occupied with another HLO, formally described as a genus *Marophrys*. These data support the idea that coverings in centrohelids were ancestrally organic. Several studied HLOs branch deeply inside genera *Acanthocystis* (3 strains) or *Polyplacocystis* (1 strain), even though they have contrasting morphology of the coverings. This unusual phenomenon may be explained as a life cycle polymorphism or a highly reproducible evolutionary event. Energy-dispersive X-ray microanalysis attributed to spicules of studied HLOs revealed that all such forms, occupying terminal branches have very little hardly traceable amounts of silica, while basally branching forms have no silica at all, which also match the idea that primitive centrohelids had organic coverings.

TETRAHYMENA GENOME ARCHITECTURE PROVIDES A BENEFIT OF SEX IN THE ABSENCE OF SEX

Rebecca Zufall (University of Houston).

The vast majority of eukaryotes alive today have experienced some form of genetic exchange, or sex, in their recent evolutionary history. While a complete explanation for this observation remains elusive, many evolutionary benefits of sex have been identified. Thus, taxa that have lost the ability to have sex, that is, asexuals, are expected to be evolutionary dead-ends. Nevertheless, some asexual lineages appear to be quite successful. These "exceptions to the rule" have the potential to provide novel insights into the evolutionary costs and benefits of sex. I hypothesize that these asexual lineages are successful because they receive benefits normally provided by sex by non-sexual means. Asexual lineages are common in the ciliate genus *Tetrahymena*. The genomic features of these lineages appear to be related to their ability to reproduce sexually. Results of a model of *Tetrahymena* biology support the hypothesis that *Tetrahymena* genome architecture can provide the benefits of sex in the absence of sex.

Last-minute Abstract

IMAGING GIARDIA IN VIVO METABOLISM AND INFECTION DYNAMICS

Scott Dawson (Department of Microbiology, UC Davis, Davis, USA).

Giardia lamblia infects more than one billion people worldwide. *Giardia* has two life cycle stages: a flagellated trophozoite and an infectious cyst that persists in the environment. Environmental cues present in the large intestine are believed to be the primary triggers of encysytation. While encystation can been mimicked by changing in vitro culture conditions, this method does not faithfully generate viable cysts. To assess and quantify *Giardia* infection and encystation dynamics in living hosts, we developed and used non-invasive in vivo and ex vivo bioluminescent imaging (BLI). Mice were imaged after infection with *Giardia* strains expressing Firefly luciferase either constitutively or only during encystation. BLI gives us unprecedented access to host-parasite interactions in real-time, and has permitted us to update and revise decades-old assumptions of the infection and encystation dynamics of *Giardia* in living hosts. First we observed that *Giardia* primarily colonizes the proximal rather than the distal small intestine small intestine. Also, challenging the prevalent belief that *Giardia* encysts primarily in the distal large intestine we imaged a high number of encysting parasites the proximal small intestine early in infection using BLI, and confirmed this by quantifying encysting specific vesicles (ESVs) in trophozoites colonizing the proximal small intestine. Lastly, transcriptomic analysis of in vivo parasites sampled by BLI indicate significantly distinct carbohydrate, lipid, and amino acid metabolic expression profiles as compared in vitro grown parasites.

ABSTRACTS

Posters

COMPARISON OF CELL DIVISION PATTERNS IN THE TINTINNID GENERA FAVELLA AND SCHMIDINGERELLA (ALVEOLATA, CILIOPHORA, SPIROTRICHA)

Sabine Dr. Agatha (**Dept. Ecology and Evolution, University of Salzburg, Austria**), **Rene Eggers** (**Dept. Ecology and Evolution, University of Salzburg, Austria**).

The classification of tintinnid ciliates is mainly based on lorica features, which are influenced by intrinsic and extrinsic factors and often display a huge intraspecific variability and interspecific similarity; hence, they are mostly of low taxonomic value (Agatha et al. 2013 in Dolan et al: The Biology and Ecology of Tintinnid Ciliates – Models for Marine Plankton). This assumption is supported by the cladistic analyses of the few cytologically studied species and the genetic phylogenies. Both kinds of trees reveal that hyaline and agglutinated loricae do not represent distinct lineages and that several families, genera, and even species are not monophyletic. The evolutionary development of the somatic ciliary pattern has, however, shown to mirror rather well the topology of the gene trees (Agatha & Strüder-Kypke 2013 in Dolan et al: The Biology and Ecology of Tintinnid Ciliates – Models for Marine Plankton), and there are some further morphological features that might be of taxonomic significance. For instance, Foissner (1996 in Hausmann & Bradbury: Ciliates – Cells as Organisms) emphasized the need of refined studies of ontogenesis and stomatogenesis specifically for inferring relationships. In the related hypotrich ciliates, differences in cell division patterns are actually used to characterise genera and families [e.g., Berger 2011: Monograph of the Gonostomatidae and Kahliellidae (Ciliophora, Hypotricha)]. Accordingly, in the present study, cell division is investigated in species that were previously congeneric owing to very similar loricae, but now belong to the only distantly related genera *Favella* Jörgensen, 1924 (Family Ptychocylididae Kofoid & Campbell, 1929) and *Schmidingerella* Agatha & Strüder-Kypke, 2012 (Family Rhabdonellidae Kofoid & Campbell, 1929). Protargol-impregnated material of the two species from the east coast of the USA has morphometrically been studied in great detail, and the findings are compared with the mainly anecdotal observations in other tintinnids. The study was financially supported by the FWF (Austrian Science Foundation; Project P20461-B17).

HOST-PARASITOID INTERACTIONS: PARVILUCIFERA SINERAE INFECTING TOXIC MARINE DINOFLAGELLATES

Elisabet Alacid (Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain), Albert Reñé (Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain), Myung Gil Park (Chonnam National University, Gwangju, Korea), Marta Turon (Centre dEstudis avançats de Blanes (CEAB-CSIC), Girona, Spain), Esther Garcés (Institut de Ciències del Mar (ICM-CSIC), Barcelona, Spain).

Parvilucifera sinerae is a parasitoid that infects dinoflagellates, including toxic-bloom-forming species. *P. sinerae* life cycle consists of a free-living zoospore that penetrates a host cell and then develops a trophocyte while destroying the host cytoplasm. The trophocyte undergoes schizogony to form hundreds of new zoospores inside a sporangium. This sporangium remains dormant until, in response to an activation signal, the zoospores are released into the marine environment, where they are able to infect a new host. We identified dimethylsulfide (DMS), produced by many marine microalgal species, as one of the chemical signals involved in zoospore activation. This fact is consistent with the results obtained in a previous study, where we found that *P. sinerae* is a generalist parasitoid, based on its broad host range among dinoflagellate species. Nevertheless, this parasitoid showed infection preferences for certain species. Under laboratory conditions, highest prevalences of *P. sinerae* (over 90%) were reached in the preferred host species like the toxic *Alexandrium minutum*, causing the complete extermination of the host population after 2 parasitoid generations. These interactions may alter the growth and mortality rates of a particular host, having consequences for the microbial community composition in the field. Laboratory results contrast with those obtained during a winter bloom of *A. minutum* in Arenys de Mar harbor (NW Mediterranean Sea) where low to intermediate infection levels of the host by *P. sinerae* were quantified.

DIVERSIFICATION IN DIPLOMONADS: REDUCTION, ACQUISITIONS AND GENOMIC COMPLEXITY

Jan Andersson (Uppsala University), Feifei Xu (Uppsala University), Jon Jerlström-Hultqvist (Uppsala University), Alejandro Jiménez González (Uppsala University), Staffan Svärd (Uppsala University), Elin Einarsson (Uppsala University), Ásgeir Ástvaldsson (Uppsala University).

Diplomonads are heterotrophic protists found in oxygen-poor environments. The group includes the human intestinal parasite *Giardia intestinalis*. However, there is a large diversity within the group. For example, *Spironucleus salmonicida* is a fish parasite, and *Trepomonas* is found in marine sediments. We study diplomonad diversity using comparative genomic tools in combination with functional studies. Here we present the main finding of this ongoing project.

The fish parasite was found to have a much larger capacity for regulation of gene expression and a larger metabolic capacity compared to the human parasite, possibly a reflection of their different lifestyle. *S. salmonicida* is causing systemic infection in the fish and encounters a fluctuating environment. However, the free-living *Trepomonas* has the largest metabolic repertoire due to acquisition of a large number of bacterial genes since the divergence from the parasitic lineages. Many of the introduced genes are coupled to an independence from the host, suggesting that *Trepomonas* has adapted to a free-living lifestyle secondarily.

Hallmark proteins of hydrogenosomes were localized to the mitochondria-related organelles of *S. salmonicida*, indicating that the ancestral diplomonad possessed hydrogenosomes, which has been reduced to mitosomes in the lineage leading to *Giardia*. The ability to form cysts is another ancestral trait; homologs to the functionally characterized cyst-associated *Giardia* proteins were detected in all studied diplomonads.

G. intestinalis cells have two nuclei with two copies of the genome each. Genomic and population genetic data suggest that *Giardia* has a sexual or parasexual life cycle. Genomic data from *G. intestinalis* has shown large variations in the frequencies of allelic sequence heterogeneity. Similarly, *S. salmonicida* has low levels of allelic sequence heterogeneity, while preliminary genome data of the closely related *S. barkhanus* indicate a very complex genome with high levels of sequence heterogeneity, likely in combination with a high frequency of repeats. Understanding the origin and dynamics of such genome complexity and the relationship to a sexual life cycle is needed for a deeper understanding of this fascinating group of protists.

ERYTHRINS, NEW TOXIC METABOLITES FROM THE EURIALINE CILIATE PSEUDOKERONOPSIS ERYTHRINA USED AS CHEMICAL DEFENSE AGAINST PREDATORS

Andrea Anesi (**University of Trento**), **Federico Buonanno** (**University of Macerata**), **Claudio Ortenzi** (**University of Macerata**), **Graziano Di Giuseppe** (**University of Pisa**), **Graziano Guella** (**University of Trento**).

Marine protozoa are known for their ability to produce a vast and chemically diverse array of secondary metabolites that are involved in different ecological functions. Morphospecies belonging to genus *Euplotes* have been extensively studied for their ability to produce chemically diverse secondary metabolites and, interestingly, it was found that strains belonging to same genetic clades were characterized by a different profile of bioactive compounds.

From the genus *Pseudokeronopsis* only two classes of pigments have been so far isolated, keronopsins as defensive molecules of *Pseudokeronopsis rubra* and, more recently, keronopsamides from cell culture of the marine ciliate *Pseudokeronopsis riccii.* We report here on the characterization of new secondary metabolites, erythrins, produced by cell cultures of *Pseudokeronopsis erythrina* (Ciliophora, Hypotricha). Their structure have been elucidated by extensive NMR and high resolution MS measurements and are characterized by a central 4-hydroxy- unsaturated delta-lactone ring bearing an alkyl saturated chain at C(2) and a butyl -benzenoid group at C(5). The simultaneous presence of the corresponding 4-sulphate analogues has also been ascertained and a reasonable proposal of their biosynthesis will be reported. Cold-shock treatment has been performed to induce the discharge of these metabolites from cell pigment granules. The analysis of cytotoxic activity on a panel of free-living ciliates and micro-invertebrates, together with some observation on the defensive behavior by *P. erythrina,* indicated that erythrins are very effective for its chemical defence.

PROTEOME PROFILES OF PHYTOPLANKTONIC PROTIST SPECIES DISCRIMINATED BY MALDI-TOF MASS SPECTROMETRY TO ASSESS THE AQUATIC ECOLOGICAL QUALITY

Lucía Arregui (Departamento de Microbiología III, Universidad Complutense de Madrid), Cristina Gutiérrez (CAI Espectrometría de Masas, Universidad Complutense de Madrid), Antonio Santos (Departamento de Microbiología III, Universidad Complutense de Madrid), Humbert Salvadó (Departamento de Biología Animal, Universitat de Barcelona), Susana Serrano (Departamento de Microbiología III, Universidad Complutense de Madrid).

The requirements of the European Union and, in particular, of the Water Framework Directive 2000/60/EC demand the monitoring of the physical-chemical quality and the use of biological indicators for the control of the ecological state of waters. Phytoplankton is one of the biological quality elements required since it serves for the detection and monitoring of certain physicochemical pressures such as organic pollution, eutrophication, changes in the mineralization of the water and thermal pollution. The application of the WFD involves the identification of the phytoplankton composition and abundance. Phytoplankton includes a group of microorganisms (mostly photosynthetic) that live suspended in the water mass. Protists are important members of this community. The identification of taxa is currently supported by keys and guides and can only be performed by qualified professionals. The aim of this work was to investigate the usefulness of the MALDI-TOF spectrometry in discriminating between protist to genus or species level. MS analyses were conducted using a mass spectrometer MALDI-TOF/TOF Ultraflex (Bruker Daltonik GmbH, Bremen, Germany) with the Flex-Control software v. 2.4 (Bruker Daltonik GmbH, Bremen, Germany) for the control of the instrument and with Flex Analysis 2.4, for the treatment of the spectra. Mass spectra were acquired operating in linear positive ion mode in a mass range from 2000 to 20000 Da. Measurements either of whole microorganism cells or of protein extracts from dense cultures in JM Medium of *Chlamydomonas* (two species), *Gonium, Haematococcus, Cryptomonas* and *Eudorina,* were obtained. Ion spectra of protein profiles of each strain were generated by triplicate being identical with only minor changes. Each mass spectrum was clearly different permitting the distinction between protist genera or even species of the same genus. Consequently if a MS protist database is firstly generated, the MALDI-TOF MS approach could be applied as a rapid assay for the identification of the most abundant protist taxa to species level found in aquatic environments facilitating the determination of their ecological quality.

PARTICIPATION OF ESCRT-III PROTEINS IN ERYTHROPHAGOCYTOSIS OF ENTAMOEBA HISTOLYTICA

Yunuen Avalos-Padilla (Department of Infectomics and Molecular Pathogenesis, Center for Research and Advanced Studies of the Nacional Polytechnic Institute, Distrito Federal, México), Abigail Betanzos (Department of Infectomics and Molecular Pathogenesis, Center for Research and Advanced Studies of the Nacional Polytechnic Institute, Distrito Federal, México), Orozco Esther (Department of Infectomics and Molecular Pathogenesis, Center for Research and Advanced Studies of the Nacional Polytechnic Institute, Distrito Federal, México).

Entamoeba histolytica is the protozoan responsible for amoebiasis, disease considered the third cause of death due to parasitic infections in the world. This parasite produces damage in the colon epithelium via adhesion, cytolysis and phagocytosis. The phagocytic process refers to the engulfment of particles from the host and this requires the participation of several molecules.

Recently, it was proved the existence of the Endosomal Sorting Complex Required for Transport (ESCRT) machinery in this parasite, and moreover, the participation of some members of this machinery in the phagocytic process of *E. histolytica*.

Here, we studied in *E. histolytica* of proteins behavior of the ESCRT-III complex, which in other organisms is involved in membrane remodeling processes during endocytosis.

Using polyclonal antibodies, we performed Western blot assays in total protein extracts and confocal microscopy studies to analyze the proteins localization in basal conditions and during erythrophagocytosis.

We found that trophozoites expressed EhVps2, EhVps20, EhVps24 and EhVps32 proteins, which are localized in the cytoplasm of the parasite. We also observed that during erythrophagocytosis, the proteins changed their localization towards phagocytic vacuoles and cytoplasmic vesicles. EhVps24 and EhVps32 behaved in a similar manner; however, EhVps2 and EhVps20 changed their localization in a different way during phagocytosis. Intriguingly, EhVps2, EhVps24 and EhVps32 located in the nuclei at some time of the phagocytic process, suggesting a new nuclear function of these proteins.

Our findings suggest that ESCRT-III proteins participate during *E. histolytica* phagocytosis.

PARAMYXIDA: EMERGENCE OF AN ENIGMATIC ORDER OF INVERTEBRATE PARASITES

David Bass (NHM/Cefas), Georgia Ward (NHM/Cefas), Rose Kerr (Cefas), Martyn Bennett (Cefas), Rosaline Hulse (UCL/NHM), Grant Stentiford (Cefas), Suzanne Williams (NHM).

Paramyxids cause economically significant mortalities of bivalves, including marteiliosis in the European oyster *Ostrea edulis* and QX disease in the Sydney rock oyster *Saccostrea glomerata*. They also cause disease in crabs, and have been implicated in modification of sexual status in amphipods. Paramyxids are very poorly known: they are very genetically divergent and little is known about their host ranges or biology outside of the hosts of economic concern. However, novel paramyxid lineages are increasingly being detected in a wide range of invertebrate hosts, and their star is clearly in the ascendancy. In February 2015 the first Paramyxean Working Group Meeting was held in Spain, attracting delegates from around the world. We present a fully comprehensive paramyxid phylogeny, clarifying the taxonomy of the group, and report results from environmental DNA (eDNA) and other molecular studies to further investigate paramyxid diversity, ecology, and host affiliations. We also present the first molecular and modern microscopy data for *Paramyxa* sp., and the type species of *Paramarteilia*, *P. orchestiae*.

GENOME ANNOTATION AND ANALYSIS OF HOLOSPORA CURVIUSCULA

Alexandra Beliavskaia (Saint Petersburg State University), Maria Logacheva (Institute for Information Transmission Problems), Dmitry Malko (Vavilov Institute of General Genetics), Sofia Garushyants (Institute for Information Transmission Problems), Mikhail Gelfand (Institute for Information Transmission Problems), Maria Rautian (Saint Petersburg State University).

The bacterium *Holospora* is an endonuclear symbiont of the ciliate Paramecium. The genus *Holospora* is included in order Rickettsiales class Alphaproteobacteria. These gram-negative bacteria have complex life cycle presented by small reproductive forms (1-2 μm long) and large infective forms upto 20 μm. The infective cells have specific structure that allows them to survive in ambient conditions and then to infest new host cells.

All *Holospora* species have host-species specificity (they can inhabit only one *Paramecium* species) and nucleus specificity (macro- or micronucleus, not both).

Holospora curviuscula inhabit macronucleus of *Paramecium bursaria*.

We used Illumina HiSeq to get draft genome of *H. curviuscula*. The genome is composed of 152 contigs and has total length 1,715,500 bp. GC-content is 37,6%.

Automartic annotation at the RAST server (http://rast.nmpdr.org) found 44 rDNA genes and 1756 protein-coding genes (there are 727 genes with defined function and 1029 genes defined as hypothetical proteins).

The genome of *H. curviuscula* was compared with partial genoms of *H. undulata* and *H. obtusa,* and 62 whole genoms of 30 strains of different endosymbiotic species from order Rickettsiales (*Anaplasma, Ehrlichia, Neorickettsia, Rickettsia, Orientia, Wolbachia*). The comparison detected 433 genes that are general for all examined endosymbionts and 203 genes specific only for *Holospora* species.

We also created a map for all metabolic pathways of *H. curviuscula.*

THE ACTIN NETWORK AS A STRUCTURAL BASIS OF THE AMOEBA PROTEUS NUCLEUS

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At the present time, the existence of actin in the nucleus raises no doubts. Nuclear actin pool was demonstrable in different mammalian cell types, amphibian and bird oocytes as well as in some species of different unicellular eukaryotes (e.g., *Paramecium caudatum, Prorocentrum micans*). The presence of actin filaments in the nucleoplasm was also repeatedly discussed for *Amoeba proteus*. However, there is practically no information in the literature about spatial organization of actin in the *Amoeba proteus* nucleus. We investigated distribution of actin in the nucleus and in the cytoplasmic region in the vicinity of the nucleus of *Amoeba proteus* (strain B) with the use of immunocytochemical approaches, including immunofluorescent microscopy and immunogold labeling. In the first case, the cells were squashed gently under coverslips, fixed, and then labeled with polyclonal antibodies against the N-terminal domain of actin (A2103, Sigma), against the C-terminal actin fragment (A2066, Sigma) or with monoclonal anti-actin antibody (MAB1501R, Millipore). The nuclei saved their proper native form after the treatments. Preparations were examined in a confocal laser scanning microscope. Other samples were embedded in LR White resin for actin detecting at the ultrastructural level with the use of immunogold-labeling procedure. Post-embedding labeling of ultrathin sections was carried out with the same set of primary anti-actin antibodies. Confocal microscopy revealed that actin filaments form a regular network in the nucleus and a well-developed layer in the vicinity of the nuclear envelope. In the nucleus, the actin network coincides with the amoebae chromatin. In this fashion, nucleoplasmic actin filaments seem to constitute a structural basis of amoeba nuclei. Immunogold-labeling transmission electron microscopy also proved directly the presence of actin filaments both in the nucleoplasm and at the cytoplasmic surface of the nuclear envelope. Interestingly, we revealed no anti-actin labels in the honeycomb layer located under the inner nuclear membrane of the amoebae nucleus. Thus, the question about the nature of the honeycomb layer remains open. Our results are in agreement with the hypothesis that actin could play a role in the maintenance of nuclear structure and regulation of gene expression.

Supported by RFBR (grants N 15-04-03451 and 15-04-01857), Russia.

HIGH-RESOLUTION DAPI-BANDED CHROMOSOMES OF DINOFLAGELLATE PROROCENTRUM MINIMUM

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Prorocentrum minimum (Pavillard) Schiller is a common planktonic potentially toxic bloom-forming dinoflagellate. It is an armored, primarily photosynthetic with reported mixotrophy species. *P. minimum* reproduces asexually by binary fission. No record of sexual process has appeared to day. There is an extremely little information about this organism karyotype. We began to analyze the *Prorocentrum* minimum karyotype after DAPI-banding of interphase and mitotic chromosomes. Dinoflagellate chromosomes are permanently condensed that facilitates studying of their chromosomes significantly. We used the "high pressure squash technique" for chromosome spreading. The technique strongly increases fine chromatin structures clarity (Novikov et al., 2007; George et al., 2010). For ecdysis induction the dinoflagellate cells were centrifuged for 5 min at 10,000 rcf. A pellet was incubated for 2 h and then 15 min treated with hypotonic 75 mM KCl solution at RT. After that cells were thrice fixed in Carnoys solution (3:1 methanol: glacial acetic acid) and dropped on to the slide. Approximately 250kg/cm2 of pressure was applied through the vise for a 90-120 second interval. Slides were then placed in liquid nitrogen, and cover slips were removed. Preparations were dehydrated in a series of ethanol (70%, 80%, and 100%), air dried and kept in –20°C until time of cytochemical staining. Preparations were DAPI stained and examined in Leica DM2500 microscope. DAPI banding patterns were enhanced as earlier reported (Demin et al., 2011). The chromosome number in *P. minimum* amounts 34 that coincide with earlier report for *Exuviaellamariae-lebouriae* (*P. minimum*'s taxonomic synonym) (Dodge, 1963). Chromosome pairs were determined according to their size and DAPI bands pattern. *P. minimum* is diploid organism (2n=34). Centromeric regions were not identified that is typical for dinoflagellate chromosomes. We determined bands with 3 different intensities – high, medium and low ones. Ideograms for individual chromosomes of the set were constructed.

MONOGRAPH OF THE FAMILY EUPLOTIDAE EHRENBERG, 1838 (CILIOPHORA, SPIROTRICHEA)

Helmut Berger (**Technisches Büro für Ökologie, Consulting Engineering Office for Ecology, Salzburg, Austria**).

The Euplotidae are an important, relatively homogenous subgroup of the Euplotia, one of the three major groups (Oligotrichia, Hypotrichia) of the spirotrichous ciliates. The predominantly benthic euplotids are widely distributed in marine and limnetic habitats (including sewage treatment plants); few species live in terrestrial habitats. The euplotids are a very uniform group compared to the hypotrichs. Most species are small or medium-sized, often distinctly sculptured, have 8–10 frontoventral cirri, 5 transverse cirri, 3 or 4 marginal and caudal cirri, and 8–10 dorsal kineties. An important morphological feature is the striking silverline system of the dorsal side. The nuclear apparatus is composed of a strongly curved macronucleus and a micronucleus. The contractile vacuole is in the posterior body portion near the right cell margin. Until now, more than 160 species, subspecies, varieties, and forms have been described and more than 4000 papers have been published on this group. In recent classifications, usually four genera or subgenera are accepted, namely *Euplotes, Euplotoides, Euplotopsis,* and *Moneuplotes*. However, this morphological separation is only partly supported by gene sequence data. The present monograph will have the same structure like the Monograph of the Hypotricha (Monographiae Biologicae, Springer) by H. Berger. The project comprises, inter alia, the following points: (i) The critical revision of the available data is the major part of the project. (ii) Description of the supposed ground pattern on the basis of a detailed analysis of all relevant features as well phylogenetic analyses. (iii) Investigation of some new populations mainly from limnetic and marine (Adriatic Sea) habitats with classical morphological methods. The monograph of the euplotids will be not only an important reference book for taxonomists dealing with this group, but also a comprehensive source of information for biologists of other disciplines (e.g., ecology, molecular biology, physiology). The PDF-file of the monograph will be freely available (Open Access). However, on safety grounds a printed version will guarantee long-term availability (>100 years).

Supported by the Austrian Science Fund (FWF) Project 26974-B25.

EXPANSION OF THE 'RETICULOSPHERE': DIVERSITY OF NOVEL BRANCHING AND NETWORK-FORMING AMOEBAE HELPS TO DEFINE VARIOSEA (AMOEBOZOA)

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Amoebae able to form cytoplasmic networks or displaying a multiply branching morphology remain very poorly studied. We sequenced the small-subunit ribosomal RNA gene of 15 new amoeboid isolates, 14 of which are branching or network-forming amoebae (BNFA). Phylogenetic analyses showed that these isolates all group within the poorly-known and weakly-defined class Variosea (Amoebozoa). They are resolved into six lineages corresponding to distinct new morphotypes; we describe them as new genera *Angulamoeba* (type species *Angulamoeba microcystivorans* n. gen., n. sp.; and *A. fungorum* n. sp.), *Arboramoeba* (type species *Arboramoeba reticulata* n. gen., n. sp.), *Darbyshirella* (type species *Darbyshirella terrestris* n. gen., n. sp.), *Dictyamoeba* (type species *Dictyamoeba vorax* n. gen., n. sp.), *Heliamoeba* (type species *Heliamoeba mirabilis* n. gen., n. sp.), and *Ischnamoeba* (type species *Ischnamoeba montana* n. gen., n. sp.). We also isolated and sequenced four additional variosean strains, one belonging to *Flamella*, one related to *Telaepolella tubasferens*, and two members of the cavosteliid protosteloid lineage. We identified a further 104 putative variosean environmental clone sequences in GenBank, comprising up to 14 lineages that may prove to represent additional novel morphotypes. We show that BNFA are phylogenetically widespread in Variosea and morphologically very variable, both within and between lineages.

A COMBINED 18S-28S RDNA DATASET TO ELUCIDATE HIGHER-LEVEL RELATIONSHIPS WITHIN RHIZARIA, WITH FOCUS ON ENDOMYXA

Cedric Berney (**Station Biologique de Roscoff**), **Rachel Foster** (**The Natural History Museum, London**), **Georgia Ward** (**The Natural History Museum, London**), **Akinori Yabuki** (**Japan Agency for Marine-Earth Science Technology**), **Takashi Shiratori** (**University of Tsukuba**), **Roberto Sierra** (**University of Geneva**), **David Bass** (**The Natural History Museum, London**).

In spite of their huge evolutionary and ecological success, Rhizaria remain one of the most poorly studied eukaryotic supergroups. Current views on rhizarian evolution suggest a basal split between two major clades, Retaria (radiolarians and foraminifera) and Cercozoa. The latter have been tentatively separated into two groups, the seemingly monophyletic Filosa (e.g. cercomonads, euglyphids, chlorarachniophytes) and the possibly paraphyletic Endomyxa. The morphological, ecological and genetic diversity of Endomyxa is huge: they include at least three major lineages of amoeboid organisms (the vampyrellids, *Filoreta*, and *Gromia*), two major lineages of parasitic organisms (the phytomyxean plant pathogens and the ascetosporean invertebrate parasites), plus a collection of lineages first identified from environmental 18S rDNA surveys, some of which have now been shown to be flagellates. In the present work, we generated complete or nearly complete 18S and 28S rDNA sequences from 35 rhizarian taxa representing 15 major lineages, from either isolated strains or environmental samples, using a primer-walking approach with lineage- and sequence-specific primers. We use this combined 18S and 28S rDNA dataset to robustly elucidate higher-level relationships and discuss major evolutionary trends within Rhizaria, and show that Endomyxa and Cercozoa as currently defined are artificial assemblages. Our results agree with the scarce existing phylogenomic data on Rhizaria but are significantly more comprehensive, covering for the first time the whole known lineage diversity within that supergroup.

EXPLORING THE MITOCHONDRIAL GENOMES OF AMOEBOZOA IN SEARCH OF NOVEL MOLECULAR MARKERS: THE EMERGENCE OF A NEW BARCODE FOR ARCELLINIDA

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Molecular biology is an indispensable tool for assessing phylogenetic relationships among protists. The most commonly used barcoding marker is the 18S (or SSU) ribosomal RNA gene, a highly conserved gene that is present in many copies in the nuclear genomes of all eukaryotes. However, in order to obtain a more complete picture of taxa evolution, other markers are required and especially faster-evolving ones. Here, we applied specifically designed primers to amplify a region that includes parts of both mitochondrial NAD9 and NAD7 in a wide array of Arcellinid testate amoebae. In this group, both genes present an overlap ranging from 8 to 16 nucleotides or an intergenic portion depending on the species considered. We tested the accuracy of these primers for reconstructing phylogenetic relationships between these highly divergent organisms. We also applied these primers to environmental samples, cloned PCR products and placed the obtained sequences in a phylogenetic tree.

AMOEBOZOA-SPECIFIC GENES AS POTENTIAL DNA BARCODES TO STUDY ENVIRONMENTAL DIVERSITY OF AMOEBAE

Natalya Bondarenko (**Saint Petersburg State University**), **Anton Bondarenko** (**Saint Petersburg State University**), **Alexey Smirnov** (**Saint Petersburg State University**).

Implication of metagenomic approaches show that the amount of unexplored diversity in the environmental appears to be enormous, providing a background to the term "the dark matter of life". Amoebozoa are among the groups that are numerous in all kinds of habitats, but rarely represent more than several percent of the total number of sequences when routine DNA barcodes (SSU gene, Cox I gene) are used to study environmental diversity. To explore cryptic diversity of Amoebozoa in the natural habitats we attempted to find Amoebozoa-specific genes and gene families for future application as a DNA barcodes in environmental studies. For this we analyze the transcriptomes of 11 Amoebozoa species represented in MMETSP database (marinemicroeukaryotes.org). We assembled them de novo using Velvet assembler based on de Bruijn graphs optimization algorithm. The preliminary assemblies at the output of Velvet were forwarded to Oases transcriptome assembler. To evaluate quality of transcriptome assemblies we used both QUAST and CEGMA pipeline. In these transcriptomes we found 80-90% of about 250 ultraconservative protein families that occur in a wide range of eukaryotes. Next we performed genes prediction using AUGUSTUS. Among the output of AUGUSTUS we have got from 25 to 75 thousands of predicted possible genes for each of our 11 transcriptomes. We did all-vs-all BLAST using 24 genomes of common species belonging outside Amoebooza with known aminoacid sequences and our AUGUSTUS output. To cluster orthologs from multiple species we sent BLAST output to OrthoMCL package. As a result we have obtained about 84 thousands of orthologs groups. For each of analyzed 11 species transcriptomes about 50% of predicted genes were clustorized into the ortholog groups. OrthoMCL revealed considerable number of groups consisting solely of Amoebozoan genes; many genes are represented with a large numbers of paralogs. Those showing low level of paralogy are potential candidates to be Amoebozoa-specific DNA barcodes. We discuss the possible approaches for the selection of the potential DNA barcoding gene sequences among the predicted genes clustered specifically for the Amoebozoa.
Supported with SPSU postdoc projects 1.50.1622.2013 and MK-4853.2015.4 President grant for young PhDs.

STUDIES ON NUCLEAR AND GENOMIC BIOLOGY OF THE DINOFLAGELLATE OXYRRHIS MARINA

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Understanding the nature and the evolution of the nucleus, chromatin and genome of dinoflagellates is one of the long-standing challenges in eukaryotic biology. Early research has suggested that dinoflagellate chromatin has a different organisation than the rest of eukaryotes, probably using non-histone basic proteins as the basis of DNA packaging. But beyond the uniqueness of this feature, the possibility that the basic chromatin organisation is fundamentally different in dinoflagellates poses crucial implications for a myriad of molecular and cellular processes that should not be overlooked. To explore the characteristics and origin of some of the unusual features of the dinoflagellate nucleus we are focusing our efforts on the phagotrophic marine dinoflagellate *Oxyrrhis marina*. This species is an emerging model for protistan biology and because of its phylogenetic position at the base of dinoflagellates, an interesting subject to investigate the origin of dinoflagellates' unusual features. Here we present the recent progress achieved in our explorations of the *O. marina* genome and nuclear biology. On one side, we analyzed the variability of genes encoding the novel Dinoflagellate Viral Nuclear Protein (DVNP). In line with results on other protein-coding genes, DVNP exists in at least several dozens variants that can be grouped in a few structural types. In addition to nucleotide sequence data for this gene (i.e. genomic and cDNA), we also found evidence of two variants expressed at the protein level. From genomic data we reconstructed the organization of a tandemly-arranged multigene cluster of HSP90, which we used to generate hybridization probes for Southern-blot experiments to obtain a detailed picture of the distribution of these genes in the genome. Finally, we present abundant expression data of many genes that constitute canonical processes of DNA metabolism, such as nucleosome assembly, chromatin regulation, etc, confirming that dinoflagellates have maintained the basic eukaryotic machinery for chromatin organization and gene expression.

UNCOVERING THE MATING LOCUS IN DIATOM SEMINAVIS ROBUSTA

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Diatoms are a group of unicellular algae that influence life on Earth in several ways. They account for about one fifth of primary production, conversion of solar energy into organic compounds and production of oxygen (1). Their ability to store energy in the form of lipids is being investigated for future biofuel production. A key feature of the diatoms' haplo-diploid life cycle is a unique strategy to switch between reduction of cell size during somatic divisions and its restitution during sexual reproduction. Despite their importance in ecosystem and potential for biotechnology, diatom biology and life cycle regulation are hardly understood at a molecular level.

In our work, we focus on elucidation of the mechanism underlying sex determination and the regulation of sexual reproduction in *Seminavis robusta*, a diatom model for life cycle research. *S. robusta* life cycle displays characteristics typical for marine pennate diatoms: size reduction during mitotic cell divisions, strict size dependence of sexual reproduction capability on cell size and presence of 2 mating types (heterothallism) (2,3).

At the congress, I will present our recent progress on the characterization of the recently identified *S. robusta* mating locus.

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GLOBAL DISTRIBUTION AND VERTICAL PATTERNS OF A PRYMNESIOPHYTE-CYANOBACTERIA OBLIGATE SYMBIOSIS

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A marine symbiosis has been recently discovered between prymnesiophyte species and the unicellular diazotrophic cyanobacterium UCYN-A. At least 2 different UCYN-A phylotypes exist, the clade UCYN-A1 in symbiosis with an uncultured small prymnesiophyte and the clade UCYN-A2 in symbiosis with the larger *Braarudosphaera bigelowii*. We targeted the prymnesiophyte–UCYN-A1 symbiosis by double CARD-FISH (CAtalyzed Reporter Deposition-Fluorescence In Situ Hybridization) and analyzed its abundance in surface samples from the MALASPINA circumnavigation expedition. Our use of a specific probe for the prymnesiophyte partner allowed us to verify that this algal species virtually always carried the UCYN-A symbiont, indicating that the association was also obligate for the host. The prymnesiophyte–UCYN-A1 symbiosis was detected in all ocean basins, displaying a patchy distribution with abundances (up to 500 cells ml-1) that could vary orders of magnitude. Additional vertical profiles taken at the NE Atlantic showed that this symbiosis occupied the upper water column and disappeared towards the Deep Chlorophyll Maximum, where the biomass of the prymnesiophyte assemblage peaked. Moreover, sequences of both prymnesiophyte partners were searched within a large 18S rDNA metabarcoding dataset from the Tara-Oceans expedition around the world. This sequence-based analysis supported the patchy distribution of the UCYN-A1 host observed by CARD-FISH, and highlighted an unexpected homogeneous distribution (at low relative abundance) of *B. bigelowii* in the open ocean. Our results demonstrate that partners are always in symbiosis in nature and show contrasted ecological patterns of the two related lineages.

DESCRIPTION AND PHYLOGENETIC POSITION OF CORLISSINA MARICAENSIS GEN. NOV., SP. NOV. (KARYORELICTEA, GELEIIDAE), A NEW INTERSTITIAL CILIATE FROM BRAZIL, WITH REDEFINITION OF THE FAMILY GELEIIDAE

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Corlissina maricaensis gen. nov., sp. nov. was obtained from samples of sediment collected in a brackish lagoon of Maricá city, in Rio de Janeiro state. The morphological description was based on live observations, after protargol impregnations, and scanning electron microscopy. The new species has a cylindrical body shape that is slightly contractile, 230-550 × 35-65 µm in size, a cytoplasm with many globular inclusions, one row of irregular cortical granules between each somatic kinety, approximately 40-62 somatic kineties, two globular macronuclei measuring 9-24 µm and one micronucleus of approximately 4-9 µm. A subapical oral cavity was approximately 20-80 × 9-25 µm, with an adoral zone on the left side of the buccal field, that was composed by 32-60 polykineties and a paroral at right side that was composed by 40-57 short polykineties. The new genus is distinguished from other geleiids by a loop-shaped posterior end of the paroral ciliature, constituted by two rows of short polykineties, and the oralization of the central superior kinety (K0i), forming a row of dikinetids that internally borders the adoral zone, followed by several rows of monokinetids. In the phylogenetic analyses, the new species was recovered as the sister group of *Parduczia orbis* with full support values based on 18S rDNA gene sequences. This work also indicates some problems in the Geleiidae definitions and proposes a new diagnosis for this karyorelictid family.

CHARACTERIZATION OF THE BIOLOGICAL ROLE OF THE MULTIFUNCTIONAL EHURE1-BP PROTEIN OF ENTAMOEBA HISTOLYTICA, DIFFERENT TO THE TRANSCRIPTION FACTOR

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EhURE1-BP is an *Entamoeba histolytica* protein that belongs to the family of multifunctional Tudor and Staphylococcal Nuclease (TSN) proteins. This protein is a transcription factor that binds to the cis-activation motif URE1. Immunolocalization assays showed that this protein is found in the nucleus, where it is involved in transcription, but it is also located in the cytoplasm, possibly participating in other biological processes. Using, immunoelectron microscopy we found that EhURE1-BP is localized in small cytoplasm vesicles and by pull-down assays we identified some proteins that interact with EhURE1-BP. These proteins are involved in different cellular processes, such as transcription, metabolic processes and the organization of the cytoskeleton, and others. Based on these results it is suggested that EhURE1-BP is a multifunctional protein similar to proteins of the TSN family.

MOLECULAR PHYLOGENY OF THE FAMILY OPHRYOSCOLECIDAE WITH EMPHASIS ON VALIDITY OF THE GENUS EODINIUM (ENTODINIOMORPHIDA, OPHRYOSCOLECIDAE)

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The genus *Eodinium* includes species of ophryoscolecid ciliates without skeletal plates, with two ciliary zones in the anterior end of the body and rod shape macronucleus. The validity of this genus is disputed by several authors due to the morphological similarities between *Eodinium* and *Diplodinium*. However, studies of oral infraciliature in ophryoscolecid ciliates showed that *Eodinium posterovesiculatum* has infraciliary arrangement exceptional in family Ophryoscolecidae which supports the validity of the genus *Eodinium*. Phylogenetic studies on family Ophryoscolecidae, based on information present in the 18s rRNA gene are incipient and the phylogenetic position of *Eodinium* remains unknown, because there's no sequences of this genus in the database. In order to check the phylogenetic relationships among *Eodinium* ciliates and other ophryoscolecid, the small subunit rRNA genes were sequenced for four morfotypes of *Eodinium posterovesiculatum:* posterovesiculatum-type, lobatum-type, monolobosum-type and bilobosum-type from four Brazilian cattle. To identify the ciliates, we performed morphometry on 20 specimens of each morfotype stained with the Lugols solution and impregnated with silver carbonate. The sequences of *Eodinium posterovesiculatum* morfotypes were aligned with other previously reported sequences of ciliates in the class Litostomatea. Phylogenetic trees were construted using two different analyzes: maximum likelihood (ML) and Bayesian inference (BI). Morphometric characteristics and the alignment of the sequences showed great genetic and morphological similarities between *Eodinium posterovesiculatum* morfotypes, suggesting that the types constitute a single species with polymorphisms. In both phylogenetic analyzes, the species *E. posterovesiculatum* has been positioned as brother group of *Polyplastron multivesiculatum* and other clade which includes seven species of the genus *Ostracodinium* with high support values. These results corroborate the hypothesis of the validity of the genus *Eodinium*, as the clade formed by the species *E. posterovesiculatum* remained phylogenetically distant to the clade consisting of *Diplodinium* ciliates. Furthermore, the analysis suggests that this species had a loss of skeletal plates, as brothers groups consist of species with plates. This work demonstrated the importance of molecular studies of ophryoscolecid ciliates in order to better understand the phylogenetic relationships within the family.

COMPLETE NUCLEAR GENOME SEQUENCE OF GONIOMONAS AVONLEA, A PLASTID-LACKING CRYPTOMONAD

Ugo Cenci (Dalhousie University).

The cryptomonads are eukaryotes comprising both photosynthetic and non-photosynthetic species. While cryptomonads such as *Guillardia theta* harbor a plastid of secondary endosymbiotic origin, members of the so-called Goniomonadea lack plastids. A long-standing question in the field of plastid evolution is whether the Goniomonadea are ancestrally non-photosynthetic or whether they lost their plastid secondarily. To address this and other issues, we have sequenced the genome of the newly described species, *Goniomonas avonlea*, and compared it to that of *Gu. theta*. The draft genome of *Go. avonlea* is ~96 Mbp in size, encoding ~32,000 proteins. Interestingly some metabolic pathways present in the *Gu. theta* plastid and periplastidial compartment are also present in *Go. avonlea*, suggesting that these cytosolic pathways were relocated during the course of secondary plastid integration. In contrast, other cytosolic pathways found in *Go. avonlea* are not in *Gu. theta;* these pathways could have been lost in *Gu. theta* or recently gained in *Go. avonlea*. The *Go. avonlea* genome is a valuable tool for elucidating the physiology of heterotrophic cryptomonads, as well as the metabolic 'rewiring' that took place during plastid integration.

BACTERIAL-LIKE MITOCHONDRIAL GENOME FOR PLANKTONIC FORAMINIFERA GLOBIGERINELLA AEQUILATERALIS

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The sequenced mitochondrial genomes of protists indicate that the structures and content of genes, as well as organizations of the mitochondrial genomes in this group are more diverse than in multicellular enkaryotes. Not only did the sequence unravel the evolution of the mitochondrium, but also serve as a practical tool to access protist's diversity. In this study we present the preliminary result of the first sequenced mitochondrial genome retrieved from planktonic foraminifera *Globigerinella aequilateralis*. The verified genome size is about 45kbp with 80% of A+T contents. We are able to identified fifteen protein-coding genes, including seven NADH dehydrogenases, three cytochrome oxidases, three ATPases, a protein translocase and cytochrome b by using standard genetic code. In addition to the identified protein-coding genes, the mitochondrial genome has five unknown open reading frames (ORFs) longer than 200 amino acid sequences. The protein-coding genes and the unidentified ORFs use all 64 amino-acid codons; and they are transcribed in the same direction except for the protein translocase. In the non-protein coding sequences, we detected 20 out of the 22 typical tRNA genes predicted from their secondary structure. Further investigation focusing on the different levels of divergence among genes and species is needed to provide the insight into the mitochondrial genomes' application in foraminiferal diversity and phylogeny.

INTEGRATING MORPHOLOGICAL, ONTOGENETIC AND MOLECULAR DATA TO EVALUATE THE PHYLOGENY OF CILIATES: A CASE STUDY ON THE HIGHLY CONTROVERSIAL ORDER UROSTYLIDA (PROTISTA, CILIOPHORA)

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Among ciliates, the order Urostylida is one of the most confused and diverse and is increasingly attractive for the researchers working on morphology, morphogenetic and molecular phylogeny fields. Previous phylogenetic analysis and systematic arrangement on the highly controversial order Urostylida were mainly based on morphological data or marker-gene information. In the present work, the morphological characters of 28 genera, ontogenetic patterns of 38 species from 22 genera, and molecular phylogenetic trees based on small-subunit ribosome gene sequences of near 60 species are used to investigate the evolutionary relationships among the urostylids further. The main lineages of the cladistic system constructed from the apomorph and plesimorph of combined morphological and morphogenetic information correspond well with the phylogenetic trees based on SSU rDNA sequences. The following conclusions could be drawn: (1) the families Epiclintidae and Parabirojimidae are confirmed to be valid and supported to be monophyletic; (2) the transfer of the genera *Thigmokeronopsis* and *Apokeronopsis* from the family Pseudokeronopsidae to the family Urostylidae is supported; (3) the family Pseudokeronopsidae including the genera *Apoholosticha* and *Heterokeronopsis* should take the intrakinetal origin of the marginal cirral rows and dorsal kineties as the diagnostic character instead of the non-fusion of multiple macronuclear nodules during the ontogenetic process; (4) the genera *Pseudoamphisiella*, *Anteholosticha* and *Diaxonella* are not supported to be included in the family Holostichidae; (5) the genus *Anteholosticha* is extremely polyphyletic; and (6) the genus *Metaurostylopsis* has its own morphological characteristics and morphogenetic pattern, which is far away from the genus *Bakuella*, and may be excluded from the family Bakuellidae. This work was supported by the Natural Science Foundation of China (Project numbers: 31430077 and 31401954).

TOXICOLOGY RESEARCH ON ULTRASTRUCTURE OF CILIATES

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With first transmission electron microscope was invented in 1931, and first scanning electron microscope was invented in 1937, all kinds of samples have been observed by SEM and TEM involving abiotic materials and organisms. Pitelka summarized ultrastructure features of Protozoa till 1963. From then on, many protozoa structures have been disclosed on submicroscopic level. Firstly, we will briefly review the study history of protozoa ultrastructure and conclude the new development and new trend in this field. Secondly, this report will focus on illustrating our recent results about toxicology effects of nine kinds of compounds on ultrastructures of five species of ciliates. These compounds involve nanomaterials, heavy metals, phosphorus pesticides and a biological agent. Results show that different compounds with minimum effective concentration will cause change of ultrastructures of ciliates. The main change appears on macronucleus, mitochondria, membrane structure and microtube. These results might reveal some mechanism of toxicology and suggest that ultrastructures of ciliates can be used to test and assess the longtime biological toxicity of various compounds. We will be devoted to this study field in the future. This work was supported by the National Science Foundation of China (NO.31471950, 31101613, 30970311)

PHYSIOLOGICAL ROLE OF MITOCHONDRIAL CALCIUM UNIPORTER (MCU) IN THE CAUSATIVE AGENT OF CHAGAS DISEASE, TRYPANOSOMA CRUZI

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Calcium ion (Ca2+) serves as second messenger for a variety of cell functions including host cell invasion by intracellular trypanosomatids. The mitochondria of these organisms possess a uniporter (mitochondrial calcium uniporter or MCU), which in *Trypanosoma brucei* is essential for its survival. Moreover, the AMP/ATP ratio regulates the survival mechanism of autophagy in trypanosomatids, whereas mitochondrial Ca2+ overload generates mitochondrial reactive oxygen species that are involved in cell death. Therefore, mitochondrial Ca+2 uptake could result in life-death decisions for these parasites. In order to determine the role of mitochondrial Ca2+ in autophagy and cell death in *T. cruzi*, the etiological agent of Chagas disease, we obtained mutant cell lines where the gene encoding MCU (TcMCU) has been either knocked out or overexpressed. The ablation of TcMCU by CRISPR/Cas9 system led to a marked decrease in mitochondrial calcium uptake without affecting the membrane potential of digitonin-permeabilized *T. cruzi* epimastigotes, whereas the overexpression of the TcMCU using the pTREX vector caused a significant increase in the ability of mitochondria to accumulate Ca2+, without altering the mitochondrial membrane potential. Mitochondrial localization of TcMCU was confirmed by C-terminal tagging with green fluorescence protein (GFP) and fluorescence microscopy. Other phenotypic features evaluated in these mutant cell lines include cell growth, autophagy and cell death pathways.

Work funded by FAPESP N° 2013/50624-0, 2011/50400-0 and 2014/13148-9.

PROTEOMIC ANALYSIS OF ATRAZINE STRESS RESPONSE IN CHLAMYDOMONAS REINHARDTII

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Atrazine is one of the most frequently organic pollutants of agricultural soils and ground and surface waters due to its widespread use. Recently, this herbicide has been catalogued as new hazardous substance in the aquatic environments by the European Union (Directive 2013/39/EU), being necessary to assess its environmental risks, including its effects on non-target organisms, such as freshwater microalgae, which are in the base of the trophic chain. Nowadays, with the development of the omics, new alternatives emerge for the study of the effects of these contaminants on microalgae. Particularly, proteomic studies have a great potential for investigating subcellular mechanisms of stress and responses that affect the growth and the physiological and biochemical features of microalgal cells.

The main purpose of this study was the analysis of changes in the cellular proteome of *Chlamydomonas reinhardtii* between control cells and cells exposed for 3 and 24 h to one atrazine concentration.

Cultures with increased concentrations of atrazine were established, and their growth and cell viability were determined by flow cytometry (FCM). Protein extractions were carried out, followed by iTRAQ labeling and MALDI-TOF/TOF Mass Spectrometry (MS) analysis. Differentially expressed proteins (p).

Based on FCM results, the atrazine concentration chosen for proteomic analysis was 0.25 µM, close to the 96 h EC50 for growth, and which cell viability remained above the 96%. Proteomic analysis indicated a subexpression of 12 proteins in cultures exposed to atrazine after 3 h of treatment, whereas after 24 h of herbicide exposure, only 7 proteins showed a differential expression pattern, 4 proteins were subexpressed and 3 overexpressed. Transketolase and phosphoglycerate kinase, subexpressed after 3 h were overexpressed after 24 h. This may be related to the process of adaptation of *C. reinhardtii* to the stress situation, where an increase in the abundance of the proteins is necessary for the maintenance of cell viability.

Financial support from Spanish MINECO (CGL2010-15993/BOS). M. E. and M. S. have Xunta de Galicia predoctoral grants (Spain).

MOLECULAR CHARACTERIZATION AND EXPRESSION OF THE APICAL MEMBRANE ANTIGEN-1 (AMA-1) FROM BABESIA BIGEMINA KAYSERI/TURKEY STRAINS

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Babesia bigemina is an apicomplexan parasite that infects red blood cells of its vertebrate host and cause babesiosis which is an important tick borne disease and endemic in different parts of the world. Apical Membrane Antigen-1 (AMA-1) is a microneme protein that exists in all apicomplexan parasites and plays an indispensable role in invasion into host cell and also has been described as an excellent vaccine candidate. This study was carried out to determine molecular characterization and expression of AMA-1 of an in vivo passage 1 and an in vitro passage 1 *B. bigemina* Kayseri/Turkey strains and supported financially by Erciyes University Research Fund with the project number TDK-2014-5030. For this aim, total RNA was extracted from the *B. bigemina* in vivo and in vitro cultured strains which have 4.1% and 11% parasitemia, respectively. The cDNA was prepared from the obtained RNA samples. PCR was carried out with specific AMA-1 primers which amplified full-length *B. bigemina* AMA-1 (BbigAMA-1) gene. The PCR products were purified and cloned. The cloned isolates were sequenced and the obtained sequences were deposited in GenBank with the accession numbers KP000032-33. Pairwise analyses of the sequences and multiple alignments with some other BbigAMA-1 isolates available in GenBank were performed and phylogenies were investigated. The BbigAMA-1 fragments were amplified by PCR using predesigned expression specific primers and cloned into the expression plasmid vectors. The plasmid was subsequently transformed into *E. coli* BL21 and expressed. The expressed samples were analyzed by SDS-PAGE and target protein weights were calculated. Pairwise alignment of the sequences from Kayseri/Turkey IV1 strain from in vivo passage 1 and Kayseri/Turkey IT2 strain from in vitro passage 1 of *B. bigemina* showed 99.8% identity to each other. According to the phylogenetic comparisons Kayseri/Turkey IV1 strain and Kayseri/Turkey IT2 strains showed most similarity to "Turkey" isolate with 99.9% and 99.8% identities, respectively. BbigAMA strains were expressed with a molecular mass of 73 kDa. In conclusion this study is the first report of comparative molecular characterization and expression of AMA-1 from two different strains of *B. bigemina* from cattle in Turkey.

DISTRIBUTION OF SOIL FREE-LIVING AMOEBAE' TROPHIC GROUPS AROUND ROOTS OF ZEA MAYS MICORRIZED BY GLOMUS INTRARRADICES.

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Species diversity is dependent on both quantity of resources and their distribution in a soil volume. As different amount of resources produce different species richness and interactions, the soil volume or sampling effort has to be made before comparing soil treatments or planted vs non-planted soils. In this sense, we wonder how species richness around the *Zea mays* rhizosphere is affected by *G. intrarradices* after 20 days of mycorrhization. We analyzed 72 g of soil sample in order to produce the rarefication curve and calculated 15 g of soil as the minimum soil volume for comparison between micorrhized and non micorrhized *Zea mays.* MPN counts of the amoebae morph types yielded 11961 individuals/g of fan shape amoeba, 11798/g acanthamoeba like and 572/g reticulated- type amoebae were found in the non-micorrhized environment. There were no clear differences between MPN numbers of micorrhized and non-micorrhized treatments. We identified 77 species distributed as 21 dominant, 22 common and 34 rare ones in both control system and treatments. Protozoan eater amoeba was the best represented group (33%) followed by bacterivorous (23%) amoeba, algivorous and fungivorous with 16% respectively, omnivorous 8% and yeast eater only amount for 4%. Contrary to what was expected, we find out similar proportion of fungivorous amoebae in the micorrhized treatment but the community composition showed some degree of differences between the rhizosphere of *Zea mays* alone and the one with the mycorrhiza. However, these similarities in numbers and species richness as well as dominance of trophic groups may be clearer and significant as the maturation of the relationship plant-mycorrhiza proceeds and the successional change of these amoebae communities may take longer.

EPIBIONT CILIATES ON NEOTROPICAL LIMNIC GASTROPODS: NEW RECORDS, COMPOSITION AND STRUCTURE OF THE COMMUNITY ASSEMBLY

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Studies on epibiotic relationship involving molluscs and ciliates in limnic environments are scarce, even if molluscs present many characteristics favorable to the establishment of this relationship, as the occupation of substrates commonly used by ciliates, as well as the preference for environments with good oxygenation and great concentration of organic matter. We analysed the composition and structure of the community of peritrich epibionts on five species of freshwater snails. We registered epibionts on *Biomphalaria peregrina; Pseudosuccinea columella; Physa acuta; Physa marmorata* and *Gundlachia cf. lutzi. Gundlachia lutzi* showed greater values of prevalence (36.80%), followed by *B. peregrina* (17.40%), *P. marmorata* (7.70%), *P. columella* (22.60%) and *P. acuta* (9.61%). We registered 16 peritrich species: *Carchesium polypinum, Epistylis plicatilis, E. chrysemydis, Epistylis* sp.1, *Epistylis* sp.2, *Opercularia articulata, O. nutans, Opercularia* sp.1, *Opercularia* sp.2, *Vorticella campanula, V. covallaria, Vorticella* sp.1, *Vorticella* sp.2, *Vorticella* sp.3, *Thuricola* sp., *Platycola* sp., besides two suctorians, *Tokophrya quadripartita, Tokophrya cf. lenarum* and one heterotrichid, *Stentor* sp.. *Biomphalaria peregrina* showed greater values of richness (12), followed by *G. cf. lutzi* (9) *P. acuta* (5), *P. marmorata* (2) and *P. columella* (2). *Platycola cf. decumbens* (27.3%) occurred with greater values of prevalence. Greater values of intensity and mean abundance were observed for *C. polypinum* (356) and *Epistylis* sp. 2 (27.49 zooids/snails), respectively. The present study constitutes the first record of epibiosis by ciliates on snails of the family Ancylidae in neotropical region. Until now, there was only one record of epibiosis by *Platycola decumbens* on the gastropod *Ancylus fusca* in Europe. We recorded for the first time epibiosis by ciliates on the species *P. marmorata, P. columella, B. peregrina* and *G. cf. lutzi,* as well as, epibiosis on snails by *E. crysemydes,* which was found only on algae and macrophytes, *O. nutans* previously reported on macrophytes, aquatic insects and crustaceans and *V. convallaria,* found in activated sludge systems These results show that the diversity of epibiont ciliates on limnic gastropods in neotropical environments is sub estimated most probably because of the lack of studies aiming to access the diversity of molluscs and associated ciliates.

PLASMODIUM (NOVYELLA) UNALIS CF. AND PLASMODIUM (HAEMAMOEBA) LUTZI IN TURDUS SPP. (PASSERIFORMES) OF THE ATLANTIC FOREST IN SOUTHEASTERN OF BRAZIL: MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION

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This study aimed to characterize morphologically and molecularly the haemosporidian of *Plasmodium* and *Haemoproteus* species in *Turdus* spp. in fragments of Atlantic Forest in southeastern of Brazil. They were captured and examined 90 birds, 69 of species *Turdus rufiventris*, 14 *Turdus leucomelas,* 06 *Turdus amaurochalinus* and 01 *Turdus flavipes.* The morphological and morphometric characterization were made by examining blood smears. Blood samples were collected for extraction and amplification of DNA from organisms of the genus *Plasmodium* and *Haemoproteus*. With positive samples were sequenced to 479 bp fragments to carry out phylogenetic analysis. The *Plasmodium unalis* cf. infection was detected in 58 birds, being 48 of the species *T. rufiventris,* 08 *T. leucomelas,* 01 *T. amaurochalinus* and 01 *T. flavipes. P. unalis* was also described in a species of the genus *Turdus* (*Turdus fuscater*), in Colombia. This identification was a molecular basis, because the available sequence database formed a clade with the sequences of the present study. Morphologically the species found has features such as a refractive globule present in trophozoites and meronts, which have only one hemozoin granule and fan-shaped; macrogametocytes and microgametocytes have accumulation of hemozoin granules and granular cytoplasm, as in the original description of *P. unalis*. However, no consistent features with the original description: trophozoites in ring-shaped; the number of merozoites, which in most was equal to four; the width of macrogametocytes which exceeded 2.6µm; and the length of gametocytes, which fill infected erythrocytes up to their poles. It was also found the species *Plasmodium lutzi* in three specimens of *T. rufiventris* and in two *T. leucomelas.* This species was identified by morphological characteristic of hemozoin pigment clumping into a solid mass in meronts and gametocytes and the number of merozoites formed in meronts (6-26). Phylogenetic analysis confirmed the morphological identification of this species, because the sequence it is grouped with existing in databases. The results indicate that the use of combined molecular and morphological techniques is still very important in identifying avian haemosporidian species. It is also suggested that studies of co-evolution host-parasites should be conducted to better understand this relationship.

ON THE MORPHOLOGY OF A NEW SPIROSTOMUM SPECIES (CILIOPHORA, HETEROTRICHEA) FROM BRAZIL

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Spirostomum Ehrenberg, 1838 are large ciliates of the class Heterotrichea with distinctive vermiform shape, common in freshwater and low salinity (brackish) environments. Species of this genus are widely used as model organisms in ecological studies of environmental impacts and symbioses between ciliates and human pathogenic bacteria. The present study provides details of total infraciliature, nuclear apparatus and morphometric data of a new *Spirostomum* species isolated from a freshwater pond in southeast Brazil. *Spirostomum binucleata* sp. nov. was investigated using live observation and protargol impregnation. The main feature which differs *S. binucleata* sp. nov. of their congeners is the presence of two oval macronuclear nodules (avg. 35×15 µm), whereas the other *Spirostomum* species have a single or moniliform macronucleus. Additionally, *S. binucleata* sp. nov. can be identified by the following characteristics: 325-530 µm long in vivo; 42-70 µm width; brownish cytoplasm;10-18 somatic kineties on each side, parallel to the main body axis, but strongly spiraled when the organism contracts; 3-4 homogeneous cortical granule rows between each kinety pair; peristome about 1/3 of the body length; contractile vacuole up to 1/4 of the total body length, with a conspicuous collecting canal; micronuclei variable in number (3-24). Molecular characterization for this species is still under preparation.

THE CHLOROPLAST GENOME OF EUGLENA MUTABILIS AND EVOLUTIONARY IMPLICATIONS

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The chloroplast genome of *Euglena mutabilis* was sequenced and analyzed to gain further insight into variations of gene clusters during diversification of phototrophic euglenids. In the higher derived Euglenales clusters became more consistent with only a few changes in the genus *Euglena. E. mutabilis* differed from the other *Euglena* species in a mirror-inverted arrangement of 12 from 15 clusters and could be regarded as the most basic *Euglena* sequenced so far. This was supported by many similarities in cluster arrangement and orientation with *Strombomonas acuminata, Monomorphina* species, *Euglenaria anabaena* and *Cryptoglena skujae. Trachelomonas volvocina* and *Colacium vesiculosum* on the other hand showed more dissimilarities to the clade Euglena and even differed from closer relatives by deviating cluster succession. RT PCR analysis was performed to allow for the verification of the proposed 77 introns in 37 protein coding genes, their exact exon-intron boundaries and for their characterization. An examination of the introns in psbC supported an evolutionary trend from a single intron in *Eutreptia viridis* to 10 introns in *E. gracilis.* In the Eutreptiales intron 1 in psbC was identified as group II twintron that encoded mat1 (maturase-like protein) necessary for intron mobility. Intron 2 (if present) classified as group III twintron contained mat2. The distribution of introns and mat1 and mat2 was used to gain insight into evolutionary processes and intrageneric differentiation. During the emergence of Euglenales a switch occurred with the result that mat1 was situated mostly in intron 2, never in intron 1. Mat2 could be found mostly in intron 1, except for *E. gracilis* strains. Each of the identified lineages started with two introns and gained additional introns the more they diverged, except for *S. acuminata* and *T. volvocina*.

RESPONSE OF TESTATE AMOEBAE AND PLANT COMMUNITIES TO PEATLAND RESTORATION: IMPLICATIONS FOR COMMUNITY CONCORDANCE

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Up to 13% of Finnish peatlands have been altered by human landuse, such as drainage for forestry, agriculture, and peat mining. The manifold environmental importance of peatlands has led to growing attempts to restore or partially rehabilitate their original properties such as surface water retention, carbon sinking, and the maintenance of specific flora and fauna.

Monitoring and assessment of peatland ecological state is a fundamental part of conservation and restoration programs. Most bioassessment studies have focused on the responses of single taxonomic groups to environmental factors and very few have addressed whether or not, different groups show parallel responses i.e. concordance. Community concordance describes similarity in distributions and abundances of different taxonomic groups across a certain region and emerges when different groups show similar responses to the changes in environmental factors. Studies based on the assessment of the plant community composition after restoration practices have shown promising results however, only little is known about changes in the microorganism communities of restored peatlands.

Testate amoebae (TA) are a polyphyletic group of shell building unicellular protists, commonly associated with peatland plants and especially abundant in *Sphagnum* mosses. Their diversity and distribution are controlled mainly by hydrological, and to a lesser extent by water pH, oxygen concentration, and peat composition. Thus, it has been suggested to use TA communities as a tool for peatland restoration monitoring and assessment. However, to this day, TA have been sparsely used to this end.

In this study we assessed concordance of the changes in TA and plant community structures among natural, ditched, five years restored, and 10 years restored boreal peatlands. TA and plant communities were concordant when comparing all sites; however there was no concordance within treatments except for five years restored sites. Our results suggest that concordance between the two communities is scale dependent and when all sites are included, might arise from the fact that both communities are related to chemical parameters, either in a direct or indirect way. We conclude that TA and plant communities are not necessarily surrogates of each other and for a whole ecosystem perspective, different approaches should be used.

MORPHOLOGICAL CHANGES IN THE CYTOSTOME-CYTOPHARYNX COMPLEX OF TRYPANOSOMA CRUZI EPIMASTIGOTES DURING CELL DIVISION

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The cytostome-cytopharinx complex is a specialized structure involved in endocytosis in the protozoan parasite *Trypanosoma cruzi*, the etiological agent of Chagas disease. The cytostome consists of an opening at the plasma membrane surface, near the flagellar pocket, followed by a profound and helical shaped invagination called cytopharynx. In epimastigotes, the proliferative insect forms, this structure is the main site for endocytosis. Recently, we have shown that this structure varies in length from cell to cell with a mean length of eight µm and is supported by two microtubule sets: a triplet that started underneath the cytostome membrane, and a quartet whose microtubules originated from staggered positions underneath the flagellar pocket membrane and followed the preoral ridge before reaching the cytopharinx. These two microtubule sets accompanied the cytopharinx forming a `gutter` and leaving a microtubule-free side, where vesicles bud or fuse. In a preliminary analysis, we have shown that epimastigotes in G2 phase of cell cycle had a longer and less helical cytopharynx when compared with those in G1. In the present work, we investigate how the cytostome-cytopharinx duplicates during epimastigote cell division by using advanced electron microscopy. Epimastigote cell cycle were synchronized using hydroxyurea. Ten to fourteen hours after synchronization the material was processed to transmission electron microscopy and observed using FIB-SEM microscopy and electron-tomography. After the 3D reconstruction, we observed that at the end of G2, when parasites already have two flagella but a single dividing kinetoplast, the cytostome closed and the cytopharynx vesiculated. At this stage, the accompanying microtubules remained with the usual helical format and disposition. During mitosis, when cells presented two flagella, two separated kinetoplasts and flagellar pockets, and a nucleus in mitosis, the microtubules depolymerized, remaining only a small part of the quartet, near the old flagellar pocket. The new flagellar pocket also possessed a short microtubule quartet. After nuclear mitosis, the microtubules started to grow again and were directed towards the Golgi complex. A new cytostome-cytopharinx was forming in each cell during cytokinesis. We could conclude that the cytostome-cytopharynx complex is disassembled during the cell division and formed de novo during the cytokinesis.

EVALUATION OF BIOMASS AND FATTY ACID PRODUCTIVITY OF THREE MICROALGAE FOR BIODIESEL PRODUCTION IN CONTINUOUS CULTURE

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The use of microalgae as source of oil for biodiesel production is an issue of current general interest. The potential of three microalgal species (*Chlorococcum oleofaciens, Muriellopsis* sp. and *Pseudokirchneriella subcapitata*) for biodiesel production was evaluated under continuous culture in photochemostat, with assessment of biomass and fatty acid productivity. A reliable and precise measurement of actual fatty acid productivity can be performed in the steady state situation of a continuous culture, in which the productivity of biomass and its composition keep stable (Del Río et al. 2015).

The influence of temperature, pH, dilution rate and nitrate concentration in the feed medium on biomass and fatty acid productivity was analysed for the three strains in bubble-column photochemostats operating in continuous mode. Under nitrate sufficiency, maximum biomass productivity levels around 0.7 g l-1 d-1 were recorded for the three strains.

The reduction in nitrate availability resulted in an increase in fatty acid content in the three strains, but the overall evaluation of fatty acid productivity revealed the great potential of *P. subcapitata*, reaching a maximun of 160 mg fatty acids l-1 d-1, compared with 110 and 70 mg l-1 d-1 for *C. oleofaciens* and *Muriellopsis*, respectively. Moreover, nitrate limitation led to enrichment in saturated and monounsaturated fatty acids, a more suitable profile as raw material for biodiesel. An analysis of the relationship between nitrogen content of the biomass and fatty acid content reafirmed the differences in behaviour between the strains. Accumulation of fatty acids in *P. subcapitata* was triggered with moderate nitrate limitation, with a mild effect on biomass production.

These and other results highlight the potential of *Pseudokirchneriella subcapitata* as an adequate source of fatty acids for biodiesel production. To verify its potential, a preliminary evaluation of *P. subcapitata* outdoor has been performed.

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Research supported by ACCIONA Energía S.A. and ALGAENERGY S.A.

MOLECULAR PHYLOGENY OF PERITRICH CILIATES (CILIOPHORA: PERITRICHIA), WITH EMPHASIS ON THE RHABDOSTYLIDS (EPISTYLIDIDAE)

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The peritrichs are classified into two assemblages (sensu Lynn 2008): the free-living Sessilida and the parasitic Mobilida. The sessilids comprise more than 100 genera and are widely distributed. Although a comprehensive recent review is not available, these genera comprise at least 800 described species. These ciliates possess a well-developed oral ciliatura and occur in many limnetic and marine habitats. Many of the taxa are epibionts on a great variety of aquatic and semiterrestrial metazoans. Recently, molecular information from the 18S-rDNA sequences, has been used to re-evaluate phylogenetic relationships among peritrichs, however, these molecular analyses have yielded different results from morphologically-based taxonomic studies. In order to re-evaluate the phylogenetic position of rhabdostylid ciliates, small subunit rRNA gene sequences were generated for four species, collected in Brazil: *Rhabdostyla inclinans* (attached to oligochaetes, bromeliads), *Rhabdostyla* sp. nov. 1 (attached to oligochaetes, urban stream), *Rhabdostyla* sp. nov. 2 (attached to insects, urban stream) and *Orborhabdosyla bromelicola* (bromeliads). Phylogenetic reconstructions were performed utilizing three different methods: neighbor-joining, maximum likelihood and bayesian analyses. These phylogenetic trees had a consistent branching pattern. The phylogenetic analyses suggest that: (1) *Rhabdostyla* sp. nov. 1 and *Orborhabdosyla bromelicola* branched together with *Epistylis* species suggesting an epistylidid rather than an opeculariid affiliation; (2) *Rhabdostyla inclinans* and *Rhabdostyla* sp. nov. 2 branched together with vorticellids. These findings support the hypothesis that rhabdostylids (*Rhabdostyla* + *Orborhabdostyla*) is a non-monophyletic assemblage defined by plesiomorphic peritrich features.

RUMEN CILIATES IN BRAZILIAN SHEEP, WITH NEW RECORDS AND REDESCRIPTION OF ENTODINIUM CONTRACTUM (ENTODINIOMORPHIDA: OPHRYOSCOLECIDAE)

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The species composition, prevalence, and relative abundance of rumen ciliates were analyzed in 15 Brazilian sheep. 28 species of ciliates were identified belonging to 3 families: Isotrichidae, Ophryoscolecidae, and Parentodiniidae. Among these ciliates, *Entodinium alces, Metadinium esalqum,* and *M. rotundatum* were found for the first time in sheep, and other 12 species for the first time in Brazilian sheep. Different morphotypes of parentodiniid ciliates were identified in 7 of the 15 sheep analyzed and this is the second report of this family in ruminants. The species *Entodinium contractum* was redescribed based on the new data on the general morphological features; for the first time, we described the oral infraciliature, which in this species is of the *Entodinium*-type. In this species, it is noteworthy that the prominent vestibular polybrachykinety can be used as an important identifying characteristic. This work seems to be the first survey of rumen ciliate species in Brazilian sheep that involves a significant number of sampled hosts and appropriate ciliatologic techniques.

SWARM V2: HIGHLY-SCALABLE AND HIGH-RESOLUTION AMPLICON CLUSTERING

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Previously we presented Swarm v1, a novel and open source amplicon clustering program that produced fine-scale molecular operational taxonomic units (OTUs), free of arbitrary global clustering thresholds and input-order dependency. Swarm v1 worked with an initial phase that used iterative single-linkage with a local clustering threshold (d), followed by a phase that used the internal abundance structures of clusters to break chained OTUs. Here we present Swarm v2 that has two important novel features: 1) a new algorithm for d = 1 that allows the computation time of the program to scale linearly with increasing amounts of data; and 2) the new fastidious option that reduces under-grouping by grafting low abundant OTUs (e.g., singletons and doubletons) onto larger ones. Swarm v2 also directly integrates the clustering and breaking phases, dereplicates sequencing reads with d = 0, outputs OTU representatives in fasta format, and plots individual OTUs as two-dimensional networks.

PREVALENCE OF BOVINE COCCIDIOSIS IN CENTRAL ANATOLIA REGION OF TURKEY AND DEVELOPMENT OF A REAL TIME PCR ASSAY FOR DETECTION OF PATHOGENIC EIMERIA SPECIES

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This study has been carried out to determine the prevalence of bovine coccidiosis in Kayseri, Yozgat, Nevsehir and Kirsehir provinces which are located in the Central Anatolia Region of Turkey and to develop species specific Real Time PCR assay for detection of pathogenic *E. zuernii, E. auburnensis, E. bovis, E. ellipsoidalis* and *E. alabamensis* species in infected cattle. For this aim, fecal samples were collected from a total of 840 cattle were investigated for *Eimeria* oocysts using the sucrose centrifugal floatation technique. *Eimeria* oocysts from positive samples were sporulated in 2% potassium dichromate solution and morphologically identified according to the described identification keys. 407 out of 840 samples (48.5%) were found to be positive for *Eimeria* oocysts. Eleven *Eimeria* species were morphologically identified as *E. auburnensis* (67.1%), *E. canadensis* (56.5%), *E. zuernii* (53.6%), *E. bovis* (49.1%), *E. ellipsoidalis* (29.7%), *E. cylindrica* (19.7%), *E. alabamensis* (15.5%), *E. brasiliensis* (12.3%), *E. bukidnonensis* (10.8%), *E. subspherica* (10.6%) and *E. wyomingensis* (0.5%). All positive samples were found to be infected with at least two *Eimeria* species and the highest mix infections were consisted with five species. Morphological characteristics of oocysts is the common method for the identification of bovine coccidia to the species level. However misidentification of the species which have similar morphology is also common and this type of identification requires expertise. Therefore species-specific real time PCR assays targeting the ITS-1 region of nuclear ribosomal gene were also developed for the first time to identify the pathogenic species with this study. The ITS-1 region of each pathogenic *Eimeria* species had sufficient inter-specific sequence variation enough to design the primers and TaqMan probes that differentially amplified each target species. Oocysts of pathogenic *Eimeria* species in fecal samples could be sensitively detected, reliably differentiated, and identified using qPCR assay which may provide a useful tool for diagnosis and epidemiology of bovine coccidiosis. This study was supported by Scientific and Technological Research Council of Turkey with the project no 113O597.

"PROTIST X": A NOVEL ANAEROBIC SISTER LINEAGE TO METAMONADS

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Anoxic marine sediments harbour a high diversity of anaerobic eukaryotes, including many that may represent novel major lineages. A predatory anaerobic protist with four equal flagella in a cruciform arrangement was isolated and established in stable dieukaryotic culture, using *Andalucia incarcerata* as prey. This protist was previously mentioned in the literature only as Protist X; it was never formally described, and light microscopy data shed little light on its phylogenetic affinity. Preliminary electron microscopy examination reveals a highly vacuolated cytoplasm, as well as numerous pyriform membrane-bound structures with a complex substructure. These represent an apparently novel type of extrusome, presumably used in predation. No obvious canonical mitochondria have been observed to date, consistent with the anaerobic habit of the organism. We obtained transcriptome data for the organism in order to infer its phylogenetic position and basic biochemistry. Phylogenomic analyses place "Protist X" as a novel "phylum-level" lineage deep within Excavata, falling as sister to the previously studied metamonads (the major group of anaerobes that includes both *Giardia* and *Trichomonas*). The transcriptome information is consistent with the presence of an anaerobic mitochondrion-like organelle in the form of a hydrogenosome. The phylogenetic position of Protist X implies that it will be an important taxon for resolving deep-level eukaryotic phylogeny, as well as for tracing the evolutionary history of anaerobiosis in the metamonads.

PINPOINTING THE ROOT OF EXTANT EUKARYOTIC DIVERSITY: ADVANCES, CHALLENGES AND CONSEQUENCES

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Determining the root of the eukaryotic tree is of crucial importance to determine the sequence of events at the earliest stages of eukaryotic evolution. Recent studies of mitochondrion-derived genes have suggested that the root may fall between unikonts and bikonts, or alternatively, at the base of excavates, whereas a gene family evolutionary analysis indicated that the root may fall between opisthokonts and other super-groups. Clearly, the question is not settled, in particular because deep level analyses like these are plagued with artefacts. In fact, the historical signal versus systematic error in rooted phylogenomic analyses of eukaryotes have not been comprehensively examined.

We have addressed this question by carefully selecting and analyzing three classes of eukaryotic genes that can be outgroup rooted:
1. genes whose closest orthologs are from the Archaea ;
2. genes of potential mitochondrial origin, where alpha-proteobacterial homologues can be used as the outgroup;
3. genes that duplicated before the last eukaryotic common ancestor, as phylogenetic reconstruction from paralogous gene families can be used to generate reciprocally rooted trees.

In addition to the large number of genes considered, our analyses also benefit from the inclusion of several newly sequenced deep-branching eukaryotic taxa that are particularly relevant to the question of the position of root.

Orthologs from a large sample of eukaryotes from all supergroups were assembled for each gene, and sophisticated phylogenetic methods, exploratory data analysis and 'robustness' procedures were used to determine support for alternative eukaryotic root positions.

These results and analyses will also be replaced in context and a broader discussion will revolve around the difficulties of such analyses and the consequences ensuing from various potential positions of the root.

GRAZING OF BLEPHARISMA AMERICANUM ON TOXIC AND NON-TOXIC MICROCYSTIS AERUGINOSA CELLS

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Cyanobacterial blooms in freshwater, brackish and coastal marine environments represent a major ecological, economical and human health problem worldwide. These hazardous blooms can have severe implications on the biodiversity of water bodies altering biological, chemical and hydrological parameters of aquatic sources. The occurrence of cyanobacterial mass populations can have significant consequences on human water security, as many species can produce a wide range of compounds that have the potential to be extremely toxic. Moreover, these toxins can also have a significant impact on the microbial loop and therefore, on the food web and ultimately on the ecosystem as a whole. *Microcystis aeruginosa* is a common species that often dominates freshwater cyanobacterial communities and produces a number of secondary metabolites, typically the hepatotoxic microcystin. By using *Microcystis* containing the microcystin-LR gene (MC+) and a strain lacking toxin production (MC-) this laboratory study investigated the impact of a novel predator-prey interaction between Microcystis and the microphagus filter feeder *Blepharisma americanum*. Through flow cytometry the MC- strain cell count recorded a significant decrease in growth rates and higher grazing mortality when exposed to *Blepharisma americanum*. The MC- strain was also observed to sustain the grazing ciliate population. Conversely, *Blepharisma americanum* predation of MC+ *Microcystis* showed no significant reduction in cyanobacterial growth rates and a subsequent complete reduction in ciliate numbers. The results in this study indicate that microcystin toxin production plays a role in anti-grazing behaviour against *Blepharisma americanum*.

THE COLORLESS CORTICAL GRANULE OF OXYTRICHIDS (CILIOPHORA, HYPOTRICHIDA) REPRESENTS A NEW EXTRUSIVE ORGANELLE

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Cortical granules of hypotrichous ciliates have diverse morphology and play an important role in ciliates identification. However, their ultrastructure and cell function are poorly known. In recent studies, we used two oxytrichids, *Architricha indica* and *Oxytricha granulifera,* both of which have colorless cortical granules, to study these granules. By using scanning and electron microscopies, it was found that the cortical granules of these two species had almost the same structure, i.e., in resting state, they were ellipsoidal vesicles with cavities on their anterior parts; the extruded structures remained the similar structure as that in resting state and were kept on the cell surface. Cortical granules of *O. granulifera* can be labeled by alcian blue staining at low pH, which indicated they may contain acidic mucosubstances. Most cortical granules of *O. granulifera* were extruded when the cells were treated with methyl green-pyronin solution, and cells regenerated new cortical granules several days after the treatment. Through observation of ultra-thin sections of *A. indica,* it was found that these cortical granules might be originated from endoplasmic reticulum in the deep part of the cytoplasm, undergo a serious of development and migration, and finally position beneath the pellicle. The results mentioned above reveal that these colorless cortical granules are similar with typical extrusomes in other ciliate groups in terms of biogenesis, component, and most important, extrusion after stimulation, and thus should be designated as an extrusive organelle (extrusome). However, their distinctive ultrastructure in resting state and after extrusion is different from all known extrusive organelles in Protozoa. Therefore, they represent a new type of extrusomes.

THE DIATOM FLORA OF NAOLI RIVER WETLAND IN NORTHEAST CHINA

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Abstract: Naoli River Wetland Nature Reserve is a national nature reserve, which is the largest marsh distribution area in the Sanjiang Plain, northeast China. Thirty-one collections for diatom analysis from four habitats were made in 2011 at different locations in Naoli River Wetland Nature Reserve. The total species richness in swamp was 103 taxa, in Naoli River 31 were found, in bogs 64 taxa were observed, and in streams 95 were observed. Dominant species differed in each habitat. Only nine species were found in all habitats which were more tolerant to various trophic conditions.

The biodiversity of diatoms in Noali River wetland is relatively high with 165 taxa. The most common genera were *Pinnualria* (43 taxa), *Gomphonema* (23 taxa), *Eunotia* (12 taxa) and *Nitzchia* (12 taxa). The diatom samples from Naoli River wetland were predominantly composed of freshwater species. A group of the species identified in Naoli River wetland can be recognized as belonging to the common swamp flora found worldwide. Three taxa distribution in the mountain habitat and some low temperature resistant species were also found.

Zhang found 80 taxa in Honghe Wetland which is a very similar size and scale to the Naoli River Wetland with similar geological complexity. We did not evidence any *Eunotia tenella*, which is usually typical for cold oligotrophic acid wetland. Likewise, *Eunotia pectinalis* var. *ventralis, Navicula graciloides* and *Navicula salinarum,* which are reported from Honghe Wetland, were not found. *Tabellaria flocculosa,* often reported as dominant or subdominant in swamps occurs in Naoli River wetland with only low abundances.

PHYLOGENETIC RELATIONSHIPS WITHIN THE CLASS HETEROTRICHEA (CILIOPHORA, POSTCILIODESMATOPHORA) INFERRED FROM FIVE MOLECULAR MAKERS AND MORPHOLOGICAL DATA

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Most of studies on molecular evolution of Heterotrichea were based solely on 18S-rDNA gene, which revealed inconsistencies from morphological classification. Due to the limitations of single locus phylogenies and the recurring problem of the lack of resolution of deeper nodes found in previous studies, we present hypotheses on the evolution of internal Heterotrichea groups based on multiple loci analyses (18S-rDNA, 28S-rDNA, ITS1-5.8S-ITS2 region, COX1 and alpha-tubulin gene) and morphological data. Phylogenetic trees based on protein coding genes are presented for the first time to Heterotrichea. Phylogenetic analyses included Bayesian inference, maximum-likelihood, maximum parsimony methods, and optimal trees were statistically compared to alternative topologies from the literature. Additionally, a Bayesian concordance approach (BCA algorithm) was used to assess the concordance factor between topologies obtained from isolated analyses. Because different loci may evolve at different rates, resulting in different gene topologies, we also estimated a species tree for Heterotrichea using the STAR coalescent-based method. The results show that (1) the single gene trees are inconsistent in relation to the position of some heterotrichean families, and alpha-tubulin trees recovered *Stentor* as polyphyletic; (2) the concatenation of all data in a total-evidence tree improved resolution of deep nodes among the heterotrichean families and genera; (3) coalescent-based species tree is consistent with phylogenies based on 18S-rDNA gene and shows Spirostomidae as a deeply diverged group of Heterotrichea; (4) Conversely, the total-evidence tree suggests that the large Heterotrichea cluster is divided in six lineages in which Peritromidae diverges at the base of tree.

INFLUENCE OF MYCOPLASMA HOMINIS ON PATHOBIOLOGY OF TRICHOMONAS VAGINALIS

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The obligate extracellular mucosal parasite *Trichomonas vaginalis* is the causative agent of trichomoniasis, the most common non-viral sexually transmitted disease worldwide. Successful colonization of the host mucosa by *T.vaginalis* is the result of multiple pathogenic mechanisms, including adhesion, secretion of cytotoxic molecules and soluble factors, interaction with of vaginal microbiome, evasion and subversion of host immune system.

An intriguing aspect of the pathology of *T.vaginalis* is represented by the symbiotic relationship with *Mycoplasma hominis*, the only one described so far involving two obligated human pathogens producing independent diseases in the same anatomical area. Studies in vitro have elucidated some aspects of this association as the ability of *M.hominis* to invade, survive and multiply in the *T.vaginalis*: the protozoan parasite could be considered as Trojan horse in mycoplasma infections. Many questions on the influence of this symbiosis over the biology of both microorganisms still remain unanswered.

We attempted to clarify some aspects of this symbiosis, examining how *M.hominis* could influence *T.vaginalis* pathobiology in vitro. We investigated the influence of *M.hominis* on parasite replication rate, on production of ATP and on ability to influence nitric oxide (NO) production by human macrophages after contact with protozoa.

Results obtained demonstrate that *T. vaginalis* stably associated with M. hominis show higher replication rate, and are able to produce larger amounts of ATP as compared to naturally mycoplasma-free protozoa. In addition, we demonstrated that *M.hominis* could modulate host immuno-response to *T.vaginalis*, by influencing the production of nitric oxide (NO) by human macrophages.

Our data demonstrate that *M.hominis* parasitism increases intracellular ATP of *T.vaginalis* and modultes protozoan virulence and ability to escape to macrophage killing, suggesting that symbiosis between *T.vaginalis* and *M.hominis* can be considererd as a mutually beneficial relationship.

RELEASE OF HOLOSPORA-LIKE BACTERIA IN DIFFERENT CILIATE SPECIES

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In contrast to many endocytobionts (Ecb) of ciliates, two life forms are characteristic of *Holospora* from *Paramecium* spp.: a short reproductive form (RF) and a long non-dividing infectious form (IF). The latter is the source of new infection in the host population. The release of the *Holospora* IF from the infected nuclei is connected with particular behavior of the Ecb. They can modify the infected nucleus division, finally producing a special nuclear structure – a "connecting piece" (CP), a kind of a bridge between the two daughter macronuclei (or micronuclei), in which majority of IF are collected. The CP is usually cleaved off from the daughter nuclei, and releases the IF into the cytoplasm and then into environment via the ciliate's cytoproct. From evolutionary point of view this adaptation is rather important for an efficient accomplishment of the Ecb life cycle. We found two such forms in the life cycle both in "classical" *Holospora* (in *Paramecium* spp.), and in some other *Holospora*-like Alphaproterobacteria infecting different ciliates (Fokin, Görtz, 2009). Depending on the Ecb, the IF are released either after every division of the infected nuclei (*Holospora*) or irrespectively of the nuclear cycle phase of the host (*Holospora*-like Ecb) (Fokin, Sabaneyeva, 1997). The mechanism of the IF release was investigated in seven *Holospora*-like Ecb:*"H. caryophila", "H. bacillata", "H. curvata",* "*Holospora* sp." from *P. putrinum,* two *Gortzia* spp., which can infect the macronuclei of different *Paramecium* spp., and Ecb from *Trithigmostoma cucullulus.* The study was performed using living cell observations, Feulgen staining, FISH and TEM. In "*Holospora*-like" Ecb, this process is reverse to normal infection and involves bacterial passing through the perinuclear space of the infected nucleus into the cytoplasm. First, the IF should generate on their own surface the fine fibrous layer (FL) and then a surrounding membrane by which they communicate with the nuclear envelope. IF could be released either individually (mainly) or in groups ("*H. caryophila"*). Probably, the FL of *Holospora*-like IF is the essential structure of communication with the host cell.

EFFECTS OF GLOBAL WARMING ON GROWTH AND GENETIC DIVERSITY OF MARINE CILIATED PROTISTS

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An increasing number of short-term experimental studies show significant effects of projected ocean warming on the performance of marine organisms. For instance, rDNA copy numbers per cell were found extremely high and different among even congeners in marine protists and intra-individual polymorphism of the 18S rDNA has been reported in various protists, however, little is known about the response of high copy number and polymorphism to marine environment change in individuals of single-celled eukaryotes. Here two marine ciliates, *Strombidium sulcatum* and *Diophrys scutum* were selected as models to test the effects of ocean warming (temperature) on population growth, cell size, small subunit ribosomal DNA (SSU rDNA) copy numbers and expression levels and intragenomic polymorphisms based on single-cell analysis. When growth temperature (Tm) increased from 16 to 25 ºC, and changed back to 16 ºC, ciliates had higher population growth rate, smaller cell size following the "Temperature-size rule" at the higher temperature, and that population growth and cell size recovered when ciliates changed back to 16 ºC. We also found extremely high rDNA copy numbers per cell and different numbers between different species in this study. The copy number and expression levles of rDNA in single cells significantly decreased with the increasing Tm, and restored to previous level when Tm decreased. Intragenomic polymorphism widely occurred throughout the rDNA and rRNA sequence and the pairwise genetic distance and haplotype diversity of rRNA was significantly higher than that of rDNA, but nucleotide diversity was not significantly different between rDNA and rRNA. At rDNA level, GC% did not change significantly when Tm increased, but significantly increased when changed back to 16 ºC. At rRNA level, GC% significantly increased when Tm reached 21 ºC, and did not become lower when temperature reached 25 ºC and returned to "normal". This study indicates that ocean warming has significant impact on phenotype and physiology of marine ciliate and leads to changes in the genetic structure of genomic rDNA, highlighting high plasticity of protist genomes which allows these unicellular eukaryotes rapidly adapt to environment stress.

FUNCTIONAL CHARACTERIZATION OF THE ABCG2 TRANSPORTER FROM THE PROTOZOAN PARASITE LEISHMANIA

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The family of ABC (ATP-binding cassette) transporters in *Leishmania* include 42 genes distributed in 9 subfamilies (A-I), some of them with relevant biological functions. We are interested in the *Leishmania* ABCG2 transporter (LABCG2), a member of the ABCG subfamily. To go into detail about functional characterization of LABCG2, we have obtained: (i) null mutants for LABCG2 (LABCG2-/-), and (ii) parasites overexpressing LABCG2. We have observed that LABCG2-/- parasites have altered the metacyclogenesis, the formation of autophagosomes as well as they present a higher content of non-protein thiols, with trypanothion (T[SH]2) as the main molecule accumulated. Using BSO, an inhibitor of g-GCS involved in the formation of trypanothion, we have stablished that there was a correlation between the levels of thiols and autophagy. Additionally, the overexpression of LABCG2 confers a significant resistance to antimonals due to a reduction in their accumulation for a significant drug efflux. LABCG2 export Sb(III) conjugated to thiols, being able to transport thiols in the absence of Sb(III). LABCG2 is mainly present in intracellular vesicles of exocytic and endocytic pathways and is partially localized in the plasma membrane. In conclusion, LABCG2 could be considered as a trypanothion transporter, probably associated with other molecules as antimony that could be involved in cellular detoxification, autophagy and virulence of this protozoan parasite. Thus, LABCG2 confers resistance to antimony probably by sequestration of the metal-thiol conjugates within vesicles and further exocytosis through the flagellar pocket of the parasite.

Financiado por el Ministerio de Economía y Competitividad, Plan Nacional de I+D+i Proyectos SAF2012-34267 y por el Plan Andaluz de Investigación.

ELUCIDATING EVOLUTIONARY RELATIONSHIPS WITHIN PHYSARACEAE (AMOEBOZOA) THROUGH A MULTILOCUS APPROACH

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The family Physaraceae, which is characterized by the presence of a calcareous capillitium and granular lime in different parts of the sporophore, is one of the most species-rich amoebozoan lineages. Based on morphological data, this family has been traditionally divided into nine genera, which comprise more than 200 species widely distributed. However, the high degree of morphological diversity and phenotypic plasticity make it difficult both, identifying certain specimens and establishing relationships between accepted morphospecies. A number of molecular studies at higher taxonomic levels have reported the monophyly of Physaraceae. By contrast, the largest genus in the group, *Physarum*, has been proved to be polyphyletic. There has also been one recent attempt to clarify the phylogeny of the group. However, it is based on ITS sequences, which have been proved to be too variable to be used for phylogenetic purposes. In addition, all these studies included a limited number of species representing only a few genera, and most of them used a single-gene approach. Consequently, the phylogenetic relationships within the family have remained poorly understood. Our study complements existing molecular data by including, for the very first time, both nuclear (nSSU rDNA and EF-1α) and mitochondrial (mitSSU rDNA) genes. Moreover, in an attempt to fully resolve phylogenetic relationships among Physaraceae species, the most inclusive taxon sampling to date (comprising representatives from all genera), is presented. An extensive morphological data set has been compiled. A careful mapping analysis of these data within a phylogenetic context, could improve our understanding of the family by shedding light onto patterns of character evolution. Besides, it will allow us to determine the most taxonomically informative characters within the family. All this information could be used to propose a convenient updated classification of this species-rich group.

DIVERSITY OF CILIATES IN TWO WASTEWATER TREATMENT PLANTS IN RIO DE JANEIRO, BRAZIL

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The study of the diversity of ciliates in wastewater treatment plants is important to maintain the quality of biological wastewater treatment system. Ciliates make population control of bacteria, are agents of organic matter biodegradation and influence the agglutination of bacteria in biological flocs, responsible for separation of solid and liquid parts of the sewage. The WWTP environments are also important for the discovery of little-known or even new ciliate species. The aim of this study is to identify and the ciliates present in Alegria Wastewater Treatment Plant and Environmental Sanitation Experimental Center, two plants located in Rio de Janeiro, Brazil. Samples of raw sewage water with sediments were collected from aeration basin during the period from December 2013 to May 2015. Specimens were isolated under stereomicroscope and studied in vivo under bright field, phase contrast and DIC, and then after protargol-impregnation and scanning electron microscopy. A total of 33 morphospecies were identified: *Aspidisca cicada, Blepharisma sinuosum, Colpoda cucullus, Cyclidium* sp., *Chilodonella uncinata, Cyrtohymena quadrinucleata, Didinium nasutum, Epystilis* sp., *Euplotes aediculatus, E. eurystomus, Frontonia* sp., *Gastrostyla* sp., *Gonostomum affine, Halteria grandinella, Holophrya* sp., *Kahliella* sp., *Loxodes striatus, Metopus contortus, Oxytricha faurei, Oxytricha* sp., *Paramecium aurelia, P. caudatum, Prorodon ovum, Spathidium anguilla, Spirostomum minus, S. teres, Strombidium* sp., *Tetmemena pustulata, Uronema* sp., *Thruricola kellicotiana, Tokophrya quadripartita, Vorticella microstoma, Zoothamnium procerius.*

Supported by FAPERJ, CNPQ and PETROBRAS.

PROTISTS WITH EYE-LIKE ORGANELLES: THE DINOFLAGELLATE ERYTHROPSIDINIUM

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The origin of eyes –organs of extreme perfection for Darwin- is an evolutionary puzzle. Typical in animals, eye-like structures are also present in very distant eukaryotes. The warnowiid dinoflagellates (*Erythropsidinium, Warnowia, Nematodinium, Proerythropsis*) are the protists with the most complex photoreceptors organelles. The ocelloid of *Erythropsidinium* consist of a cornea-like surface layer, a lens-like structure, a retina-like structure with stacked membranes, and a pigment cup, all assembled in a single cell. Gehring (2005, J. Hered. 96: 171-184) proposed that the eyes of vertebrates could be derived from these dinoflagellates by gene transfer. We studied the phylogeny based on 18S rRNA genes of these dinoflagellates, as well as their ecology and ultrastructure. The latter suggested that the ocelloid is a highly modified chloroplast, arguing in favor of convergent evolution for this organelle and disfavoring homologous origin by gene transfer to metazoans. In addition, we also examined the morphology and function of other unique organelle, the piston. Although we cannot consider that *Erythropsidinium* has a 'true eye', our study shows that eye-like structures converge into the same morphology and that complex photoreceptors do have independent origins in evolution.

THE IMPACT OF ENVIRONMENTAL CHANGES ON THE DIVERSITY OF SYMBIOTIC BACTERIA ASSOCIATED WITH CILIATED PROTISTS

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There are intensive studies concerning the impact of environmental changes on free-living bacterial communities or protozoan communities. Yet, the effects of environmental changes on protists-bacterial symbiotic relationship have been seldom investigated. Symbiotic relationships between bacteria and protozoa are prevalent in various natural environments. Under the background of global environmental change (global warming, increasingly occurred ocean anoxia and water pollution events), the study of the influence of environmental factors and pollutions on symbiotic bacteria may shed new light on the ecological and evolutionary adaptation of microbes of both sides. In this work, the bacterial diversity of two ciliate species (freshwater species *Paramecium bursaria* and marine species *Diophrys scutum*) were investigated under laboratory conditions. These include aerobic and micro-anaerobic, light and dark, a range of temperatures, water with different concentrations of oxytetracycline and heavy metals. Our aims were to examine the effects of dissolved oxygen, light, temperature, antibiotic and heavy metal pollutions on bacterial diversity associated with ciliate species. A set of molecular approaches including clone librariey construction and sequencing of 16S rRNA genes, terminal restriction fragment length polymorphism (T-RFLP), fluorescence in situ hybridization (FISH), and phylogenetic analysis were used. Multivariate analyses (MDS) and statistical analyses (ANOSIM) were performed to visualize and to test the significance of bacterial community differences. In general, we found environmental changes affect assemblages of bacterial endosymbionts, but this effect also depends on host species. Our data contribute to our understanding of the interaction between eukaryotes and symbiotic bacteria, and their adaptation to environment changes.

EXPERIMENTALLY ADAPTED TETRAHYMENA THERMOPHILA STRAINS TO EXTREME METAL STRESS: DIFFERENTIAL AND REVERSIBLE CDMT GENE AMPLIFICATION

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We have maintained *T. thermophila* cultures under increasing metal concentrations during more than two years, until obtaining adapted strains to the highest tolerable metal concentration. Three experimentally adapted strains to Cd2+, Pb2+ or Cu2+ were obtained, which are resistant to 115 µM Cd2+ (~ 2.5x the wild-type LC50), 4 mM Cu2+(~12.7x the wt LC50) and 5.5 mM Pb2+ (~ 5.7x the wt LC50) in PP210 medium. Growth rates of Cd- and Cu-adapted cells are lower than that of the wild-type SB1969. However, the Pb-adapted strain presents a similar growth rate to the wt. Likewise, cross-exposure experiments and double-exposure experiments were also carried out on these adapted strains. A differential metallothionein (MT) gene amplification seems to be another strategy to protect cells from the extreme Cd stress. Calculation of copy number/microL of all MT genes was carried out in each metal-adapted and control strains by using qPCR. A differential amplification of two CdMT genes MTT1 and MTT3 (located in the left arm of the Nº4 chromosome, at 1.7 Kb one from each other) was detected only in Cd-adapted cells. This metal-induced amplification of both genes is ~5 times with regard to the control. Therefore, MTT1/MTT3 genes are represented 45n in control strain (SB1969). However, in Cd-adapted strain MTT1/MTT3 is 225n. After one month without Cd, the Cd-adapted strain still maintains a MTT1 and MTT3 gene copy number higher than the control strain, but it gradually diminishes until reaching (after 7 months) the same number of copies that the control has. We maintained Cd-adapted strain without Cd during 10 months and then we exposed it again to the Cd maximum concentration for 1 week. After that treatment, both MTT1/MTT3 genes were again amplified (~2 times with regard the control). Therefore, this adaptive response seems to be fast (~1 extra-copy /5 cell generations) and reversible. TEM analysis has shown several drastic cellular alterations in metal adapted strains. In Pb-adapted strains, we have observed numerous electron-dense inclusions which might be metal-MT complexes located in both cytoplasm and also outside the cells. An excellent elimination system of Pb-complexes might exist in these cells.

GENE EXPRESSION ANALYSIS OF METALLOTHIONEINS AND AP-1 TRANSCRIPTION FACTORS IN EXPERIMENTALLY ADAPTED TETRAHYMENA THERMOPHILA STRAINS TO EXTREME METAL STRESS: A MODEL OF GENE EXPRESSION COORDINATION

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Metallothioneins (MT) are multi-stress proteins mainly involved in binding metal ions. In T. thermophila five MT genes encoding 3 CdMTs (MTT1, MTT3 and MTT5) and 2 CuMTs (MTT2 and MTT4), have been reported (Díaz et al., 2007). Likewise, four putative AP-1 transcription factors might be involved in the regulation of the MT gene expression (Gutiérrez et al., 2011). We have analyzed by qRT-PCR the induction level of MT and AP-1 genes in different *T. thermophila* strains: the wild-type (wt) (SB1969), two strains including recombinant plasmids (pVGFMTT1 or pVGFMTT5, with reporter constructs PMTT1::GFP::MTT1 and PMTT1::GFP::MTT5, over-expressing MTT1 or MTT5 genes, respectively) (Amaro et al., 2014), and three experimentally adapted strains to extreme metal concentrations obtained after 2 years of metal adaptation process. The main conclusions obtained from these experiments are: 1)- At constitutive level (without metal stress), the ranking of MT gene expression level is: MTT1/3 > MTT2/4 > MTT5 for wt strain. 2)- However, after Cd or Pb exposure the ranking habitually change to: MTT5 > MTT1/3 > MTT2/4 in the majority of strains; after Cu exposure the ranking is: MTT2/4 > MTT5 / MTT1/3. 3)- In Cd-adapted strain, the MTT1/MTT3 gene shows the highest basal value. However, its gene expression pattern is similar to other Cd-treated strains and its gene induction values decrease under Cd stress or increase/decrease under Pb or Cu stress, with regard to other strains. 4)- In Cu-adapted strain the MTT2/4 constitutive values are the highest among other strains. 5)- MTT1/3 seems to have a leading role in Cd-adapted cells, MTT2/4 in Cu-adapted strain and MTT5 in Pb-adapted cells. 6)- MTT1/3 and MTT5 proteins might regulate differentially the expression of the rest of MT genes. 7)- The ranking of AP-1 gene expression at constitutive level is: AP1-IV > AP1-I ≥ AP1-II > AP1-III. All these results (and others not reported here) seem to show a likely connection between MT and AP-1 elements. MT proteins might participate directly or indirectly in their own gene expression regulation under metal stress. A model of the gene expression coordination is proposed.

Supported by project PR6/13-18856 (Santander-UCM).

CONSTRUCTION AND EXPRESSION OF VECTOR WITH MIP/PILE ADVANTAGES EPITOPE GENES OF LEGIONELLA PNEUMOPHILA

Jinlei He (Chengdu,China) , Jianping Chen (Chengdu,China).

Objective *Legionella pneumophila,* the most common pathogen of Legionnaires disease, widely exist in natural and artificial water and soil. Currently, there is no ideal preventive measures against the pathogen. Accordingly, our study is to select advantages epitope genes of mip and pilE which are virulence factors of *Legionella pneumophila* and to construct fusion vector of advantages epitope genes mip/pilE, and to detect its expression in prokaryotic system so as to set the basis for future research on *Legionella pneumophila* protein vaccine. Methods Following analysis of secondary structure and surface properties such as: physical and chemical properties, hydropathy, plasticity, antigen index and extracellular domain of Mip and PilE proteins were through bioinformatics methods. The region which active epitope may exist was selected as advantages epitope region, then its tertiary structure and function were predicted by PHYRE2 Protein Fold Recognition Sever. After the selection, the recombinant plasmid pET-mip, pET-pilE and pET-mip/pilE with advantages epitope genes were constructed by PCR amplification and T4 ligase connection, and induced the expression in *E.coli.* SDS-PAGE and Western Blot were test to confirm the expression, after the expressed proteins were purified through Nickel column and dialysis. Results Many potential antigenic epitopes in Mip and pilE were identified, and the tertiary structure and function of the selected advantages epitope regions were predicted. Moreover, the selected advantages epitope regions of mip, pilE and mip/pilE were cloned and expressed successfully. But the immunogenicity and protective of the expressed proteins need further validation by animal experiments in the future. Conclusion DNA Star software and Expasy online analysis system may successfully predict antigenic epitopes for Mip and PilE of *Legionella pneumophila*. And PHYRE2 Protein Fold Recognition Sever may successfully predict the proteins tertiary structure and function. Prokaryotic expression vector pET-mip/pilE with advantages epitope genes has been successfully constructed and efficiently expressed for *Legionella pneumophila* protein vaccine.

THE PHYLOGENETIC ANALYSES OF ANIMAL PATHOGENS IN ENTEROBACTERIACEAE

Yanxia He (Sichuan University).

Enterobacteriaceae include a wide range of bacterium that parasitize people, terrestrial and aquatic animals, plants and insects. The number of the genera and species in Enterobacteriaceae is increasing, and with the gradual improvement of the bacteria identification technology, the original classification system has been modified. The classification of Enterobacteriaceae is dynamic. In this study, the ribosome 16S rRNA genes of Enterobacteriaceae animal pathogens were selected to carry on the phylogenetic analyses. 194 reference sequences, who meaned 194 species or subspecies from 30 enterobacterial genera, were downloaded from GenBank and were analyzed. MrBayes v3.2 was used to conduct the Bayesian analysis and FigTree v1.4.2 was used to visualize the result. In the analysis, 21 genera were monophyletic. *Averyella* was closed to *Buttiauxella; Budviccia, Rahnella, Ewingella* and *Yersinia* also formed a clade; Besides, *Cosenzaea, Proteus, Providencia, Morganella, Photorhabdus, Leminorella* formed another one. Another 9 genera, including *Citrobacter, Cronobacter, Enterobacter, Escherichia, Klebsiella, Pantoea, Salmonella, Serratia, Shigella,* were polyphyletic and embedded each other. Totally, these data suggest that Enterobacteriaceae is complex and polyphyletic, meanwhile, 16S rRNA gene sequence phylogeny might conflict with the traditional classification results, and only 16S rRNA gene isn't enough, but plays a very important role to the phylogenetic analyses of Enterobacteriaceae.

Keywords phylogenetic analysis, Enterobacteriaceae, 16S rRNA.

THE FLAGELLAR APPARATUS OF CYANOPHORA

Aaron Heiss (American Museum of Natural History).

Glaucophytes are one of three lineages known to have primary plastids. Their plastids uniquely retain such cyanobacterial features as peptidoglycan and carboxysome-like bodies. In spite of this, glaucophytes were only recognised as a distinct lineage about twenty years ago, having been previously thought of as aberrant green algae. This has meant that most morphological studies of the group have been cursory, generally only noting deviations from a "standard" green-algal bodyplan. In particular, while microtubular roots have been identified in the flagellated genus *Cyanophora*, a full reconstruction of the flagellar apparatus has never been published. We address this deficit by investigating the ultrastructure of *C. paradoxa* through serial-section transmission electron microscopy, and present a computer-based reconstruction of its flagellar apparatus. We also investigate the slightly larger *C. biloba*. We will discuss our findings in the light of our modern understanding of *Cyanophora* as a member of a distinct lineage of algae.

SEM STUDIES ON THE MORPHOLOGY OF CERTAIN MARINE PLANKTONIC DINOFLAGELLATES (DINOPHYTA) FROM MEXICAN WATERS, INCLUDING NEW SPECIES

David Hernández-Becerril (Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, UNAM).

Despite that marine planktonic dinoflagellates form a conspicuous and important fraction of the phytoplankton in tropical areas, many species of relative low abundances remain poorly-known regarding their detailed morphology. This study is based on phytoplankton net and bottle samples collected since 1999, from the southern Gulf of Mexico (SGM) and the tropical Mexican Pacific (TMP), which were analyzed by light (LM) and scanning electron microcopy (SEM). Various species of Dinophysales were studied, including *Amphisolenia bifurcata, Amphisolenia brevicauda, Amphisolenia globifera, Amphisolenia inflata, Amphisolenia laticincta, Amphisolenia schauinslandi, Methaphalacroma skogsbergi, Oxyphysis oxytoxoides, Phalacroma expulsum, Phalacroma turbineum, Pseudophalacroma nasutum, Triposolenia bicornis, Triposolenia depressa* and *Triposolenia truncata*. Some new species of Dinophysales have been described, such as *Phalacroma gibbosum,* found in the SGM, and closely related to *Phalacroma favus* and *P. rapa, Phalacroma palmatum* sp. nov., encountered in the TMP, and *Dinophysis conjuncta*, detected in both areas. Other thecate dinoflagellates studied were *Blepharocysta paulsenii, Blepharocysta splendor maris, Centrodinium pulchrum, Podolompas bipes, Protoceratium reticulatum* and *Scripsiella trochoidea,* some of them extremely rare and with a strong tropical affinity. We also found two new records in the TMP, which correspond to recently described species in more temperate areas, *Azadinium spinosum*, a tiny species known as Azaspiracid toxins producer, and *Vulcanodinium rugosum*, associated to the production of Pinnatoxin, another potent toxin. Finally, species such as *Akashiwo sanguinea, Cochlodinium polykrikoides* and *Levanderina fissa,* are bloom-forming or potentially toxic athecate dinoflagellates and were also studied in SEM. Some particular morphological characters with taxonomic relevance were found, e.g. number and position of pores in the sulcus or joined sulcal lists in *Dinophysis* and *Phalacroma*, presumably attaching pores in epitheca and hypotheca in *Centrodinium*, whereas other characters such as plate arrangements, presence of delicate and tiny plates, and variable development of lists were confirmed.

Keywords: Dinoflagellates; Morphology; Phytoplankton; Scanning electron microscopy; Taxonomy.

FIRST RECORD FOR THE AMERICAS OF THE GIANT CILIATE LOXODES REX

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As the foundations of food webs, protozoa are essential to the success of an ecological system. These organisms are often overlooked and research in the Americas is sparse. Recent samplings conducted in freshwater canals and ponds in Florida, USA, have revealed the large, enigmatic ciliate *Loxodes rex*. Originally described as endemic to tropical Africa, *L. rex* has traditionally been considered a prime candidate for proof of microbial endemism. Our studies have shown this giant, non-encysting ciliate to be thriving in subtropical Florida. Our observations are novel, and include both the first record of occurrence for the Americas and the first high quality in vivo images for this charismatic species. Microbial populations are so large, and distribution potential at a global level is so prevalent, that protozoan dispersal may not be affected by physical barriers. *L. rex* has been found in an ecosystem containing other large-sized ciliates such as *Frontonia vesiculosa,* a species also described by some authors as having a limited geographical distribution. Studies such as these will enhance our understanding of the distribution potential for ciliate species, and by extrapolation other microbes, particularly protists.

DINOFLAGELLATE TAXONOMY AND CLASSIFICATION, A PROPOSAL

Mona Hoppenrath (Senckenberg am Meer).

A new classification scheme based on nameless ranked systematic for dinoflagellate levels above genus will be proposed. The proposal is based on the knowledge about morpho-species classification as well as molecular phylogenetic hypotheses. Core (= dinokaryotic) dinoflagellates will be separated into dinokonts and desmokonts and the dinokonts grouped into polyalveolates and oligoalveolates. Within the polyalveolates we will have non thecates and light thecates. Within oligoalveolates symmetricomorpha, mesomorpha, asymmetricomorpha, pre-sagittale, and sagittale will be distinguished. A selection of characters used for taxonomy will be evaluated and discussed.

REDUCED VERSION OF BACTERIAL SECRETION SYSTEM IN THE MITOCHONDRION OF EXCAVATES

Lenka Horváthová (Charles University in Prague, Czech republic), Vojtech Žárský (Charles University in Prague, Czech republic), Alžbeta Krupicková (Charles University in Prague, Czech republic), Marek Eliáš (University of Ostrava, Czech republic), Gerard Huysmans (Institute Pasteur, Paris, France), Mohamed Chami (University of Basel, Switzerland), Olivera Francetic (Institute Pasteur, Paris, France), Pavel Doležal (Charles University in Prague, Czech republic).

Mitochondria of all eukaryotes have originated from a single alphaproteobacterial ancestor. Evolution of the organelle was accompanied by extensive transfer of genes from the ancestral endosymbiont to the host cell nucleus, as well as dramatic renewal at the level of its proteome. The protein flow across the two membranes of the evolving mitochondrion reversed. New pathways importing proteins into the organelle had to be installed, while bacterial machineries originally used for protein secretion to the cell exterior were abandoned, but not entirely. Searching the eukaryotic genome data we have found that one group of eukaryotes (Excavata) still possesses components of one of the bacterial secretion systems: type II secretion system (T2SS). The eukaryotic version of the system is minimalist, consisting of only 4 of 12-15 different protein components (Gsp proteins) normally present in bacteria. Nevertheless it can be still functional, as each of these proteins represents a core subunit of 4 T2SS subassemblies that span both the outer and inner membranes. We show localizatition of these proteins within mitochondrion of one of the representatives of excavates, a free living amoeba *Naegleria gruberi*. Cryo-electron microscopy of GspD, a homologue of bacterial outer membrane secretin, revealed that the protein forms multimer resembling bacterial pore. We are further investigating interactions among the components of the reduced "mitochondrial secretion system" that might be still able to secrete proteins to the cytosol of the cell.

EXPANDING THE ENTAMOEBA UNIVERSE: NEW HOSTS YIELD NOVEL RIBOSOMAL LINEAGES

Alison Jacob (London School of Hygiene and Tropical Medicine).

Removing the requirement for cell culture has led to a substantial increase in the number of lineages of *Entamoeba* recognized as distinct. Surveying the range of potential host species for this parasite genus has barely been started and it is clear that additional sampling of the same host in different locations often identifies additional diversity. In this study, using small subunit ribosomal RNA gene sequencing, we identify four new lineages of *Entamoeba*, including the first report of *Entamoeba* from an elephant, and extend the host range of some previously described lineages. Additionally, examination of microbiome data from a number of host animals suggests that substantial *Entamoeba* diversity remains to be uncovered.

MOLECULAR AND PHYLOGEOGRAPHIC CHARACTERIZATION OF THE INFECTION BY TRYPANOSOMA SPP, IN CATTLE OF COLOMBIA

Jeiczon Jaimes-Dueñez (Grupo BCEI, Universidad de Antioquia, Medellín, Colombia), Omar Triana (Grupo BCEI, Universidad de Antioquia, Medellín, Colombia), Ana Mejía-Jaramillo (Grupo BCEI, Universidad de Antioquia, Medellín, Colombia).

Animal trypanosomosis is a parasitemic disease in Africa and South America that causes serious economic losses in livestock. Several factor like eco-biogeographic distribution, diversity, vectors population density and host, modulate the interaction with trypanosomes. To understand the epidemiology and interaction between vector, host and trypanosomes, we developed an epidemiologic and phylogeographic study in an important livestock state (Arauca) from Colombia. A total 240 blood cattle samples and 27 tabanids insect were analyzed by PCR using different molecular markers, to determine the prevalence of infection with trypanosomes. In addition, the ITS rDNA and Cathepsin L-like (CATL) genes were sequenced and analyzed to determine the phylogeography of the parasites.

The molecular analysis in cattle showed a prevalence of 41.6% and 11.6% to *T. theileri* and *T. evansi*, respectively, and a prevalence of 62.9% and 25.9% for the same species in tabanids. No infection with *T. vivax* was identified. The statistical analyses showed a significant correlation

Our results indicate a high prevalence of *T. theileri* and *T.evansi* in cattle and tabanids from Arauca - Colombia, suggesting a high transmission by mechanical vectors of the family Tabanidae. These infections were associated to clinical signs like low in the hematocrit in adult's animals and the presence of a single linage of *T.theileri* in Colombia.

SMALL FRESHWATER ECOSYSTEMS HARBOUR COMPLEX PROTIST COMMUNITIES CHARACTERISED BY SEASONAL DYNAMICS AND RESILIENCE

Ludwig Jardillier (**Université Paris-Sud**).

Over the last decades, most of the studies investigating the diversity and dynamics of the small protists (cells 0.2-5 µm in size) have been restricted to large freshwater ecosystems and marine environments because of the surface they represent on Earth. However, small continental aquatic systems (<0.1 km2) cover at least the same surface as larger freshwater bodies. They are numerous and characterised by a wide range of environmental conditions. We thus monitored the dynamics of the small protists for 2 years (April 2011 to April 2013) in five small freshwater systems differing in their environmental conditions and trophic status, including 4 ponds and one stream in the North-Western France. Their composition was determined based on 454-pyrosequencing of 18S rRNA genes. A large set of environmental parameters was measured concomitantly. Multivariate statistical analyses were used to explore the ecology of the small protists in these freshwater systems. The protist diversity was high with sequences affiliated to all recognized supergroups. New phylotypes were detected every month in all ecosystems. A few OTUs affiliated to taxa previously thought restricted to marine environments (e.g. group MAST-3) while other OTUs were only detected in freshwater systems (e.g. group HAP-1). The composition of the small protist community differed among the five ecosystems over the 2-years survey, with only 50 OTUs from a total of 3,742 OTUs shared by the 5 ecosystems. A clear seasonal pattern was observed in each ecosystem despite a complex temporal dynamics of the high-rank taxa detected. Low-abundance OTUs represented the vast majority of the community and showed very different dynamics, appearing occasionally, remaining at low frequencies or instead reaching high frequencies. In addition, severe drought events occurred in one of the ponds and the stream for 1 to 5 months. Each time, the protist community showed a rapid resilience that occurred within a month after the recovery of the water level, with both community composition and structure being very similar to those observed prior to the drought event.

A COMPARISON OF SOME METHODS TO QUANTIFY HETEROTROPHIC FLAGELLATES OF DIFFERENT TAXONOMIC GROUPS

Alexandra Jeuck (University of Cologne), Frank Nitsche (University of Cologne), Claudia Wylezich (IOW-Leibniz Institute for Baltic Sea Research), Olaf Wirth (Oekopol Institute for ecology and politics), Melanie Hennemann (University of Cologne), Nicole Nopper (University of Cologne), Tanja Bergfeld (Federal Institute of Hydrology), Shahla Monir (University of Cologne), Anja Scherwass (University of Cologne), Hartmut Arndt (University of Cologne).

Heterotrophic flagellates contribute significantly to the matter flux in aquatic and terrestrial ecosystems. Still today their quantification and taxonomic classification bear several problems in field studies, though these methodological problems seem to be increasingly ignored in current ecological studies. Here we describe and test different methods, the live-counting technique, different fixation methods, cultivation methods like the liquid aliquot method (LAM), and a molecular survey called aliquot PCR (aPCR). Each of the described methods has its advantages and disadvantages which have to be considered in every single case. With the live-counting technique a detection of living cells up to morphospecies level is possible. Fixation and staining methods are advantageous due to the possible long-term storage and observation of samples. Cultivation methods (LAM) offer the possibility of subsequent molecular surveys, and aPCR tools might complete the deficiency of LAM in terms of the missing detection of non-cultivable flagellates. All these methods have been tested using field samples and cultures of freshwater, marine and freshwater sediment heterotrophic flagellates. In summary, we propose a combination of several techniques to investigate heterotrophic flagellates reducing the gap between the different methodological problems.

DEVELOPMENT OF NEW NUCLEAR GENE MARKERS IN CILIATES USING GENE CAPTURE AND NEXT-GENERATION SEQUENCING

Jiamei Jiang (**Shanghia Ocean University**), **Hao Yuan** (**Shanghia Ocean University**), **Chenhong Li** (**Shanghia Ocean University**).

Ciliates are a major evolutionary lineage within the protists, which are distributed in nearly all habitats on our planet and are an essential component for functioning, processes and stability of ecosystem. While there are abundant molecular data for ciliates, most of those are limited to the 18S ribosomal RNA locus. Sequence data of nuclear protein-coding genes are scarce. Here we targeted 42 coding DNA sequences (CDS) from single-copy protein-coding genes that are shared across *Tetrahymena thermophila, Paramecium tetraurelia, Ichthyophthirius multifiliis, Oxytricha trifallax* and *Stylonychia lemnae.* We used the genomic resources available for *Tetrahymena thermophila,* sequence capture techniques and Next-Generation Sequencing to generate sequences. 26 CDS were captured in all six taxon. These newly developed markers could facilitate the molecular basis of barcoding, population genetics and phylogenetic studies.

EVOLUTION OF THE "UNCONVENTIONAL" O2-SCAVAGING SYSTEM IN DIPLOMONADS

Alejandro Jimenez-Gonzalez (**Uppsala University**), **Feifei Xu** (**Uppsala University**), **Jan O. Andersson** (**Uppsala University**).

Diplomonads are a group of unicellular heterotrophic protists. Inside this group we can find a mix of lifestyles, including parasites (e.g. *Giardia intestinalis* or *Spironucleus salmonicida*) as well as the free-living Trepomonas and Hexamita. Diplomonads live in anaerobic or microaerophilic environments, although parasites species may experience increasing level of oxygen during infection. Diplomonads lack the traditional aerobic mitochondria. Instead, they contain either hydrogenosomes (*S. salmonicida*), where energy is produced by fermentation with liberation of H2, or mitosomes (*G. intestinalis*), whose only known function is the synthesis of the Fe-S clusters used by some proteins, while the energy production takes place in the cytoplasm. Most of these enzymes are inhibited under O2 conditions, especially PFOR and Fe-hydrogenase. Functional and genomic studies have indicated that diplomonads have an elaborated enzymatic system to survive under this condition. The conventional enzymes for oxidative stress response, superoxide dismutase and catalase have not been found in diplomonads. Instead an O2-scavaging NADH oxidase, superoxide reductase, A-type flavoprotein and proteins belonging to the thioredoxin superfamily have been found to be involved in the antioxidative response. These enzymes interact creating a redox pathway well-adapted for coping with changing O2-levels during infection and transmission. In this pathway, reactive oxygen species (ROS) are reduced to H2O and oxidized proteins are reduced again avoiding structural damage inside the cell. Previous studies showed that this group of enzymes often are similar to bacterial homologs indicative of a possible prokaryote origin by lateral gene transfer (LGT).

We perform a bioinformatics study of the oxygen stress response genes in diplomonads, with the goal to understand the evolutionary adaptation to increasing oxygen levels coupled to pathogenicity. A total of 25 enzymes involved directly or indirectly in the O2-scavaging system are targeted. We use a phylogenetic approach to systematically investigate the origin of these enzymes. Preliminary trees will be presented and discussed.

INTERCLONAL VARIABILITY, AUTOGAMY, CLONAL LIFE HISTORY, SENESCENCE AND COHESION OF HISTOPHAGOUS SPECIES: TETRAHYMENA ROSTRATA

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A histopahgous ciliate *T.rostrata* was found as parasite in renal organs of two small land snails *Zonitoides nitidus* and *Cochlicopa lubrica*. Starvation medium induced encystment, which induced meiosis and autogamy followed by development of new macronuclei. The autogamy induced whole genome homozygosity. It could be expected, that small sizes of *T. rostrata* populations, their isolation, autogamy and variability of environment may enhance fixation of different alleles and speciation. Therefore mitochondrial COX1 gene was sequenced for "barcoding" of the *T. rostrata* strains. Small divergences in this sequence appeared even between these strains, that were isolated from different specimens of the same host species and collected at the same site (0.2-0.6%). The divergence in COX1 sequence between our strains isolated from *C. lubrica* and Spanish strains from *Helix aspersa* and Deroceras reticulatum (Segade P, Kher DH, Lynn DH, Iglesias R [2009] Parasitology 136:771-782) was only about 1%. The divergences between the strains from *Z. nitidus* and other *T. rostrata* strains were higher than typical intra species COX1 divergences in *Tetrahymena* (4- 4.5%), but not high enough to claim, that the strains from *Z. nitidus* constitute separate species. The inter-strain differences in cytology and life history were not found, consistent with previous reports and with the above conclusion (cohesion of species). In the PAUP phylogenetical tree of the SSUrRNA sequences *T. rostrata* does not group closely with any other species of the "T. pyriformis" complex and have distinct position in comparison with the other species within the genus *Tetrahymena*.

Cell division rate of all our *T. rostrata* clones declined linearly with a number of cell divisions from the last autogamy, until senescence. This gradual senescing in diiferent subclones was different than abrupt expressing of the Hayflick limit in mammalian cells. The senescent *T. rostrata* strains could not be rescued by expanded cell isolations, but they were rejuvenated only by another encystment induced autogamy. The senescent cells showed reduced uptake of food vacuoles deterioration of their micronucleus, failures in macronuclear development and excystment.

THE DYNAMICS OF MITOCHONDRIAL METABOLISM IN A CERCOZOAN CAPABLE OF GROWTH IN AEROBIC AND LOW-OXYGEN CONDITIONS

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In aerobic eukaryotes mitochondria produce ATP by oxidative phosphorylation using molecular oxygen as a terminal electron acceptor. However, many eukaryotes living in low oxygen conditions possess anaerobic mitochondria or mitochondrion-related organelles (MROs) such as hydrogenosomes and mitosomes. However, the detailed evolutionary trajectories that occur during the early stage of mitochondrial adaptation to anaerobiosis remain unclear. In this study, we isolated and established a culture of cercomonad strain KY003 capable of growth in aerobic and low-oxygen conditions. Phylogenetic analyses of 18S rRNAs show that KY003 is a close relative of *Brevimastigomonas anaerobica*. Whereas *B. anaerobica* possesses mitochondria with tubular cristae in transmission electron micrographs, KY003 contains double membrane-bound organelles resembling hydrogenosomes. We characterized the complete mitochondrial genome (mtDNA) sequence of KY003 and found that it is a circularly-mapping molecule roughly 26 kb in length. The mtDNA-encoded genes are composed of genes for complexes I, III-V, two rRNAs, and 10 tRNAs. Quantitative RT PCR analyses revealed that all the protein-coding genes and rRNA genes were lower expressed in low-oxygen conditions relative to aerobic condition, suggesting that both transcriptional and translational activities are down-regulated in response to hypoxia. We hypothesize that this down regulation of electron transport complexes under hypoxia likely leads accumulation of NADH and deficiency of NAD+. Comparative transcriptome analyses between aerobic and low-oxygen conditions strongly suggests that genes for substrate-level phosphorylation, NAD+-independent pyruvate/acetyl-CoA metabolisms, Hydrogen production, and amino acid metabolisms were more highly expressed in low-oxygen conditions. Mitochondria of KY003 likely adapt to the deficiency of NAD+ in hypoxia by up-regulation of oxygen- and NAD+-independent pathways for ATP generation. We propose that this pattern of adaptation of mitochondrial functions during hypoxia has occurred in the early stages of mitochondrial adaptation to anaerobiosis in many distinct protistan lineages.

REVISING AMOEBOZOA SYSTEMATICS USING PHYLOGENOMICS FROM BROAD SET OF TAXA

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Amoebozoa is the largest eukaryotic supergroup that primarily contains amoeboid cells. Although amoebae have been known for centuries, there was little evidence before molecular phylogenetics to suggest these diverse organisms would group together. Little is known about the deep evolution of the Amoebozoa, which appear to have diverged around 1.2 billion years ago. The primary issue with our understanding of amoebozoan evolution stems from a historical lack of deep sequence data. Most of conceptions of amoebozoan phylogenetics are solely based on 18S rDNA data. Many attempts to build a robust Amoebozoan phylogeny, have all but failed. To date, there are no phylogenomic studies that include more than a handful of amoebozoans. Here, we strategically sampled 40 amoebozoan species to evenly cover most amoebozoan lineages based on previous 18S rDNA phylogenies. Using RNA-seq transcriptome data, we have constructed a robust phylogenomic tree of Amoebozoa employing a dataset of 351 orthologous proteins. This tree shows that Amoebozoa are monophyletic. Our analyses strongly recover nine Amoebozoa lineage groups: Macromycetozoa, Variosea, Tubulinea, a novel group consisting of *Pessonella* spp. and *Sapocribrum*, *Himastimentia*, and a second novel group consisting of Pelltida, Acanthamoebida and Stereomyxa. Our tree strongly shows that Macromycetozoa and Variosea form a well-supported sister lineage. Dactylopodida and Stygamoeba form a single group with maximum support and are sister to Vannellidae. We have recovered maximum support for Thecamoebida group which forms a sister lineage to a novel group consisting of Vannellidae, Dactylopodida, and Stygamoeba. Our amoebozoan phylogenomic tree offers a major overhaul of the problem with taxon sampling and deep sequence data of Amoebozoa. These well-supported deep-level clades are a major development in the systematics of Amoebozoa.

COMPARATIVE ANALYSIS OF THE MONOCERCOMONOIDES SP. AND TRIMASTIX PYRIFORMIS – INSIGHT INTO THE EVOLUTION OF METAMONADA

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Metamonads are a group of protists consisting solely of anaerobes or microaerophiles possessing metabolically diverse mitochondrial derivates without cristae and genomes. While the parasitic species are relatively well studied (e.g. *Giardia* or *Trichomonas*), free-living and commensal metamonads are one of the most neglected protists. *Monocercomonoides* and *Trimastix pyriformis* represent one of the three main lineages of Metamonada and have non-parasitic lifestyle, thus their genomes could theoretically be more plesiomorphic in the gene content than those of the parasitic lineages. The comparative analysis of parasitic, endobiotic and free-living Metamonada genomes may provide an insight into the evolution of parasitic lifestyle and anaerobic lifestyle.

We have recently sequenced transcriptome and genome of *Monocercomonoides* sp. - an oxymonad that lives commensally in the guts of Chinchilla. The genome of *Monocercomonoides* sp. contains a high number of introns and high number of genes originated from LGT. Many of these genes are important enzymes playing role in basic metabolism. Analysis of metabolic pathways and oxygen stress response enzymes revealed many similarities between *Monocercomonoides* and parasitic metamonads. We are currently analyzing data from draft genome and transcriptome of *Trimastix* – the free-living representative of metamonads. In our comparative analyses we are especially interested in the impact of lateral gene transfer on Metamonada genomes, origin of transferred genes as well as lineage of Metamonada where these events have happened. Comparative analysis of the main metabolic pathways will help us to decipher how the metabolism changed due to parasitic lifestyle.

CHLOROPHYLL CATABOLISM GENERATING CYCLOPHEOPHORBIDE ENOLS GENERATED BY AUTOTROPHIC AND HETEROTROPHIC EUGLENOIDS

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The phototoxic effects of chlorophylls on living cells present a potential risk not only to photosynthetic organisms but also to those who feed on them. We have reported that the metabolic conversion of chlorophylls a/b to 13(2),17(3)-cyclopheophorbide a/b enols (cPPB-aE/bE, or CPEs) is a major detoxification mechanism in phycophagic protists. Furthermore, CPEs are known to be produced by algae such as phototrophic dinoflagellates and phototrophic euglenoids. We investigated on the production of CPEs by various phototrophic and phagotrophic protists that cover the entire group of euglenoids as well as a lineage of heterolobosea. Almost all of the euglenoid and heterolobosean phagotrophs examined converted chlorophylls into CPEs after ingestion of algal/cyanobacterial cells, which were also indicated by disappearance of the autofluorescence from chloroplasts of the diets in relatively early stage of their digestion. Phototrophic euglenoids, including members of Eutreptiales and Euglenales, generally accumulated CPEs within the cytoplasm in concomitant with the degradation of chloroplasts under unfavorable growth conditions. Time course observations by TEM and fluorescence microscopy, together with chemical analyses of cell fractions, indicated that endogenous degradation of the thylakoid structure proceeded along with the generation of CPEs from chlorophylls, resulting in a formation of brown-colored granules. The isolated brown granules exclusively contained CPEs, but neither chlorophylls nor proteins, suggesting that these granules are final disposal forms of chlorophylls in these organisms. When mixotrophic euglenoid *Rapaza viridis* ingested prey cells of *Tetraselmis* sp. cells, CPEs became detectable only in the late stages of the complex decomposition processes of the *Tetraselmis* chloroplast. We therefore infer that the CPE metabolism of phototrophic euglenoids for detoxifying endogenous chlorophylls has been inherited from the ancestral heterotrophs where it was originally targeted on the exogenous chlorophylls from their diets.

MORPHOLOGY AND MOLECULAR PHYLOGENY OF TWO NEW ZOOTHAMNIUM SPECIES (CILIOPHORA, PERITRICHIA, ZOOTHAMNIIDAE) AND A SUGGESTION OF GUIDELINE TO DESCRIBE ZOOTHAMNIIDAE

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We discovered two new *Zoothamnium* species which collected from eutrophic waters in South Korea. The *Zoothamnium* species were studied based on live and silver impregnated observations and small subunit (SSU) rRNA gene sequences. *Zoothamnium* sp. 1 is diagnosed by funnel-shaped colony, pot-like zooid outline with double layered peristomial lip, parallel three rows of infundibular polykinety 3, and number of silverlines 53-61 between peristomial lip and trochal band, 21-28 lines between trochal band and scopula. *Zoothamnium* sp. 2 is diagnosed by fan-like colony, single layered peristomial lip, infundibular polykinety 3 diverged row 2 and 3 from row 1 and number of silverlines 62-117 between peristomial lip and trochal band and 46-55 lines between trochal band and scopula. The molecular phylogenetic trees are also reconstructed with SSU rDNA sequences of these two *Zoothamnium* species using the algorithms of Maximum Likelihood and Bayesian Inference. We discussed the relationships between morphology and molecular results. Moreover, we suggest a guideline of description for species of Zoothamniidae which is one of difficult taxa taxonomically.

TAXON-RICH MULTIGENE PHYLOGENY OF THE PHOTOSYNTHETIC EUGLENOIDS (EUGLENOPHYCEAE)

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To establish taxonomy and understand phylogenetic relationships among strains and species of the photosynthetic euglenoids, we performed phylogenetic analyses based on a four gene sequence dataset (nr SSU and LSU rDNA, and pt SSU and LSU rDNA) from 343 taxa (including three outgroup). The phylogenetic tree based on the combined dataset was split into two major clades: Euglenaceae and Phacaceae. The family Euglenaceae was a well-supported monophyletic group containing eight genera (*Colacium, Cryptoglena, Euglena, Euglenaformis, Euglenaria, Monomorphina, Strombomonas,* and *Trachelomonas*), each representing a monophyletic lineage, except for the genus *Euglena*. The genus *Euglena* was divided into three subclades (A1, A2, and A3) and was paraphyletic due to *Euglena* archeoplastidiata being grouped with the genus *Euglenaria* and *E. cf. velata* with the genus *Colacium*. The family Phacaceae was supported as a monophyletic group and contained three genera (*Discoplastis, Lepocinclis, and Phacus*). The genus *Phacus* contained traditionally defined members as well as the non-traditional *P. warszewiczii* and *P. limnophila,* which support the generic concept of Linton et al. (2010).

COMPARISON OF MORPHOLOGICAL AND MOLECULAR DATA BETWEEN TWO POPULATION OF STROMBIDINOPSIS MINIMA (CHOREOTRICHIA:CILIOPHORA) OF KOREA

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Strombidinopsis minima was collected from tidal flat, Seocheon, Korea in June, 2015, and cultured in laboratory. Single cell PCR was performed, and the SSU, 5.8S, ITS1, ITS2 and partial of LSU rDNA are obtained. Based on their SSU rDNA, phylogenetic trees of *Strombidinopsis* were analyzed. Live observation and protargol staining were used. Morphological and molecular data of *S. minima* were compared with those of previous study of Gangwha population. And the phylogenetic position of two populations suggest new genus for *S. minima*. Also, updated data of Seochoen population will be help to understand of cryptic species of choreotrichs. This work was supported by the Basic Research for Sustainable Use of Marine Bioresources, funded by National Marine Biodiversity Institute of Korea (MABIK).

OBLIGATE MIXOTROPHY OF THE PIGMENTED DINOFLAGELLATE POLYKRIKOS LEBOURAE (DINOPHYCEAE, DINOFLAGELLATA)

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The marine sand-dwelling dinoflagellate *Polykrikos lebourae* possesses obvious gold-brown pigmented plastids as well as taeniocyst-nematocyst complex structures. Despite of the presence of the visible plastids, previous attempts to establish this species in culture all failed and thus the unavailability of cultures of this species has posed a major obstacle to further detailed exploration of ecophysiology of the dinoflagellate. Here, we isolated *P. lebourae* from sandy sediment of an intertidal flat on Korean western coast, successfully established it in culture, and have been maintaining the stock culture over the past 3 years. Using this stock culture, we explored phagotrophy and potential prey resources of *P. lebourae*, growth and grazing responses of *P. lebourae* to different prey organisms, the effect of prey concentration on growth and grazing rates and gross growth efficiency (GGE) of *P. lebourae* when fed three different prey organisms, and the growth kinetics of *P. lebourae* under different light regimes. *P. lebourae* captured prey cells using a tow filament and then phagocytised them through the posterior end. The dinoflagellate was capable of ingesting a broad range of prey species varying in size, but not all prey species tested in this study supported its sustained growth. GGE of *P. lebourae* was extremely high at low prey concentration and moderate or low at high prey concentrations, indicating that *P. lebourae* grows heterotrophically at high prey concentrations but its growth seems to be more dependent on a certain growth factor or photosynthesis of plastids derived from the prey. In the presence of prey in excess, *P. lebourae* grew well at moderate light intensity of 40 µmol photons m-2 s-1, but did not grow at dim and high (10 or 120 µmol photons m-2 s-1) light intensities. Our results suggest that the benthic dinoflagellate *P. lebourae* is an obligate mixotroph, requiring both prey and light for sustained growth and survival.

BENTHIC FORAMINIFERA AS AN INDICATOR OF ENVIRONMENTAL STRESS

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Monitoring the status of marine environments is traditionally based on macrofauna surveys. Benthic foraminifera are also a good indicator of environmental stress because of their fast turnover rates and a high degree of specialisation. The advantage of foraminifera as an indicator is their preservation in the fossil record. Fossil assemblages can be traced back to pre-impact times, and thus unstressed assemblages can be reconstructed. The aim of the FOraminiferal BIo-MOnitoring (FOBIMO) initiative was to develop a foraminiferal bio-monitoring tool. There were three FOBIMO workshops (in 2011, 2012 and 2013). The first published outcome was a suite of standardized methods with respect to sampling devices, storage and treatment, faunal analysis and documentation, of which we present here a selection. The second step was to suggest a sensitivity index based on foraminifera. An internationally well-established marine biotic index, AMBI, commonly applied to assess ecological quality status was adapted for use on benthic foraminifera. As required by the AMBI formula, species were assigned to one of five ecological groups according to their sensitivity/tolerance to conditions along an increasing stress gradient (here increasing organic matter enrichment). For the assignments, we used 19 published data sets on NE Atlantic continental shelf and slope assemblages for which total organic carbon (TOC) data were available. Assignments were based on the relative abundance of the different species along associated TOC gradients. Of the 128 assigned species, the majority was assigned to Groups I-III dominating in unpolluted to slightly polluted environments with a high to good ecological quality status. Groups IV and V, representing polluted environments with a moderate to poor ecological quality status had 1 and 2 species, respectively. The resulting foraminifera-based Foram-AMBI was calculated using the AMBI formula and tested on four independent foraminiferal data sets from the same geographical region. This first attempt to apply the AMBI formula on benthic foraminiferal data shows promising results. However, to improve the applicability of Foram-AMBI, there is a need to assign more species and obtain data from studies along wide organic carbon pressure gradients, particularly from the southern North Sea and southwards.

PHYTOMONAS NORDICUS: THE MONOXENOUS TRYPANOSOMATID DESCENDED FROM PLANT PARASITES

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Trypanosomatid *Phytomonas nordicus* parasitizing predatory bug *Troilus luridus* was described at the twilight of the morphotype-based systematics. Despite its monoxenous life cycle, this species was attributed to the dixenous genus *Phytomonas* due to the presence of long twisted promastigotes and development of flagellates in salivary glands. However, these characters were considered to be insufficient to prove phytomonad nature of the species and therefore its description remained virtually unnoticed. Here we performed molecular phylogenetic analyses using 18S rRNA gene and ITS1/ITS2-containing region and convincingly demonstrated the affinity of *Phytomonas nordicus* to the genus *Phytomonas*. In addition, we scrutinized the most phytomonad part of its life cycle, i.e. development in the salivary glands. We argue that in many aspects the life cycle of monoxenous *Ph. nordicus* resembles that of its dixenous relatives exemplified by tomato-parasitizing *Ph. serpens*.

THE GENUS COCHLIOPODIUM HERTWIG ET LESSER, 1874 (AMOEBOZOA, DISCOSEA): PHYLOGENETIC RELATIONSHIPS, CURRENT STATE OF TAXONOMY AND FURTHER CHALLENGES

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The genus *Cochliopodium* comprises discosean amoebae that are enclosed dorsally by a layer of scales (tectum). Scales are considered to be species-specific and have been used as the most reliable character for identification of the species in this diverse taxon comprising around 20 species. With this contribution, we perform an overview of the current state of *Cochliopodium* biodiversity, taxonomy and phylogenetic relationships. Phylogenetic analysis of this genus based on the SSU rRNA and cytochrome C oxidase subunit 1 (Cox1) genes shows that *Cochliopodium* consists of several subclades each characterized by several variants of the scale structure. Structural features of the scales are only partly correlated with the molecular phylogenetic tree topology. Only basal branching of *Cochliopodium* into two major clades correlates with particular structural features of their scales. Scales in the more divergent groups of species are too diverse to suggest any groupings based on their structure that might correlate with the phylogenetic tree topology. Nearly identical types of scales can evolve in several independent clades; at the same time, contrasting scale types may be present in the species with nearly identical genotypes. Moreover, several studied species demonstrate the presence of at least two distinct scale types that may occur within the same clonal culture, thus undermining the idea of scale characteristics as a marker for morphological species identification. The problem of evaluation of the phylogenetic relationships within *Cochliopodium* is further complicated by the incongruence between the SSU rRNA and Cox1 gene phylogenies. At the same time, both markers are in agreement in placing the marine *Ovalopodium* and *Parvamoeba* spp. at the base of the cochliopodiid phylogenetic tree permitting insights into the evolution of the cell coat in the whole family Cochliopodiidae. Finally, the data obtained show that the diversity of *Cochliopodium* spp. is still significantly underestimated. Even a modest sampling effort consisting of several independent samples from the same local habitat may result in immediate isolation of new species that may strongly differ from sample to sample.

This study was partially supported by the grants 12-04-01835-a, 15-04-06239-a and 15-29-02749 from RFBR, and 1.50.125.2014 from the St-Petersburg State University.

CRISPR/CAS9 GENE DISRUPTION IN TRYPANOSOMA CRUZI: AN APPROACH TO STUDY PROTEINS INVOLVED IN CALCIUM HOMEOSTASIS

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Calcium ion (Ca2+) is an important second messenger in trypanosomatids, essential for their survival through their complex life cycle. The recent development of the CRISPR/Cas9 system for gene disruption in *Trypanosoma cruzi*, the causative agent of Chagas disease, is facilitating the functional analysis of proteins in this parasite. We used the CRISPR/Cas9 system for disruption of three genes encoding proteins involved in *T. cruzi* calcium homeostasis: inositol 1,4,5-trisphosphate receptor (TcIP3R), mitochondrial calcium uniporter (TcMCU) and proline dehydrogenase (TcPRODH). TcIP3R is involved in calcium release from intracellular stores in response to IP3 signaling. TcMCU is a channel located in the inner mitochondrial membrane, with low affinity and high capacity for Ca2+ uptake that is required to provide reducing equivalents to support oxidative phosphorylation through activation of intramitochondrial dehydrogenases. In *T. cruzi*, one of these dehydrogenases is TcPRODH, an enzyme that catalyzes the oxidation of L-proline to Δ1-pyrroline-5-carboxylate (P5C), the first enzymatic step in the L-proline oxidation to glutamate. Here we report the use of CRISPR/Cas9 system using pTREX vector for the expression of Cas9 and a specific single guide RNA (sgRNA) to target TcIP3R, TcMCU and TcPRODH genes, plus a DNA donor for homologous recombination to rapidly generate mutant cell lines in which these genes have been disrupted. We demonstrate that genome editing of these endogenous genes in *T. cruzi* is successful without detectable toxicity of Cas9. Our results evidence the usefulness of this methodology for the study of metabolic pathways and physiological processes in *T. cruzi*. Work funded by FAPESP (Processes 2013/50624-0, 2011/50400-0 and 2014/08995-4).

CHANGES IN CILIATE POPULATIONS IN AN INTERTIDAL MICROBIAL COMMUNITY WITH CHRONIC EXPOSURE TO PETROLEUM HYDROCARBONS ON PRUDENCE ISLAND IN NARRAGANSETT BAY, RHODE ISLAND

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Petroleum byproducts released into shallow coastal bays and estuaries can lead to significant changes in ecosystem dynamics, including reduced biodiversity and alterations in species distribution patterns. The damaging effects of these organic compounds, some of which are known toxins, may be heightened in soft mud sediments. This study of an intertidal microbial community at a contamination site in Narragansett Bay located at the south end of Prudence Island compares the ciliate fauna at a contaminated site with an adjacent non-oiled site. Releases of breakdown products of gasoline and diesel fuel persist even after cleanup and mitigation efforts at this former military site. Observations and assessments of field samples included light and fluorescence microscopy (Zeiss Axioscope and Axiovert), video imaging, SEM (Jeol 6010A), along with DNA extraction and analysis. Changes in population dynamics, trophic relationships, species composition, and predation patterns observed in samples collected over several years suggest that the response of a marine microbial community to chronic, low-level exposure to petroleum hydrocarbons demonstrates a shift to an altered, but stable ecological community.

CONTROL OF FORAMINIFERA BY TEMPERATURE, SALINITY AND DEPTH IN THE YELLOW SEA SEDIMENTS: A CROSS SYSTEM COMPARISON FROM INTERTIDAL ZONE TO CONTINENTAL SHELF

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The environmental implications and ecological distribution of foraminifera in continental shelf of the Yellow Sea of China left much to be clarified, which have restricted the studies on paleoenvironmental reconstruction in this region. Temporal dynamics of foraminifera from a tidal flat at low and high intertidal areas were studied from 2010 to 2012 based on 17 months' samplings. Spatial distributions of foraminifera at surface sediments from 24 stations of the Yellow Sea were investigated in autumn of 2012. Species composition and community parameters of the total and living foraminiferal fauna were analyzed. Temporally study revealed a unimodal-type seasonal dynamics of foraminifera in the tidal flat. Foraminiferal community parameters were more closely coupled with environmental temperature and salinity at low intertidal area than those at high intertidal. Foraminiferal abundance, species richness and Margalef index were positive correlated to salinity, and species richness was significantly negatively correlated with temperature. Spatial study indicated that the community composition and distribution of continental foraminifera were significant controlled by environmental temperature, salinity and depth. Different species show different restrains from different environmental factors. Four typical functional groups were recognized including intertidal fauna, offshore shallow water fauna, cold-water-mass fauna and Yangtze estuary fauna. Based on the temporal and spatial studies and statistical analysis results, in comparison with the season, habitat was supposed the more significant contributory factor in regulating benthic foraminiferal faunas in the Yellow Sea (supported by 2014FY110500; NSFC41176132, 41476043; GZH201100202; GASI-03-01-03-01).

TOXOPLASMA GONDII MOB1 SUB-CELLULAR LOCALIZATION AND ASSESSMENT OF ITS POTENTIAL ROLE IN PARASITE REPLICATION

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Mob1 is a component of the core kinase module of Hippo and MEN (mitotic exit network) pathways that are involved in the control of accuracy of cell division and proliferation. Therefore, Mob1 is an excellent target to study the control of protozoan parasite replication, a key matter in parasite/host interaction. *Toxoplasma gondii* presents one gene putatively coding for this protein. A phylogenetic analysis using its predicted aminoacid sequence, as well as Mob1 proteins from model organisms throughout the eukaryotic tree of life shows that apicomplexan parasites form a clade and are distant from other protozoan parasites like the Trypanosomatida. We confirmed that this gene in *T. gondii* is expressed and, interestingly, our data show that its transcript levels dramatically decrease (94%) during the parasite replication inside the host cell. In addition, we have constructed a transgenic parasite strain that overexpresses Mob1 and these parasites show a significant delay in the replication process. Using an in house polyclonal antibody against *T. gondii* Mob1 heterologously expressed in *E. coli,* we observed a very clear localization of the protein in the parasite posterior pole, where the basal complex, a structure involved in cytokinesis in *T. gondii*, is localized. Additionally, we observed a dot localized in the middle of the cell. However, this Mob1 signal did not co-localize with the centrosome, in contrast with what has been observed for other organisms. Experiments are in progress to characterize the Mob1 loss of function. Altogether, the data presented above support that Mob1 is involved in the control of *T. gondii* replication. The identification of proteins involved in the regulation of parasite replication and the establishment of their interactions network can be a platform to investigate the control of parasite replication inside the host.

A NEW REPRODUCIBLE METHOD FOR FAST AND EFFICIENT CONVERSION OF CHINESE LEISHMANIA SC10H2 PROMASTIGOTE FORMS INTO AMASTIGOTE FORMS IN VITRO

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Background.
Leishmania, one of the protozoan parasites, is responsible for a major zoonotic diseases called leishmaniasis, in 88 countries all over the world. Chinese *Leishmania* isolates SC10H2 (L. SC10H2) was proved to be an undefined species closely related to *Leishmania tarentolae*. L. SC10H2 can cause fatal visceral leishmaniasis in human being with neither optimal treatments nor extensive studies so far. Moreover, it has been found that the transformation rate of L. SC10H2 amastigotes was low in vitro. Thus, it is essential to seek for a new reproducible method which is able to converse L. SC10H2 promastigote forms into amastigote forms in an expressing and efficient way in vitro.

Methods.
Different culture conditions for L. SC10H2 promastigotes transformation were assessed including three medium (M199, RPMI1640 and Schneider Drosophila medium), four pH levels (4.6, 5.5, 6.4 and 7.2), FCS concentrations (10%, 20% and 50%), three temperatures (28°C, 32°C and 37°C) and two CO2 concentrations (5% and none). The conversion efficiency of amastigote-like forms was recorded in all groups. After conversion, the viability test as well as the morphological and protein identification of amastigote-like forms were carried out in order to guarantee the success of the conversion.

Results.
L. SC10H2 with the highest proportion (94%) of amastigotes were observed in Schneider's medium with 50% FCS, at pH 6.4 maintained at 32°C in 13-day culture. The amastigotes obtained were morphologically similar to intracellular amastigotes, even at the ultrastructural level. Furthermore, the axenic amastigotes still remained their viability; Amastigotes and promastigotes differed in terms of their SDS-PAGE and Western-blot profiles. Three different proteins of 100KDa, 90KDa and 50KDa were found only in axenic promastigotes, while three different proteins of 75KDa, 60KDa and 45KDa only in amastigotes. 30kDa and 45kDa proteins were recognized by specific antibodies only in axenic promastigotes.

Conclusion.
A new reproducible method for fast and efficient conversion of L.SC10H2 promastigotes into amastigote in vitro was preliminarily determined by this study.

Keywords.
New, Reproducible, Efficient, conversion, *Leishmania* SC10H2 , Amastigotes, in Vitro.

AMOEBORADIX SPP. REPRESENT A HIGHLY DIVERGENT LINEAGE OF PARASITIC EUKARYOTES POTENTIALLY RELATED TO FUNGI

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In recent years, our knowledge on the diversity of microbial eukaryotes has immensely progressed thanks to the increased description of new species and genera and also to culture-independent molecular approaches. Despite so, our knowledge on protist diversity is still very fragmentary and the discovery of new eukaryotic lineages remains possible, especially among less-readily accessible protists parasitizing other protists. Some years ago we described a new genus and species, *Amoeboradix gromovi*, a parasite of the yellow-green alga *Tribonema gayanum* (Mamkaeva et al. 2007), but its phylogenetic position on the eukaryotic tree based on morphological traits was uncertain. *A. gromovi* is superficially similar to chytridiomycetes, but has amoeboid zoospores possessing a pseudocilium with a very long kinetosome composed of microtubular singlets. Rounded cysts form rhizoids inside the host; thick-walled sporangia are rounded to pear-shaped. We have subsequently isolated additional strains with the same morphological characters (strains X-44, K-1, K-2 and K-23) from different freshwater habitats. Here we present a morphological study and molecular phylogeny based on SSU and LSU rRNA genes of *Amoeboradix* sp. (strain K-1). The amoeboid zoospore is 4 µm long and 3 µm wide and exhibits few prominent lipid globules; it produces thin and rarely branching granulated filopodia during movement. Most prominent organelles and structures of zoospore are: big lipid globules, which seem to be fused in one huge curved rosary chain, and long kinetosome (1.8-2 µm) composed of microtubular doublets/singlets. The nucleus occupies a central position in the cell, surrounded with relatively dense cytoplasm filled with scattered ribosomes. Mitochondria possess lamellar cristae; a small thinly granulated microbody is closely associated with lipid globules forming microbody-lipid complex (MLC). The Golgi body is present in the nucleus vicinity. Oval sporangia contain several nuclei with centrioles and Golgi bodies, many lipid globules, microbodies, and mitochondria with flat cristae. Zoospores release sporangia via inoperculated pores. Molecular phylogenetic analyses show that *Amoeboradix* represents an extremely divergent lineage of protists, likely related to fungi.

PHYLOGENOMIC ANALYSIS OF NASSULA SP., NASSULA CITREA, AND PSEUDOMICROTHORAX DUBIUS PROVIDES HIGH SUPPORT FOR A NASSOPHOREAN CLADE

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The monophyly of the nassophoreans has been assumed by morphologists since these ciliates all share a complex cytopharyngeal basket or nasse (Eisler. 1988. Eur. J. Protistol. 24:75) and some also have cortical alveolocysts (Eisler & Bardele. 1983. Protistologica, 19:95). However, this monophyly has been undercut by sequence data for the 18S rRNA gene, which strongly suggests that microthoracids are separated from nassulids (Gong et al. 2009. J. Eukaryot. Microbiol. 56:339). Therefore, we have undertaken this phylogenomic analysis to determine whether a multigene alignment will provide resolution to this question.

Single or several cells of *Nassula* sp., *Nassula citrea*, and *Pseudomicrothorax dubius* were isolated from cultures, placed in duplicate PCR tubes, lysed, and their transcriptomes amplified and sequenced following the protocol outlined by Kolisko et al. (2014. Current Biol., 24:R1081). The duplicate libraries were concatenated after confirmation that the amplified 18S rRNA genes were identical to the assigned species. Contigs were assembled, aligned, and paralogs selected following Gentekaki et al. (2014. Mol. Phylogen. Evol., 78:36).

Analysis of the 158 single-gene trees generated by RAxML showed the following: for 85/158 genes, all three species belonged to the same clade; for 92/158 genes, the two *Nassula* species were sister taxa; and for 41/158 genes, *Pseudomicrothorax* was sister to one of the *Nassula* species; and for 61/158 genes, these species were associated with other clades. Preliminary analysis of the resulting phylogenomic dataset has shown highest support for the clade of the two *Nassula* species and *Pseudomicrothorax* confirming the monophyly of nassophoreans.

TRICHODINA DOMERGUEI CF. DIAPTOMUS. IN A WARM-MONOMICTIC MAAR-CRATER LAKE: A VEGETARIAN ECTOPARASITE?

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Trichodinas have been supposed obligatory parasites that are not able to survive without a host; however, bacteria are declared their food source and their occurrence within the plankton has been repeatedly reported. During the microbial-loop analysis of the Mexican Plateau water bodies, trichodinas (up to 2 cells/mL) were found feeding upon picocyanobacteria at the metalimnetic bottom / oxycline; in the cultures of isolated diaptomid copepods, trichodinas were apparently responsible for their perish (cladocerans survived). To confirm the trichodinas' behaviour during an annual cycle, we studied a warm-monomictic maar-crater lake La Preciosa (Puebla, Mexico).

Using Quantitative Protargol Stain, *Trichodina domerguei cf. diaptomus* parasitizing on *Leptodiaptomus sicilis* was identified. Feeding experiments upon Fluorescently Labelled cyanoBacteria (*Synechococcus* sp.) mimicting the lake picocyanobacteria size distribution were performed in water taken from the representative layers (epilimnion = 8 m , metalimnion or 16 m, oxycline or 24 m and bottom =40 m during the stratification and mixing, respectively), enriched with zooplankton cropped there with Schindler-Patalas plankton trap.

The ciliate numbers roughly followed the distribution of zooplankton. Except for the samples from the oxycline with a sudden drop to the zero level (October-November 2012 and 2013), T. domerguei was very scarce in the unenriched samples. In the end of study (from October 2014 to February 2015), trichodinas surprisingly disappeared even from the zooplankton samples. All trichodinas possessed picocyanobacteria in the vacuoles. Clearance rates varied by an order of magnitude within the water column (400 to 4000 nL (cell.h)ˆ(-1) and throughout the year. However, due to the pronounced picocyanobacteria distribution pattern, the ciliates´ uptake rates were well comparable; consistently, maximums varied from 1300 to 2000 cells (cell.h)ˆ(-1). Maximum average uptakes were found in the epilimnion during mixing and at the metalimnetic bottom / oxycline during the stratification period. Within the layer where apparent anoxygenic photosynthetic bacteria prevailed, trichodinas were not already found.

Trichodina domerguei cf. diaptomus specific feeding rates upon picocyanobacteria were similar to those of plankton ciliates but *Halteria* spp. or minute oligotrichs. It seems trichodinas might grow upon picocyanobacteria while depending on the copepods as vectors within the water column.

MICROAEROBIC SCUTICOCILIATES IN A SALINE MONOMICTIC MAAR-CRATER LAKE ALCHICHICA (MEXICO)

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Since the last decade, a hyposaline lake Alchichica is under microbial ecology studies including Quantitative Protargol Stain (QPS) evaluation of abundance, CARD-FISH and Fluorescently Labelled Bacteria estimation of feeding activity of ciliates. The water column ciliate assemblage is dominated by vorticellids during mixing while during stratification, euplotids and gymnostomes are biomass- and the scuticociliates number-dominated. However, fine-scale stratification studies (0.5 m) covering metalimnion / oxycline layers revealed much higher differences in the assemblage composition that those found using regular sampling. In particular, morphologically distinct scuticociliates not resembling any known species were observed within the oxygen gradient during the late stratification. Only *Isocyclidium globosum* was identified in the top of the anaerobic hypolimnion and in the very bottom. Tentatively, five more species of scuticociliates were registered but three of them were of uncertain genus. There is no doubt that not strictly anaerobic species also could migrate to the anoxic layers searching their prey bacteria. Apparently, the scuticociliate reproductive stages like vacuolated cells were also found abundant there.

In order to separate the life cycle stages of present scuticociliates, fine scale samples were taken throughout the metalimnion / oxycline during a pronounced stratification period and the ciliates were pre-isolated using a dilution method on microwell plates. Cultivation in the corn whole grain-infusion enriched with the lake bacteria was applied upon both aerobic and hypoxic conditions. The best results were obtained in the "Candle Jar" cultivation (adopted from plasmodia's method). The cultures were characterised using epifluorescence microscope (DAPI staining), registering nucleus form and general morphology. In total, eight distinguishable morphotypes (supposing different stages of more than one species) were defined; however, it was impossible to confirm their validity using QPS and/or Taxol (Paclitaxel; Invitrogen) staining.

In the second step, one cell isolation was applied and the isolates were DAPI and QPS characterised. Only two species remained well defined morphologically and the living cycle of them is under construction. However, the taxonomy of the species is still unclear and we are looking for collaboration in their molecular characterization.

SIMILARITIES AND DIFFERENCES OF PROTEINS INVOLVED IN INORGANIC POLYPHOSPHATE METABOLISM IN BACTERIA AND PHOTOSYNTHETIC PROTISTS

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Inorganic polyphosphates (polyP) are linear polymers composed by orthophosphate residues (Pi) linked by high energy phosphoanhydride bonds. Important regulatory and stress-tolerance roles have been recently assigned to polyP [1]. Several enzymatic proteins are involved in their metabolism in bacteria, including exopolyphosphatase (PPX, EC 3.6.1.11), which sequentially hydrolyzes polyP to Pi, and polyphosphate kinase (PPK, EC 2.7.4.1), which reversibly synthesizes polyP from NTPs. This study provides the first systematic survey of genes encoding proteins involved in polyP metabolism in photosynthetic protists. In our lab, we had previously cloned and functionally validated genes involved in polyP metabolism in photosynthetic prokaryotes (anoxygenic photobacteria and cyanobacteria) [2, 3]. Based on these findings, possible orthologs of bacterial ppx and ppk genes were identified in a number of photosynthetic protists (green and red algae), such as *Osterococcus tauri, Volvox carteri, Cyanidioschyzon merolae, Porphyra purpurea*. Interestingly, we further confirmed that most of these orthologs of cyanobacterial ppx and ppk genes are expressed and the corresponding enzymatic proteins produced at significant levels in phylogenetically diverse photosynthetic protists, suggesting that they have been functionally preserved during the evolution of the photosynthetic lineages. Thus, the colonial green alga *Volvox carteri* possess a bacterial-like ppx gene (a multi-domain PPX containing a Ppx-GppA domain and an extra HD-phosphohydrolase domain) what makes this result noteworthy, since the only member of this family of phosphatases described so far in eukaryotes was Rtg2p, a key component of the retrograde signaling mitochondrial pathway in budding yeasts. Furthermore, we have identified and functionally validated a number of genes of certain microalgal groups (Prasinophyceae and Dinophyceae) encoding proteins of the DHH-DHHA2 phosphoesterase superfamily similar to *Saccharomyces cerevisiae* exopolyphosphatase PPX1, and the Mn-dependent Family-II soluble pyrophosphatases so far exclusively described in prokaryotes. Overall, this study suggests that some proteins of the polyP metabolism were preserved across the photosynthetic evolutionary lineages.

Supported by MICINN and PAIDI BIO-261 (partially found by FEDER-EU program).
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SODIUM-TRANSLOCATING MEMBRANE PYROPHOSPHATASES, A NOVEL STRATEGY FOR IONIC HOMEOSTASIS IN PHOTOSYNTHETIC MARINE PROTISTS

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Ion (H+/Na+)-translocating membrane pyrophosphatases (m-PPases, PF03030) are integral proteins of the prokaryotic cell membrane and eukaryotic vacuo-lysosomal membranes, which couple hydrolysis of energy-rich pyrophosphate (PPi), a ubiquitous subproduct of cell anabolism, to the generation of an electrochemical gradient useful in cell bioenergetics. Genomes of plants and many protists have multiple genes encoding functionally diverse m-PPase paralogs. Our aim is to identify and functionally characterize the putative sodium-translocating pyrophosphatases (Na+-PPases) that, according to sequenced genomes, a number of phylogenetically diverse marine microalgae with key roles in major biogeochemical cycles - prasinophytes, stramemopiles (diatoms, pelagophytes), haptophyceae, chlorarachniophytes, rhodophytes - could possess. These m-PPases would be the first Na+-PPases reported in eukaryotes, their amino acid sequences being similar to their functional orthologs of archaea and bacteria. Given their close relation to Na+-PPases of prokaryotes, they probably arose via HGT from prokaryotic sources. Their wide distribution among marine protists suggests an important role in saline environments. Our preliminary results with the diatom *Phaeodactylum tricornutum* and the prasinophycean *Ostreococcus tauri* indicate that their membranes do contain catalytic-competent Na+-PPases, which is consistent with the abundance of their transcripts in EST libraries. Determining the subcellular location of microalgal Na+-PPase is of particular relevance because if located within the cell it would be the first primary sodium pump found so far in cell endomembranes, while a plasma-membrane localization would reveal this m-PPase as the only sodium pump of the eukaryotic cell membrane able to use an energy-rich substrate alternative to ATP. Na+-PPases should play a role in alkali cations homeostasis of marine photosynthetic protists, many of which employ sodium-dependent co-transporters for absorption of most nutrients (sugars, urea, nitrate, phosphate), and additionally have a PPi-based bioenergetics different to fungi/animals which allows them using this metabolite as an alternative chemical-energy source under stressful conditions, as was previously proposed by our group for photosynthetic prokaryotes.

Supported in part by PAIDI-BIO-261 grants (JA, partially founded by EU FEDER program).

ACANTHAMOEBA GENETIC DIVERSITY INSIDE DRINKING WATER TREATMENT PLANTS

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Acanthamoeba is a free-living amoeba that has the ability to act as an opportunistic human parasite capable of causing amoebic keratitis (AK) or Granulomatous Amoebic Encephalitis (GAE) among other infections. It is also known as a vector of other pathogens. *Acanthamoeba*'s subgenus classification based on morphology is being replaced by a classification based on the sequences of the 18S rRNA gene with a total of 20 different genotypes (T1-T20) that have been isolated worldwide.

In order to know their genetic diversity in the environment, a study was conducted with a total of 40 water samples from three Drinking Water Treatment Plants from the central area of Spain. Samples included water from the inlet and outlet of the plant as well as at intermediate points of the purification system. Water was collected following the recommendations of the American Environmental Protection Agency (EPA) and concentrated them by IDEXX Filta Max System. Later, concentrated samples were cultured in nonnutritive agar plates seeded with inactivated *Escherichia coli*. Once the amoebae were isolated by dilution, DNA was extracted by heat shock,purified and sequenced with Jdp1/Jdp2 primers that amplified for the GTSA.B1region that allows a genotype analysis.

The results showed the presence of *Acanthamoeba* genotype T4 in 100% of water samples, however, when DF3 region, that is a hypervariable region of the 18S gene, is analyzed, a great diversity of subtypes is found. Some of them presented increased resistance to purification treatments as in the case of subgenotypes T4/16 and T4/1, which were isolated at the input and output of two of the plants or T4/36 which was isolated in treated water. Because some of these types have been found in clinical samples, susceptibility of *Acanthamoeba* to the treatments currently used in DWTP should be studied in order to improve them.

Finantial Support: Funded by grant PI12/02725 from FIS, FEDER, and by grant USPCEU-PC07/2013 and USP-PC07/2014 of the Fundación Universitaria San Pablo-CEU. Thiago Gomes was supported by EADS CASA-Brasil and also by CAPES Foundation grant (DF 70040-020), Lucianna Vaccaro by FPI USP-CEU.

GETTING INSIDE IN THE ACANTHAMEOBA – LEGIONELLA RELATIONSHIP

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Acanthamoeba and *Legionella* are two waterborne microorganisms that can infect humans. *Acanthamoeba*'s life cycle alternates between an active form, the trophozoite and a latent or resistant form, the cyst. This later structure is able to survive to different water treatments, biocides, changes in temperature, pH or osmolarity. Because of this, their distribution is wide and has been isolated around the world and in different environments. *Legionella* is a gram negative bacterium that can infect human macrophages causing the human respiratory disease legionellosis. *Legionella* despite showing a long-term survival in sterile tap water, its proliferation has been described as dependent on its relationships with other microorganisms such as *Acanthameoba. Legionella* intracellularly multiplies in this amoeba being protected from harsh environmental conditions like biocides, antibiotics, acid and osmotic stress or temperature changes, thus hindering their removal from water systems. Moreover, *Legionella*'s virulence, growth and survival in the environment are enhanced by their ability to form symbiotic relationship with other microorganisms. In order to better understand this relation, *Acanthamoeba castellani* ATCC 30234 proteins were biotinylated, extracted and faced to alive *Legionella pneumophila* NCTC 1181. After interaction, unlinked *Acanthamoeba* proteins were washed. To retrieve the linked ones, the bond with *Legionella* was broken by heat shock. Then, *Acanthamoeba*'s biotinylated proteins were purified with Dynabeads ® M-280 Streptavidin (Invitrogen) and analyzed by LC-MS/MS. Western blot analysis of surface biotinylated membrane proteins revealed 4 strong bands of approximately 20, 30, 58 and 112 kDa. The results of the LC-MS/MS showed that some of those proteins selectively attached to *Legionella*. Because the mechanism of interaction of *Legionella* to human macrophages is similar to that of *Acanthamoeba*, knowledge of these proteins opens the door for future studies both for the treatment of legionellosis and to improve its elimination of man-made water systems, main focus of *Legionella* infection.

CHLAMYDOMONAS ACIDOPHILA: A POLIEXTREMEMOPHILE PHOTOSYNTETIC PROTIST ISOLATED FROM TINTO RIVER WITH A HIGH RESISTANCE TO HEAVY METAL(OID)S

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Chlamydomonas acidophila is a well-known poliextremophilic microalgae isolated from the natural acid extreme habitat Tinto River (Huelva, Spain). Acid aquatic environments usually contains high concentrations of heavy metal(oid)s because low pHs increase remarkably bioavailable metallic concentrations. Under these high metallic levels eukaryotic biodiversity is usually low since cells have to be adapted to these biotoxic extreme conditions. First, we have studied the cytotoxicity of several heavy metals (Cd2+, Zn2+, Cu2+, Ni2+, Co2+, Fe2+) and metalloids (As3+, As5+), using flow cytometry and the iodine propidium fluorophore. Obtained lethal concentrations for 50% of populations (LC50), after 24h of treatment, were: 1.98 microM (Cd), 163.91 mM (Zn), 10.85 microM (Cu), 97.18 mM (Ni), 61.78 mM (Co), 10.91 mM (As3+), 41.63 mM (As5+) and 143.7 mM (Fe). Therefore, as far as we know, this strain is the most resistant eukaryotic microorganism at present detected. Sublethal concentration exposures of these toxic agents caused important ultrastructural alteractions, which were specific of each treatment. The main biotoxicity cell targets were organelle involved in photosynthesis; including thylakoids from the unique big chloroplast, the pyrenoid and the pigmented photoreceptor (stigma). Treated cells also presented a vacuolised cytoplasm with numerous lipid droplets. Some modifications in nuclear organization have been also observed after certain metallic exposures (Cd, Co, Ni). According to our results, *C. acidophila* has, at least, two different mechanisms of metal resistance; bioaccumulation and biosorption. Vegetative cells have a polysaccharide capsule, whose thickness increased remarkably after exposure to As5+, Cu, Cd or Zn. Embedded into this structure discrete osmiophilic small particles were detected. These micro- nanoparticles also appeared into the cell cytoplasm exposed to Cd or As3+. At present we are elucidating the chemical nature of this electrondense nanostructures by XDES (X-Ray Energy Dispersive Spectroscopy). And, in the next future, we will carry out a molecular analysis of these mechanisms to explain the high metal resistance of this protist.

PRESENCE OF THREE ANTIOXIDANT-SYSTEMS (GSH/GR, TRX(SH2)/TRXR AND TRY(SH2)/TRYR) IN TETRAHYMENA THERMOPHILA: AN INTEGRATED VIEW OF THE STRESS RESPONSE TO METAL(OID)S

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Three different, but not exclusive, antioxidant systems may exist in prokaryotic or eukaryotic cells; GSH/GR (glutathione/glutathione-reductase), Trx(SH2)/TrxR (thioredoxin/tioredoxin-reductase) and/or Try(SH2)/TryR (trypanothione/trypanothione-reductase). GSH is a tripeptide (gamma-L-glutamyl-L-cysteinyl-glycine), present in all aerobic organisms (from bacteria to animals), it is the most abundant non-protein thiol against oxidative stress. Trx(SH2) is another antioxidant molecule, a small protein with redox activity, present in all living systems (from bacteria to human). Try(SH2) is an antioxidant molecule present in trypanosomatids, which lost both; GSH/GR system and TrxR. *T. thermophila* responses to Cd2+ stress using two main molecules with thiol groups; metallothioneins and GSH as a part of the antioxidant system GSH/GR, which constitutes a reduction source against the oxidative stress induced by Cd2+. In this process are also involved enzymes such as; GPxs (at least 5 GPx genes are over-expressed) and TrxRs (at least 2 isogenes are over-expressed). GSH is one of the first cellular defense line against As5+ stress, which acts as a reduction source for enzymes involve in offset the oxidative stress originated by this metaloid. Likewise, GPxs and TrxRs (2 isogenes of each type) are over-expressed under As5+ stress. For the first time in a free-living ciliate, the existence of Try(SH2) in *T. thermophila* has been reported, by HPLC and mass spectrometry. Its macronuclear genome has 4 genes similar to GspS (glutathionyl-spermidine synthetase), and the only presence of synthetase domain in these TtGspS constitutes an unique feature with regard the rest of organisms with TryS (trypanothione synthetase) or GspS. In addition, in this ciliate an enzymatic activity similar to TryR has been detected, corroborating the existence of Try(SH2). At present, *T. thermophila* is the only free living protist in which three different antioxidant systems coexist. According to global results under metal stress, the ranking of activity levels of these three systems is; GSH/GR>Trx(SH2)/TrxR >Try(SH2)/TryR. Therefore, in this ciliate the Try[SH2]/TryR system might have a secondary function in maintaining the cell redox equilibrium, though the three systems might also act coordinately in the cellular antioxidant defense. An integrated view of the main antioxidant systems, under Cd2+ or As5+ stress, is reported.

SELECTION AND CHARACTERIZATION OF SCFV ANTIBODIES AGAINST PNEUMOCYTIS JIROVECII FROM PHAGE DISPLAY LIBRARIES.

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Pneumocystis jirovecii is an extracellular opportunistic microorganism that infects the pulmonary alveoli of humans and causes pneumonia. Diagnosis of *P. jirovecii* pneumonia (PcP) is made by a selective combination of biological, serological, histological and molecular methods. Phage display technology can be used as a rapid diagnostic tool by producing specific recombinant antibodies against *P. jirovecii.* In this study three phage display libraries: the human synthetic scfv Tomlinson I+J libraries, (MCR, UK) and Griffin scfv library (Griffin.1 library) were used to select *P. jirovecii* strains presenting scfv binding phages. As samples of antigens were used a purified fragment of *P. jirovecii* Major surface glycoprotein (Mgs) and human respiratory specimens previously tested positive for *P. jirovecii*, due to difficulties reproducing this microorganism in vitro. In the panning protocol, washing steps were made with a high salt concentration buffer (500 mM NaCl) to remove the low binding and unspecific phages. Phage elution was performed with respiratory specimens, following the infection of *E. coli* TOP10F for 1 hour at optimal temperatures. This process was repeated two more times. A selection of 92 clones from the 3rd pannings (all scfv-phage libraries) were tested by indirect-ELISA. The specificity and the usefulness of these scfv-phages were investigated by ELISA and immunofluorescent assays.

COMPARISON OF ULTRASTRUCTURE AND CHEMICAL COMPOSITION OF THE CELL WALL OF CHLORELLA IN FREE-LIVING AND ENDOSYMBIOTIC CONDITIONS

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Morphological and chemical properties of the cell wall of symbiotic *Chlorella* sp. (strain Kb1) were examined in a free-living state and in the endosymbiotic condition in the cytoplasm of *Paramecium bursaria* (strain PB-Kb1). Cell wall of *Chlorella* was stained with Calcofluor white M2R, FITC-WGA, or FITC-LFA. Calcofluor is a fluorescent dye that stains β-D-glucopyranose polysaccharides such as cellulose, N-acetylglucosamine, sialic acid, and glycosaminoglycans. WGA is a lectin that binds N-acetylglucosamine and sialic acid residues, and LFA is a sialic acid-specific lectin. Cell wall of the free-living *Chlorella* was stained well with Calcofluor, while it showed a decreased stainability (~50%) after the same strain of *Chlorella* cells were introduced to the aposymbiotic *P. bursaria* to re-establish the symbiotic relationship between *Chlorella* and *P. bursaria*. This result suggests that some changes in the polysaccharide constituents of the cell wall might have occurred during the course of the establishment of endosymbiosis. Cellulase treatment did not diminish the Calcofluor staining, indicating that cellulose is not the major saccharide responsible for the Calcofluor staining. The cell wall was stained well with either WGA or LFA, irrespective of the endosymbiotic conditions, suggesting that N-acetylglucosamine and sialic acid are not responsible for the decrease in Calcofluor staining after endosymbiosis. Taken together, these results suggest a possible decrease in the amount of glycosaminoglycans on the surface of the cell wall under the endosymbiotic condition. Transmission electron microscopy with quick-freezing and freeze-substitution showed that thickness of the cell wall of free-living *Chlorella* was 20-30 nm, while that of symbiotic *Chlorella* was 7-12 nm, indicating that the cell wall of symbiotic *Chlorella* changes in both structure and chemical properties with the establishment of *Chlorella-Paramecium* symbiosis.

MITOCHONDRIAL RESPIRATORY CHAIN OF AN OYSTER PARASITE *PERKINSUS MARINUS*

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Perkinsus spp. are notorious marine unicellular protists, which parasitize commercially important bivalve species like clams and oysters worldwide. In their lifecycle, propagating and thus pathogenic trophozoite develops into an enlarged dormant hypnospore in an anaerobic condition, and then into small dispersal stage zoospore when returned to aerobic condition. The mitochondrial respiratory chain is therefore supposed to have important role in the parasites' physiology and ecology, but there has been little study. The draft genome shows that *Perkinsus marinus* harbors five dehydrogenases transferring electron to quinone and two quinol oxidase systems. Here we studied biochemical activities of these predicted enzymes and analyzed quinone species using in vitro culture of *P. marinus* trophozoite. Type 2 NADH dehydrogenase, succinate-quinone reductase (SQR), dihydroorotate dehydrogenase, and malate-quinone oxidoreductase were active; the specific activity of SQR was predominant among them. In contrast, glycerol-3-phosphate dehydrogenase activity was not detected. Both cyanide-sensitive and -insensitive systems almost evenly contributed for quinol oxidation by molecular oxygen. In addition, NADH-fumarate reductase activity was prominent, suggesting that *P. marinus* has an ability of anaerobic malate dismutation as mussels and oysters do. Total quinone analysis showed that the major quinone species were ubiquinone-8 and a variant of menaquinone-7, which is a low-potential quinone suitable for mediating electron transfer from NADH to fumarate. All these results indicate that *P. marinus* is able to efficiently perform redox reactions both in aerobic and anaerobic conditions. We speculate that this is an adaptation to the tidal environments in which the host organism lives, and a key process on the evolutionary course of the parasites.

SOME DETAILS OF THE LORICA APERTURE OF LAGENOPHRYS DISCOIDEA (PERITRICHIA: LAGENOPHRYIDAE) WITH SCANNING ELECTRON MICROSCOPY, AND NOTES ON ITS GEOGRAPHIC DISTRIBUTION

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Genus *Lagenophrys* includes 62 ectosymbiont species of crustaceans hosts. *Lagenophrys discoidea* Kellicott, 1887 has been reported in Neartic, Neotropical and Palaeartic regions, attached to the carapace of ostracods. Available morphological data includes characters only observed under optical microscopy. The aim of this contribution is to provide new cytological data with scanning electron microscopy to reveal details of the lorica aperture of *L. discoidea* and also its first record on Ukrainian hosts. Samples were obtained from Dnieper River by manual collection, and the ostracods of the Cyprinidae family carried lagenophryid individuals attached to the external surface of the carapace. Standard methods were used for scanning electron microscopy. We observed the shape and disposition of smooth anterior and posterior lips, and the collar of aperture, characters that correspond to the species diagnosis. The rounded lorica, its thickness and the base of the lorica attached to host, were also observed with detail. These observations complement the knowledge of this specialized species of *Lagenophrys*.

Acknowledgements. To Programa de Apoyos para la Superación del Personal Académico (PASPA-DGAPA-UNAM) for the grant to RME for a short stay at NCCU and Secretaría de Intercambio Académico and Dirección General de Cooperación e Internalización, UNAM, for the grant to RME for a short stay at Schmalhausen Institute of Zoology, NAS, Ukraine. Biól. M. Reyes and M. C. Maricela Vicencio, Facultad de Ciencias, UNAM are thanked for their technical support.

CYTOLOGICAL CHARACTERIZATION OF EUPLOTOIDES OCTOCARINATUS (CARTER, 1972) FROM MEXICO, WITH DATA OF ITS WORLD GEOGRAPHIC DISTRIBUTION AND 18S RDNA SEQUENCE

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Genus *Euplotoides* contains 11 species, and for *E. octocarinatus,* a poorly known species, few records concerning its geographical distribution data have been published. Moreover, its 18S rDNA sequence has been obtained only for two populations. The goal of this contribution deals with some cytological attributes observed in a Mexican population, and some notes about its 18S rDNA sequence. Samples were obtained from a small pond in Oaxaca state. *Euplotoides octocarinatus* was impregnated with Klein technique for cytological characterization. We confirmed the species identity and then the sequence was obtained. Based on previously published records, we plotted all this data to represent its geographic distribution. Our results indicate that this species has a predominant American distribution, with records in the USA and Brazil, and now in Mexico.

DIVERSITY AND SYSTEMATICS OF THECAMOEBID AMOEBAE (AMOEBOZOA: DISCOSEA: THECAMOEBIDAE)

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Amoebae of the family Thecamoebidae are widely distributed in the environment. These organism are relatively easy to isolate and cultivate, also the frequency of occurrence of species is very different. There are rather common species like *Thecamoeba quadrilineata, T. striata* and *T. orbis* as well as numerous species known from few findings or never re- isolated since initial description. Many of these species were studied only at the light-microscopic level and require investigation with modern methods, including electron microscopy and molecular data (this especially concerns the genus *Thecamoeba* and genera of unclear systematic position like *Pseudothecamoeba* and *Thecochaos*). Our studies show that "hotspot" of *Thecamoeba* diversity is terrestrial habitats – soil, grass, dry leaves and surface of trees. During our studies we isolated 15 strains of *Thecamoeba*; some were identified as known species (*Thecamoeba aesculea, T. similis* and *T. quadrilineata*). Among studied strains there are representatives of no less than two *Thecamoeba* species, which are new for science. We have found two strains of amoebae belonging to the genus *Sappinia*. In contrast, amoebae of the genus *Stenamoeba* were never found in terrestrial samples. Our data show that species diversity of thecamoebid amoebae remains considerably underexplored.

Supported with Russian Science Foundation grant 14-14-00474.

ALTERATIONS IN THE CYTOPLASMIC STRUCTURES AND CYST WALL DURING DIFFERENTIATION OF GIARDIA INTESTINALIS

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Differentiation from one life cycle stage to another is an adaptation used by many parasites to ensure their transmission and survival. *Giardia intestinalis* is a major cause of water-borne diarrheal disease. Nonetheless, the basic biology of this protist is not completely understood. It exhibits two developmental stages during the life cycle, trophozoite and cyst. Trophozoites quickly become rounded early in the encystation process, the cells can no longer attach to surfaces and begin a huge transformation. Moreover, the changes that happen in the cyst wall (CW) during the excystation process are poorly studied. Encystation and excystation are crucial processes for establishment and maintenance of *Giardia* infection. Thus, the aim of this work is to better understand the ultrastructural modification during the differentiation process of *G. intestinalis*. Here, we induced the differentiation process in vitro, analyzed the cyst formation and changes in the CW of the parasite by complementary techniques, such as scanning and transmission electron microscopy. The 3D reconstruction using a Dual-Beam microscope was performed aiming to better analyze the cell below the CW. A field emission scanning electron microscopy (FESEM) compared the mature cysts and the beginning of excystation process. Interestingly, the parasite's flagella are internalized into the cyst body without a specific region, and were observed either in the peritrophic space or in the cytoplasm. The fragmented ventral disk was found nearby the four nuclei. A cell tail was observed at late phase of encystation by 3D reconstruction, and the caudal flagella were surrounded by the CW until the peritrophic space connection. During the excystation, changes in the cyst shape and presence of electron-dense vesicles localized near the CW were observed. The FESEM allowed detailed images of the CW. In mature cyst, the wall microfibrils were seen in a tighter arrangement. However, early in the excystation this tight arrangement of the wall microfibrils was lost. Damage in the CW was seen by transmission electron microscopy. In conclusion, our results show for the first time a 3D reconstruction of cysts and structures remodeled during encystation. Moreover, the modification of CW fibrils arrangement is a step in the excystation process.

INTESTINAL PARASITOSIS IN RELATION TO CD4+T CELLS LEVELS AND ANEMIA AMONG HAART INITIATED AND HAART NAÏVE PEDIATRIC HIV PATIENTS IN MODEL ART CENTER, ADDIS ABABA, ETHIOPIA

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Background: Intestinal parasites (IPs) are major concerns in most developing countries where HIV/AIDS cases are concentrated and almost 80% of AIDS patients die of AIDS-related infections. In the absence of highly active antiretroviral therapy (HAART), HIV/AIDS patients in developing countries unfortunately continue to suffer from the consequences of opportunistic and other intestinal parasites.

Methods: A comparative cross-sectional study was conducted among HAART initiated and HAART naive pediatric HIV/AIDS patients attending a model ART center at Zewditu Memorial Hospital between August 05, 2013 and November 25, 2013. Stool specimen was collected and processed using direct wet mount, formol-ether concentration and modified Ziehl-Neelsen staining techniques. A structured questionnaire was used to collect data on socio-demographic and associated risk factors. CD4+ T cells and complete blood counts were performed using BD FACScalibur and Cell-Dyn 1800, respectively.

Results: The overall prevalence of IPs was 37.8% where 27.8% of HAART initiated and 45.5% of HAART naive pediatric HIV/AIDS patients were infected ($p < 0.05$). *Cryptosporidium* species, *E. histolytica*/dispar, Hook worm and Taenia species were IPs associated with CD4+ T cell counts <350 cells/μL in HAART naive patients. The overall prevalence of anemia was 10% in HAART and 31.7% in non-HAART groups. Hook worm, *S. stercoralis* and *H. nana* were helminthes significantly associated with anemia in non-HAART patients [AOR, 95% CI: 4.5(1.3, 15.2), P< 0.05]. The prevalence of IPs in non-HAART patients was significantly associated with eating unwashed/raw fruit [AOR, 95%CI: 6.3(1.2, 25.6), P<0.05], open field defecation [AOR, 95%CI: 9.3(1.6, 53.6), P<0.05] and diarrhea [AOR, 95%CI: 5.2(1.3, 21.3), P<0.05]. IPs significantly increased in rural residents [AOR, 95%CI: 0.4(0.1, 0.9, P<0.05)].

Conclusion: The overall prevalence of intestinal parasites significantly differed by HAART status and cryptosporidium species were found only in HAART naïve patients with low CD4+ T cell counts. Anemia was also more prevalent and significantly associated with IPs in non-HAART patients. This study identified some environmental and associated risk factors for intestinal parasitic infections. Therefore, Public health measures should continue to emphasize the importance of environmental and personal hygiene to protect HIV/AIDS patients from infections with intestinal parasites and maximize the benefits of HAART.

HYDROLOGICAL CONNECTIVITY DETERMINING METACOMMUNITY STRUCTURE OF PLANKTONIC HETEROTROPHIC FLAGELLATES

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Species distribution patterns are regulated by a combination of abiotic factors, biotic interactions and dispersal processes. In aquatic environments, the dispersal potential of the organisms is directly related to the hydrological connectivity among habitats. In this study, we approach three types of environments of the Upper Paraná River-floodplain system, which differ in their degree of connectivity: lotic environments, connected lakes and isolated lakes. We aimed to investigate if the relative role of the environmental and spatial components in structuring the heterotrophic flagellates depends on the degree of hydrological connectivity. We expect that communities in isolated lakes would be more subject to dispersal limitation, while in connected lakes and lotic environments the communities would be regulated mainly by environmental variables (species sorting). We sampled in the planktonic region of 22 environments during the low water period in 2014. We determined the relative importance of the assembly mechanisms using variance partitioning and evaluated changes in beta diversity and environmental heterogeneity in each type of environment. Our results reinforce the relationship between beta diversity and environmental heterogeneity, which were both higher in the isolated lakes, connected lakes and lotic environments, respectively. The greater contribution of the environmental variables in structuring the hetrotrophic flagellates metacommunity, regardless of the hydrological connectivity, may be related to the elevated dispersal capacity of those microorganisms. The spatial component was also significant, however only in the isolated lakes, a species sorting mechanism partially constrained by dispersal limitation. In summary, our results support the idea that microorganism communities are mainly structured by environmental factors, even considering environments with distinct connectivity degree.

GAUSE WAS WRONG: A PRACTICAL USE OF FUNCTIONAL AND NUMERICAL RESPONSES

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For ~80 years, data obtained by Gause on *Paramecium* (predator) and yeast (prey) have been used to evaluate and develop models of predator-prey dynamics. This model system, however, has not been empirically examined since the 1930s. We have empirically evaluated if *Paramecium aurelia* ingests and grows on six yeast strains. Recognising that *P. aurelia* ingested these strains but could not grow on them, we then parameterised the Rosenzweig-MacArthur predator-prey model, which relies on ingestion not growth measurements; model simulations were compared to time-series data of *P. aurelia* and the yeast *Saccharomyces cerevisiae,* provided by Gause in his original work. It was hypothesised that if the model produced simulations of predator-prey dynamics that mimicked the original data, then it was likely that *P. aurelia* could grow on yeast, but an appropriate strain had not been found; in contrast, if the model could not simulate the data, then it was unlikely that *P. aurelia* can grow on yeast. We reveal the latter to be the case, and a critical review of the original experiments revealed two issues: 1) Gause manipulated his data by adding yeast and ciliates, and, therefore, his data set should not be considered as a self-sustaining time-series, and 2) the system was undoubtedly contaminated with bacteria, allowing *P. aurelia* to survive on bacteria rather than yeast. For these reasons, we conclude that the data on yeast and *Paramecium* produced by Gause should not be used to evaluate predator-prey dynamics, and studies that have relied on it should be regarded with caution. Furthermore, this study indicates that future work should not pursue empirical studies on *Paramecium* and yeast as a model system.

CRYTOSPORIDIUM AND GIARDIA IN RAW AND TREATED SLUDGE FROM WASTEWATER TREATMENT PLANTS

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Cryptosporidium oocysts and *Giardia* cysts,(oo)cysts, are common water contaminants and have been detected in irrigation water, effluents and sludge from wastewater treatment plants. They are excreted in large numbers that are infectious at minimal exposure doses, hardy in the environment, and resistant to oxidizing disinfection.

Both oocysts and cysts are precipitated in sewage sludge during wastewater treatment by flocculation and activated sludge. Numerous pathogens remain associated with particulates, thus concentrating them in the sludge. Subsequent spreading of sludge onto agricultural land can spread the circulation of these protozoan parasites in the ecosystem, posing a threat to human and animal health through the contamination of pastures and raw water sources of drinking water. Sludge treatments as biological digestion, lime stabilization and composting can be employed to reduce pathogens in sewage sludge.

The objective of this study was to collect quantitative background data on (oo) cysts in the raw and treated sludge from wastewater treatment processes in eastern of Spain.

Sludge from 5 wastewater treatment plants (WTP1, WTP2, WTP3, WTP 4 and WTP5) with different stabilization processes (aerobic and anaerobic digestion, lime stabilisation) have been analysed for the presence of *Cryptosporidium* and *Giardia* before and after the sludge treatment. A composting treatment plant (CTP) has also been assessed.

After a sedimentation step, samples were processed and (oo) cysts were isolated by Immunomagnetic Separation (IMS) and detected by Immunofluorescence (IFA) (USEPA, 2005).

Results obtained in this study show that oocysts of *Cryptosporidium* and *Giardia* cysts were present in 26 of the 30 samplings of raw sludge samples. In treated sludge samples, presence of both cysts and oocysts has been observed in all WTP's analyzed (25 samples) with different stabilization treatment except in samples from the compost plant where no (oo) cysts were detected.

This study provide evidence that (oo) cysts are present throghout the wastewater processes and in end-products with the negative consequences for public health.

FREE-LIVING AMOEBAE IN WATER SOURCES BY PCR AND SEQUENCING IN SPAIN

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Free-living amoebae (FLA) are ubiquitous protozoa widely isolated from water. Among them, *Acanthamoeba* species are the most common FLA in those environments and can cause rare but severe infections of the eye (*Acanthamoeba* keratitis, AK), skin, and central nervous system (Granulomatous Amebic Encephalitis, GAE). FLA belonging to the genus *Naegleria* and *Balamuthia* are also important human pathogens, although its presence in water is infrequent. Identification of different FLA in sources able to reach humans is of great importance.

A total of 50 water samples were analyzed for the presence of FLA. Nineteen drinking and 31 wastewater samples were filtered. Membranes were placed in Non-Nutrient-Agar (NNA) and the culture was maintained until amoebae growth was observed. FLA were purified individually using a micromanipulator and incubated in NNA. Cultures were harvested, and after DNA isolation, were subjected to Multiplex PCR (Le Calvez et al., 2012). Results were confirmed by 18S rRNA PCR plus sequencing (Thomas et al., 2006).

FLA growth was observed in 22/31 and 5/19 wastewater and drinking water samples respectively. In total, 39 FLA were purified using the micromanipulator, 31 from waste water and 8 from drinking water samples. Multiplex PCR yielded specific fragments of *Acanthamoeba, Naegleria, Vannellidae, Vermamoeba* and *Echinamoeba*, being *Acanthamoeba* the most common FLA isolated in both types of water. In some cases, although the micromanipulator was used to isolate a single amoeba, unspecific fragments were observed after Multiplex PCR analysis of the cultures, showed that an axenic culture was not achieved. By 18S rRNA PCR and sequencing, *Vannellidae* spp., *Naegleria* spp. and the *Acanthamoeba* species *A. castellanii, A. tubiashi, A. polyphaga, A. rhysodes* and *A. mauritaniensis* were identified. To our knowledge, it is the first time that *Vannellidae* spp. is identified in wastewater. The fact that those human pathogens were detected from water sources even after disinfection treatment could be a risk concerning Public health.

This study was partially supported by a grant of the "Recovery and joint Resources from I+D+i of VLC/CAMPUS program" funded by the MECD (International Excellence Campus Program). The authors thank the Generalitat Valenciana for FEDER financial support 2007-2013 (T0104000).

INVESTIGATING MINISOG AS A PROTEIN LOCALIZATION TOOL IN OXYGEN-SENSITIVE PROTOZOAN PARASITES

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*Trichomonas vaginali*s and *Tritrichomonas foetus* are flagellate protozoan parasites that cause venereal disease of humans and cattle, respectively. *Trichomonas vaginalis* is currently the most common, non-virally transmitted venereal disease of humans. Even with significant medial and veterinary burden, little is known of protein-protein interactions and localization in these parasites. Genetically encodable fluorescent protein tags, such as the green fluorescent protein (GFP), has enhanced the resolution of light microscopy expanding our understanding of sub-cellular protein localization. These tags, however, require oxygen for chromophore maturation and are of little use as a protein tracker in oxygen-sensitive parasites, such as the trichomonads. A potential solution to this problem is miniSOG, a novel, oxygen-independent fluorescent protein derived from phototropin2 of plants. In addition to fluorescence, miniSOG offers an added advantage of over GFP as an ultrastructural tag at the transmission electron microscopy (TEM) level. Therefore, fluorescence signals can be translated into TEM signals without the need for antibody localization, surpassing the current achievable resolution for ultrastructural protein localization. In an attempt to expand the parasitologists toolbox, we use *T. foetus* as a model to investigate the capacity for miniSOG to perform as a genetically encodable tag in oxygen-sensitive parasites. We report on the first lipid based transfection in *T. foetus* and investigate miniSOG fluorescence and the TEM signals in *T. foetus*.

COMPARATIVE GENOME ANALYSIS OF PEDINOPHYTE PLASTIDS AND THE PEDINOPHYTE-DERIVED PLASTIDS IN TWO DINOFLAGELLATES LEPIDODINIUM CHLOROPHORUM AND STRAIN MRD-151

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An undescribed dinoflagellate strain MRD-151 was isolated from seawater sampled in Japan. Analyses of plastid genes and pigment compositions of strain MRD-151 suggested that the original (peridinin-type) plastid was substituted by a pedinophyte-derived plastid containing chlorophylls a+b. Besides strain MRD-151, a single case of plastid replacement involved in a pedinophyte has been known for other dinoflagellate *Lepidodinium* spp., which are apparently distantly related to strain MRD-151 in the host phylogeny. Thus, pedinophyte-derived plastids were most likely established (at least) twice in dinoflagellate evolution. In this study, we conducted comparative analyses of the plastid genomes of strain MRD-151, *L. chlorophorum*, and three free-living pedinophytes (*Pedinomonas minor, P. tuberculate,* and *Marsupiomonas* sp. NIES 1824) to investigate how the plastid genomes of pedinophytes were altered during endosymbioses in dinoflagellate cells. We newly sequenced the complete plastid genome of strain MRD-151. The MRD-151 plastid genome was mapped as a circular molecule of ~100 Kbp in length. The gene repertory of the MRD-151 plastid genome was found to be a subset of those of the three pedinophyte plastid genomes, being consistent with the pedinophyte-origin of the MRD-151 plastid. Intriguingly, the repertory of conserved protein genes in the MRD-151 plastid genome appeared to be very similar to that in the *L. chlorophorum* plastid genome, albeit the two genomes were evolved separately in the distantly related host lineages. Our comparative analyses provide two insights into the genome evolutions of the MRD-151 and *L. chlorophorum* plastids derived from two distinct pedinophyte endosymbionts. Firstly, the MRD-151 and *L. chlorophorum* plastid genomes were likely derived from two ancestral plastid genomes carrying similar sets of conserved protein genes. Secondly, independent but similar evolutionary pressures toward genome reduction/gene loss likely shaped the gene repertories of the MRD-151 and *L. chlorophorum* plastid genomes.

TESTICULAR MYXOSPORIDIASIS AND ULTRASTRUCTURAL CHARACTERISTICS OF MYXOBOLUS BUFONIS (MYXOBOLIDAE) INFECTING THE EGYPTIAN TOAD BUFO REGULARIS (BUFONIDAE). A LIGHT AND ELECTRON MICROSCOPIC STUDY

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The phylum Myxozoa comprise more than 2180 species, almost all of which are considered to be obligate parasites of aquatic fishes and amphibians. They are dangerous pathogens responsible for severe economic losses. From March to September 2014, forty adult male *Bufo regularis* (Bufonidae) captured from different areas at Giza province, Egypt were surveyed for myxosporean parasitic infection. Of these, 22 (55%) were infected by histozoic plasmodia, which produced spores after rupture belonging to Myxosporidia. The present investigation introduced a new data for the recorded parasite observed by light and transmission electron microscopy. The infection was detected as large clusters of macroscopic plasmodia embedded in the testicular tissue causing distortion at their site of infection. The host reaction was manifested by the encapsulation of the plasmodia with a thick layer of connective tissue. Plasmodia were whitish in color, elliptical to ovoid in shape measuring 0.54±0.2 (0.34–0.63) mm in diameter. The spores were subspherical, reaching 7.1±0.2 (6.2–8.4) µm in length and 6.3± 0.2 (5.8–7.0) µm in width with two equal sized polar capsules regularly arranged at the anterior pole of each spore. They were 3.4±0.2 (3.0–4.2) µm in length and 1.9±0.2 (1.6–2.4) in width with 6-8 turns of polar filaments. Ultrastructural analysis showed that the plasmodia were surrounded by a plasma membrane with numerous projections and pinocytotic channels extended toward the host cell. The generative cells and the different developmental stages were arranged at the periphery of the plasmodia while immature and mature spores were centrally arranged. Sporogenesis, capsulogensis, valvogenesis and spore maturation of the present parasite were also described.

EXPERIMENTAL AND OBSERVATIONAL EVIDENCE OF PHENOTYPIC PLASTICITY ON LEIDY'S BUTTERFLY, HYALOSPHENIA PAPILIO

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Untangling relationships between morphology and phylogenetic position is key for building a reliable taxonomy. Yet, finding relevant synapomorphic traits for separating taxa is especially difficult in those groups that are composed of small organisms that lack diagnostic traits. This includes typically small metazoan such as protists. Observational and experimental studies have revealed evidence for morphological variation driven by environmental conditions in Arcellinid testate amoebae. Thus, discriminating taxonomically-relevant traits from pure phenotypical plasticity is not straightforward and contributes to current confusion in taxonomy, with detrimental consequences on inferences on biogeogeography or the use of these protists in applied ecological studies.

One of the most abundant testate amoeba in oligotrophic Northern Hemisphere peatlands, *Hyalosphenia papilio*, has been shown to include at least 12 genetic lineages based on COI, several of which having geographically limited distributions. This morpho-species is also polymorphic with respect to shell dimensions and number of pores. This variability has been interpreted as possibly due to phenotypic plasticity, but this has not yet been formally tested.

Here, we aimed at determining to which extent the morphological variation of *Hyalosphenia papilio* was the product of phenotypic plasticity. We investigated if the morphology of *H. papilio* varied in function of (1) environmental condition and (2) phylogenetical lineage.

We analyzed the morphology (shell shape, size and pore number) of *H. papilio* specimens collected from 1) a European-wide sampling with field-measured water-table depth and 2) a mesocosm experiment in which water table depth was controlled to assess to what extent morphology was correlated to environmental conditions. We then isolated individuals over time in the mesocosm study and from environmental samples and sequenced them for COI, to assess if morphological variation was due to phenotypic plasticity or if it was genetically determined.

The results show a response of pore number to water table level, and demonstrate that the shape of *H. papilio* is explained mainly by temperature and geographical distributions. Finally, no morphological feature appeared to be correlated with the phylogeny of the observed specimens.

COMPARATIVE GENOMICS OF NEPHROMYCES COMMUNITIES FROM TUNICATE RENAL SACS

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Nephromyces is a divergent apicomplexan that lives as an endosymbiont inside the renal sac of molgulid tunicates. The nature of *Nephromyces* as an apicomplexan remained enigmatic for long time, mostly due to: (i) its peculiar habitat, (ii) the presence of cytoplasmic bacterial endosymbionts, (iii) a complex life-cycle with unusual morphologies, and (iv) a presumably mutualistic association with its animal host. Moreover, several lines of evidence suggest that the renal sac of an individual tunicate host harbors a complex community of diverse *Nephromyces* lineages: the multiple-infection hypothesis. We decided to further investigate this hypothesis by using deep sequencing of renal sacs in order to better understand the developmental dynamics of this symbiosis. Our initial analyses revealed the presence of eleven apicoplast genomes in one single renal sac. Surprisingly, there was considerable sequence divergence among the apicoplast genomes, although their gene content and order was highly conserved. We then used the phylogenetic information contained within 27 apicoplast proteins to infer *Nephromyces*' phylogenetic placement within Apicomplexa. Finally, we set out to characterize the complex renal sac metagenome of another individual tunicate host in order to compare the diversity of *Nephromyces* between different hosts in nature. These preliminary results support the idea that molgulid renal sacs are complex ecosystems inhabited by a diverse community of different *Nephromyces* lineages. Future efforts will focus on elucidating the metabolic contribution of each partner to the dynamics of this complex symbiotic system.

THE GUIDED ENTRY OF TAIL-ANCHORED PROTEINS PATHWAY IN GIARDIA INTESTINALIS

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The special class of membrane proteins, so called tail-anchored (TA) proteins, carry a single C-terminal transmembrane domain that anchors them to organellar membranes. TA proteins mediate interactions among membrane bounded compartments by their N-terminal domains during various processes such as vesicular transport, regulation of apoptosis or protein translocation. In some eukaryotes, the specific pathway controls precise post-translational insertion of tail-anchored proteins into the endoplasmic reticulum membrane – Guided Entry of Tail-anchored proteins (GET) pathway.

Our bioinformatics analyses revealed the absence of most of the GET proteins in majority of the eukaryotic lineages except opisthokonts. However, one of the components of GET pathway (Get3) is conserved in all eukaryotic groups excavates included. We are using *Giardia intestinalis* in order to characterize its GET machinery. We have shown that *Giardia* Get3 is a cytoplasmic protein with affinity to the endoplasmic reticulum. Using chemical cross-linking followed by affinity purification of biotinylated Get3, the specific set of interacting proteins has been identified.

In addition to giardia-specific information, our general aim is to define the evolution of GET pathway in eukaryotes.

ACCUMULATION OF CESIUM IN LIPID DROPLETS OF PARAMECIUM BURSARIA

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A large amount of radioactive cesium was released into the environment by the Fukushima Daiichi Nuclear Power Plant accident in 2011. Bioremediation of radioactively contaminated land has been attempted, but the result is not satisfactory so far. It is because cesium has been strongly absorbed on clay minerals, and extraction of cesium from soil particles is difficult under natural environments. Recently, however, we found that *Paramecium bursaria* has a strong ability to dissociate cesium from soil particles, and to accumulate it inside the cytoplasm. Cesium chloride was absorbed into experimental soil particles (kaolin particles of 1 um in diameter), and the particles were extensively washed with water before mixed with the cell suspension of *P. bursaria*. After 4 days, paramecia were collected by using cathodal galvanotaxis, and the average concentration of cesium in the cytoplasm was measured by ICP-MS. The concentration of cesium inside the cytoplasm of *P. bursaria* was found to be ~30 mM, which was 300 times as high as the extracellular soil suspension (0.1 mM). Aposymbiotic strain of *P. bursaria* without endosymbiotic algae did not show any accumulation of cesium. Localization of accumulated cesium in *P. bursaria* was analyzed with the STEM-EDS image mapping technique. The intracellular cesium signals were found in lipid droplets of both symbiotic *Chlorella* and host *P. bursaria*, suggesting the presence of some kind of sequestering mechanism of cesium into the lipid granules.

DIVERSITY OF PLEURONEMA SPP. (CILIOPHORA, SCUTICOCILIATIA) IN THE HANGZHOU BAY ESTUARY, WITH REPORTING FOUR NEW SPECIES

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During the recent survey on diversity of ciliates in the estuary of Hangzhou Bay, seven *Pleuronema* species were isolated, including four new ones. They are *Pleuronema binucleatum* spec. nov., *P. parawiackowskii* spec. nov., *P. orientalis* spec. nov., *P. rariseta* spec. nov., *P. marinum, P. coronatum,* and *P. czapikae.*

P. binucleatum spec. nov. can be identified by possessing two macronuclei, six to eight preoral kineties, 35-41 somatic kineties, and posterior end of the anterior fragment of membranelle 2 hook-like. *P. parawiackowskii* spec. nov. is characterized by six to eight preoral kineties, 23-29 somatic kineties, posterior portion of the anterior part of membranelle 2 (M2a) slightly curved but non- hooked, and macronucleus sausage-like. Compared with congeners, *P. orientalis* spec. nov. is diagnosed by two or three perioral kineties and 42–50 somatic kineties, membranelle 1 (M1) three-rowed, hook-like posterior end of M2a. *P. rariseta* spec. nov. is clearly separated by smaller size about 55–85 × 25–55 μm in vivo, four or five perioral kineties and 21–23 somatic kineties, and posterior end of M2a hooked-like.

After comparison with other population of *P. marinum*, it is suggested that many misidentifications present in previous studies. And an improved diagnosis of *P. marinum* was supplied: cell about 95-120 × 30-50 μm in vivo, elongate or elliptical in outline; two to four preoral kineties and 53-70 somatic kineties; both membranelle 1 and membranelle 3 three rowed; posterior end of M2a straight; contractile vacuole characteristically positioned in the middle near dorsal side. Phylogenetic analyses based on SSU rRNA gene sequences indicate that the monophyly of the genus *Pleuronema* is still ambiguous although all members cluster together but with very low supporting value.

RESTING CYST MORPHOLOGY AS A GENERIC MARKER IN THE TOVELLIACEAE: A REASSESSMENT

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Tovellia is a genus of freshwater dinoflagellates established in 2005 to receive seven thin-walled species of the polyphyletic genus Woloszynskia sensu lato. The species originally transferred to *Tovellia* have greenish-brown chloroplasts and some accumulate red pigments in the cytoplasm. They have an extraplastidial, lipidic eyespot, an apical line of narrow plates and, where known, they produce a characteristic resting cyst, with axial horns and a narrow equatorial constriction surrounded by protuberances or short spines. This cyst type was thought to be unlike other known cysts and used as a marker for the genus, in contrast with the smooth, round cysts of *Jadwigia* and *Bernardinium*. However, a similar cyst type was found in *Opisthoaulax* vorticella, type species of another recently recognized member genus of the Tovelliaceae. The phylogenetic relationship between *Opisthoaulax* and *Tovellia* has yet to be examined, but it seems unlikely that the colourless and strongly asymmetric species of *Opisthoaulax* will fall within the range of *Tovellia*. On the other hand, two recently described species with vegetative cell morphology and LSU rDNA-deducted phylogeny well within *Tovellia*, added a new cyst type to the genus: ellipsoid, with a little-marked, wide (cingulum-like) equatorial constriction, no axial horns and broader, sometimes long and ramified, spines. In addition, an as yet undetermined species of *Tovellia* has been reproducing sexually and producing cysts in culture that show unique morphological features: they are generally ellipsoid, with a moderately marked, wide sub-equatorial constriction and covered with groups of hair-like spines with tips converging like the poles of a wigwam.

MULTIGENE ANALYSIS OF ARCHAMOEBAE (AMOEBOZOA: CONOSA) SHOWS THAT ENTAMOEBIDAE REPRESENTS A DEEP LINEAGE OF THE GROUP

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Archamoebae is an understudied group of anaerobic, free-living or endobiotic protists that constitutes the major anaerobic lineage of the supergroup Amoebozoa. So far, the phylogeny of Archamoebae has been based solely on SSU rRNA and actin genes that are unable to resolve relationships among main lineages of the group; several different scenarios on the evolution of Archamoebae were proposed. In this study, we present the first multigene phylogenetic analysis that includes members of all four families of Archamoebae: Entamoebidae, Mastigamoebidae, Pelomyxidae, and Rhizomastixidae. The analysis clearly shows that the latter three families form a clade of mostly free-living, amoeboid flagellates, here called Pelobiontida. Predominantly endobiotic and aflagellated Entamoebidae represents a separate, deep-branching lineage, Entamoebida. Based on the results, plesiomorphic features and convergent evolution within the Archamoebae are discussed, and a revision of higher-level classification of Archamoebae is proposed.

PAULINELLA LONGICHROMATOPHORA SP. NOV., A NEW MARINE PHOTOSYNTHETIC TESTATE FILOSE AMOEBA CONTAINING THE CHROMATOPHORE

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The freshwater testate filose amoeba *Paulinella chromatophora* is the sole species in the genus to have plastids termed the "chromatophores" of a *Synechococcus*/*Prochlorococcus*-like cyanobacterial origin. Here, we report a new marine phototrophic species, *Paulinella longichromatophora* sp. nov., using light and electron microscopy and molecular data. This new species contains two blue-green U-shaped plastids reaching up to 40 µm in length. Further, the new *Paulinella* species is characterized by having five oral scales surrounding the pseudostomal aperture. All trees generated using three nuclear rDNA datasets (18S rDNA, 28S rDNA, and the concatenated 18S + 28S rDNA) demonstrated that three photosynthetic *Paulinella* species congruently formed a monophyletic group with robust bootstrap and Bayesian supports (≥99% RAxML and 1.0 Bayesian support), but their relationships remained unresolved within the clade in all trees. The *P. longichromatophora*, nevertheless, clustered consistently together with *Paulinella* strain FK01, but with very poor supported. Phylogenetic analyses inferred from plastid-encoded 16S rDNA and the concatenated dataset of plastid 16S + 23S rDNA demonstrated that chromatophores of all photosynthetic *Paulinella* species formed a monophyly and fell within cyanobacteria clade with a close relationship to α-cyanobacterial clade containing *Prochlorococcus* and *Synechococcus* species with very robust supports of 100% bootstraps and 1.0 Bayesian posterior probabilities. Additionally, phylogenetic analyses of nuclear 18S rDNA and plastid 16S rDNA showed divergent evolution within the photosynthetic *Paulinella* population after a single acquisition of the chromatophore. After the single acquisition of the chromatophore, ancestral photosynthetic *Paulinella* appears to diverge into at least two distinct clades, one containing marine *P. longichromatophora* and freshwater *Paulinella* strain FK01, the other *P. chromatophora* CCAC 0185.

THE PREVALENCE OF CANINE ORAL PROTOZOA AND THEIR ASSOCIATION WITH PERIODONTAL DISEASE

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Periodontal disease is currently one of the most important health concerns for companion animals. Previous studies have demonstrated that at least half of all dogs will have some form of the disease within their lifetime which, without early intervention, can lead to painful periodontal ligament destruction, alveolar bone loss, and eventual loss of teeth. The recent focus of research in canine forms of periodontitis has been the identification and characterisation of the bacterial communities present. However, other microorganisms are known to inhabit the oral cavity and could also influence the disease process.

The objective of this study was to screen for the presence of protozoa within canine plaque samples and to examine their distribution in relation to periodontitis. We employed a novel, broad spectrum 18S PCR designed to target the identification of protists, in conjunction with next generation sequencing analyses. Organisms from the genera *Trichomonas* and *Entamoeba* were identified in pooled and categorised samples of canine plaque. The prevalence of these oral protozoa was monitored in a total of 92 healthy and diseased animals attending a veterinary dental referral practice in the UK. Through PCR the overall prevalence of Trichomonads and Entamoebae detected was 56.52 % (52/92) and 4.34 % (4/92) respectively. Using next generation sequence analysis the proportion of Trichomonad sequences found in each of a series of pooled healthy, gingivitis, early stage periodontitis and severe periodontitis samples was 3.51 %, 2.84 %, 6.07 % and 35.04 % respectively. The proportion of Entamoebae found in each of the same pooled samples was 0.01 % (healthy), 0.01 % (gingivitis), 0.80 % (early stage periodontitis), and 7.91 % (severe periodontitis). Binomial statistical analysis concluded both detected genera of protozoa were statistically associated to animals with periodontal disease.

These findings provide the first conclusive evidence for the ubiquitous presence of canine oral protozoa in dog plaque and suggest a possible role for protozoa in the periodontal disease process.

TESTATE AMOEBAE ASSOCIATED TO BIOLOGICAL SOIL CRUST OF AN INTERTROPICAL DESERT IN MEXICO

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Drylands are fragile systems because they have large spaces devoid of vegetation. However most of these areas are covered by associations of mosses, lichens and cyanobacteria known as biological crusts. Biological soil crusts are microhabitats for a large number of microorganisms and it is interesting to understand more about the dynamics of these systems. In this work, the testate amoebae community associated with biological soil crusts were studied on the southwest side of The Cutha hill, between 1462 to 1757 m a. s. l., in the Tehuacan desert, Mexico because these organisms easily respond to environmental changes. The sampling was stratified random dividing the study area in altitudinal levels (12 levels in total). The altitude levels were divided according to vegetation and was determined the coverage of biological soil crust on each level. The biological soil crusts were collected by triplicate on each level, and environmental and soil parameters were measured. A total of 11 testate amoebae genera were identified across all altitudinal levels. The dominant genus was *Centropyxis*, found on each level being also the most abundant. Other genera found were *Ciclopyxis, Trigonopyxis, Phryganella, Heleopera, Euglypha, Difflugia, Arcella* and *Trinema*. The soil with the highest genera richness was at 1462 m a. s. l. The ecological analyzes indicate high dominance and low diversity in the testate amoebae community. The levels with greater diversity were related to crusts dominated by *Pseudocrossidium* moss and the vegetation was dominated by legumes and columnar cacti. Regarding environmental parameters, the temperature ranged from 32 to 39 °C, moisture from 24 to 42 % and the wind speed from 0.6 to 1.8 m/s. About soil parameters, the temperature ranged from 29 to 37 °C, the gravimetric moisture from 20 to 38 % and the electrical conductivity was around 100 millimhos g-1.

FREE-LIVING AMOEBAE OF AN INTERTROPICAL MEXICAN DESERT: DRIVING THE DISTRIBUTION OF THESE COMMUNITIES IN THE SOIL?

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Arid landscapes are characterized by large open areas and areas of vegetation in patches. In these areas the plants are very important as they modify the conditions of the soil in the root zone and under the canopy. In the roots of plants a lot of free-living amoebae inhabit which have a key role in controlling the cycle of soil nutrients, so understanding the spatial dynamics of this important component of the arid zones is essential. The aim of this study was to determine the spatial distribution of the community of free-living amoebae in response to physical and chemical factors in a gradient of 0-30 cm depth of soil under the canopy of *Prosopis laevigata* and *Parkinsonia praecox* in an alluvial terrace degraded in Zapotitlan Salinas, Puebla during a rainy season and drought. In total 11 families, 17 genera and 27 species of free-living amoebae were found, and it was observed that most of them have bacterivorous eating habits. The Vahlkampfiidae family showed the highest frequency and the highest species richness. Other genera present in soil samples were *Filamoeba, Mayorella, Nuclearia, Stachyamoeba, Vampyrella* and *Vanella*. *Prosopis laevigata* and *Parkinsonia praecox* during drought have the highest species richness of free-living amoebae while the interspace showed the lowest during the drought. The species with greater frequency in different microenvironments were grown and an amplification and sequencing of the SSU 18 S gene was performed, identifying them as *Acanthamoeba, Filamoeba* and *Nuclearia*. In relation to spatial analysis, during the season of drought effect of water holding capacity, organic matter content and distribution arenas in the communities on the ground were found.

CHARACTERIZATION OF PROTIST COMMUNITIES FROM GRANITE WEATHERING PITS IN A SPANISH NATIONAL PARK

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We are developing a multidisciplinary project (Microepics) within a granite mountain National Park in Central Spain (La Pedriza, Parque Nacional Sierra de Guadarrama). La Pedriza has a unique feature of strongly eroded, and irregular granite rocks that create a distinct landscape with a huge variety of micro and mesoscale landforms that act as niches for microbial species. The complexity of granite formations makes accessing the many water reservoirs in the area tricky, thus much of La Pedriza is pristine for the study of microbial communities. The most iconic landforms of the area are granitic weathering pits; these are basins created by differential erosion on the surface of granitic outcrops which go through alternating states of desiccation and inundation from surface runoff by rains or snow melt. Therefore, these habitats dry up periodically from evapotranspiration for most of the year and are partially filled with water, snow or ice for the remainder, mainly in winter and autumn. The microbial communities within these granitic landforms are subjected to extreme changes in temperature and water content both daily and through the year.

The long term goal of this project is to explore protist communities, especially of ciliates, associated with these particular habitats and relate them to weathering conditions, environmental variables and human influence.

This study initially involved sampling dried sediments from granitic pits at 1,250 m altitude, re-hydratation of samples and their microscopic study during several weeks tracking the succession of the communities to assess cryptic diversity. Preliminary results indicate that communities show very different composition from one pit to another in type, number and abundance of species per g of dry weight. Some of the pits show rich testate amoeba and hypotrich ciliate populations while others do not held any at all. Diverse scuticociliate populations are very typical from most sediments. Some very small species of *Cyclidium* are the dominant ciliates. These complex environments offer a great opportunity to describe hitherto unknown ciliates resting cysts, and to comprehensively investigate the life cycle of rare and new ciliate species.

*Funded by Project Microepics- MINECO-CGL 2013-40851-P.

TWIN-ARGININE TRANSLOCASE IN THE MITOCHONDRIA OF THE EUKARYOTIC ORGANISM NAEGLERIA GRUBERI

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Formation of mitochondria by the conversion of the bacterial endosymbiont is one of the most fundamental moments in the evolution of eukaryotic organisms. While the organelle became genetically dependent on the nucleus of eukaryotic cell, new protein translocases were created in both mitochondrial membranes. This enabled the proteins synthesized on the cytosolic ribosomes to be efficiently transported into the organelle. Original complexes were lost or became a part of newly developed eukaryotic machineries. Therefore, only few similarities can be found among current bacteria and mitochondria. From the functional and the evolutionary perspective *Naegleria gruberi* (Excavata:Heterolobosea) represents unique model organism for the studies of the proteins transport, as its mitochondria contain bacterial as well eukaryotic transport systems. This project focuses on the twin-arginine translocase (TAT) widely present in bacteria and plant chloroplasts, whose part, homolog of TatC (NgTatC), was found in *N. gruberi* mitochondria. We endeavour to find the function of NgTatC in the *N. gruberi* mitochondria. In addition to the reconstitution of the mitochondrial TAT translocase in the bacterial membranes, we test the participation of NgTatC during the transport of naegleria Rieske protein, a known TAT substrate of the plastid translocase. We also focus on finding the interaction partners of NgTatC, as the main channel forming subunits TatA / TatB have not been found in naegleria.

EVALUATION OF THE SENSITIVITY TO ZINC OF THREE MOST COMMON BENTHIC CILIATES AND THEIR NATURALLY ASSOCIATED BACTERIA FROM A POLLUTED TROPICAL BAY

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Ciliates are an essential component of microbial food webs, connecting biomass production to higher trophic levels and providing substrates for bacterial growth. Bacteria are widespread in sea sediment and the first to metabolize organic matter and may form associations with ciliates. Heavy metals are toxic and are accumulated throughout trophic web, mainly in environments with high microbial activity. The aim of this study was to evaluate the Zn sensitivity of *Euplotes vannus, E. crassus* and *Diophrys appendiculata,* and their naturally associated bacteria sampled from sediments in the northwest and east regions of a polluted Guanabara Bay, Rio de Janeiro, Brazil. These ciliates and their associated bacteria were exposed to 0, 0.001, 0.009, 0.05, 0.1, 1.0 and 5.0 mg Zn L-1 in sterile 24-well polystyrene plates without supplementary nutrients along 0, 24, 48, 72, 96 h of assay. The unexposed ciliates and bacteria did not appear to be negatively affected during assay. In control group, *E. vannus* exhibited an increase in the biomass content from 2.3x102 to 2.3x103 µg C cm-3 between 0-96 h, *E. crassus* showed higher biomass with 7.07x102 µg C cm-3 at 48 h, and *D. appendiculata* showed biomass variation between 1.24x102-2.47x103 µg C.cm-3 (0-96 h). With Zn exposition, the maximum biomass was pointed by *E. vannus* 3.32x102 µg C cm-3 (0.009 mg Zn L-1 72 h), *E. crassus* with 1.33x103 µg C cm-3 (0.05 mg Zn L–1, 96 h), and *D. appendiculata* with 2.18x103 µg C.cm-3 (0.009 mg Zn L-1, 24-48 h). The bacterial biomass were higher after Zn exposure, mainly at 96 h with 2.40x10-1, 4.00x10-2 µg C cm-3 (1.0 mg Zn L–1), interacting with *E. vannus* and *E. crassus*, but with *D. appendiculata* the higher bacterial biomass content was at 48 h with 1.62x10-1 µg C.cm-3. The growth of *E. vannus*, *E. crassus* and *D. appendiculata* showed concentration dependent manners, but *E. vannus* is more sensitive to zinc. Naturally associated bacteria showed better adaptation to living in increasing concentrations of Zn, and the ANOVA and Dunnett test showed that previous environmental selection is important. These results show that new bioremediation tools are necessary.

WHEN A LAKE STOPS MIXING – THE FATAL EFFECTS OF WARMING ON THE PROTISTAN COMMUNITY

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Here we present the striking consequences of lake warming on the composition of algal and ciliate assemblages in Lake Zurich (136m deep), Switzerland. The exceptional warm winter in 2014 caused an incomplete water turnover, only reaching down to 60m. In consequence, there was no measurable mixis of phosphorus rich deep water with surface layers during winter. Due to this nutrient limitation, the annually reoccurring phytoplankton spring bloom (mainly cryptophytes and centric diatoms) did not develop at all. Notably, algal mass developments in spring are the major basis for the annual successions of various consumers within the entire food web in deep temperate lakes. Increased primary production usually induced a rise in various bacterial taxa and bacterivorous protistan predators, but also served as resource for algivorous ciliates and metazoans in Lake Zurich. The 'absence' of an algal bloom in 2014 seemed to propagate and even aggravate along all trophic levels, causing drastic quantitative decreases and changing evenness of consumer assemblages. Especially algivorous ciliates (Prostomatea and Spirotrichea) were negatively affected and appeared in quite low numbers. In general, the succession of taxonomic ciliate groups differed strongly from patterns observed during former spring bloom events. We highlight, that a further series of warm winters will indeed cause an additional oligotrophication of the lake and drastic changes in protistan communities.

MORPHOLOGY, PHISIOLOGY AND MOLECULAR PHYLOGENY OF TWO NEW HALOPHILIC HETEROLOBOSEAN AMOEBO-FLAGELLATES

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Halophilic protists inhabiting continental saline waters are very curious for protistologists. In this regard taxon Heterolobosea is especially remarkable because of presence of obligate halophilic species unable to grow at salinity level under 35‰, such as *Pharyngomonas kirbyi, Pleurostomum flabellatum, Tulamoeba peronaphora, Euplaesiobystra hypersalinica*. Different reports and our data show occurrence of heterolobosean amoebo-flagellates in saline water bodies of all continents except for Antarctic. In present work we describe features of lifecycle, morphology and SSU gene sequences in two new halophilic heterolobosean amoebo-flagellates. They were isolated from temporary saline ponds with mineralization more than 100‰ nearby the mouth of the saline river Chernavka flowing into Elton lake, the largest saline lake in Europe (Volgograd region, Russia). The first strain HML-9 is a halophilic amoebo-flagellate with three-stage lifecycle comprised of amoeboid, flagellated vegetative stages and resting cysts. Range of salinity supporting growth of the culture is 30-150‰. Optimal salinity is approximately 90-120‰. The flagellated cells are of spherical shape. They have two isocont and isomorphic flagella and poorly distinguishable slit-like cytostom. The amoeboid cells are heterolobosean with very short eruptive semispherical shaped pseudopodia. Sequence of SSU gene is the most similar to *Euplaesiobystra hypersalinica*, but the similarity degree 83% is rather low for identification. Another strain HML-11 represents halophilic heterolobosean amoebo-flagellate too. Lifecycle is similar to the strain HML-9. The flagellated cells looked like *Triflagellum diaphanum* Ruinen, 1938. They have two anterior and two posterior apical flagella. One of them usually passes through a ventral groove. So the flagellum is invisible under microscope and the flagellated cell appears to have only three flagella. The longitudinal ventral groove is asymmetrical and has a marked funnel-shaped cytostome passed into intracellular channel. Amoeboid cells are of lobosean and heterolobosean types. In spite of remarkable morphological differences between this strain and *Pharyngomonas* sp. strain RL we revealed high similarity of SSU genes at 98%. Possibly the strains represent new taxa, thus their morphology, physiology and some genetic features should be described in detail and their taxonomical position should be established.

The work was supported by RSF (project 14-14-00515) and RFBR (projects 13-04-00740, 14-04-97069, 15-29-02518, 15-29-02749).

CENTROHELID HELIOZOA FROM SALINE AND BRACKISH INLAND WATER BODIES OF RUSSIA

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The species composition and distribution of centrohelid heliozoa in saline and brackish inland water bodies of Russia with salinity 2.0-42.2‰ was studied with scanning electron microscopy. Centrohelids are known to be a common component of benthic and periphyton microbial communities in both freshwater and marine ecosystems. Currently the taxon Centrohelida counts approximately 100 species. Centrohelids are revealed in different biotopes, being a part of microbial food chains, providing the transformation of matter and energy in water ecosystems. In the communities they play a role of predators grazing on other microorganisms. At present data on species diversity of centrohelids in saline and brackish continental waters are presented in few reports. All these reports about fauna of Centrohelida in inland saline and brackish water bodies describe only six species *Heterophrys marina, Parasphaerastrum marina, Raineriophrys erinaceoides, Choanocystis kareliensis, C. aculeata, C. perpusilla.* Related to this circumstance, the aim of this research was to assess species composition and distribution of centrohelids in saline and brackish inland water bodies in Russia. Brackish water bodies in the South Urals and saline rivers in Elton region of subarid zone of Russia were studied. Thirteen centrohelid species *Heterophrys marina, Polyplacocystis coerulea, Polyplacocystis ambigua, Raineriophrys erinaceoides, Pterocystis foliacea, Heteroraphidioprys australis, Acanthocystis pectinata, A. turfacea, A. dentata, A. myriospina, A. astrakhanensis, A. taurica and Choanocystis ebelii* were revealed. Three species *Heteroraphidiophrys australis, Polyplacocystis coerulea* and *Choanocystis ebelii* were found for the first time in natural sites of Russia. Five species *Polyplacocystis ambigua, Pterocystis foliacea, A. pectinata, A. dentata u A. taurica* were revealed in saline and brackish water bodies for the first time and so were described as eurihaline. It seems *Heteroraphidioprys australis* is halophilic. Based on our data an upper limit of salinity making survival of centrohelids possible is not sea level (about 35‰) but 42.2‰.

The work was financed partially by Russian Science Foundation (project 14-14-00515), by Russian Foundation for Basic Research (projects 13-04-00740, 15-44-02467, 15-34-50255, 15-29-02749), and by grant of the Government of Orenburg region in 2015 (project 16).

ION CHANNELS IN DINOFLAGELLATES REVEALED BY PATCH-CLAMPING AND ANALYSIS OF TRANSCRIPTOMES

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Ion channels are transmembrane protein complexes that conduct ions down their electrochemical gradient across cell membranes. These proteins are tightly involved in cell signaling, thus playing a crucial role in various physiological processes in a cell. Electrophysiological and genomic studies revealed plethora of ion channels in animals, fungi and plants. Nevertheless, little is known about ion channels in dinoflagellates, an ecologically important group of marine microorganisms. Two main reasons underlie the lack of knowledge in this area: (1) native dinoflagellate cells are not accessible to patch-clamping due to complex cell covering, and (2) genomes of free-living dinoflagellates have not been sequenced. We developed an approach to obtain spheroplasts of the armored dinoflagellate *Prorocentrum minimum* that can be effectively used in patch-clamp studies. Our method is based on inhibition of cellulose synthesis by 2,6-dichlorobenzonitrile. Using this approach, we recorded the activity of high-conductive cation-channels in the membrane of *P. minimum*, i.e. voltage-gated potassium channels, inwardly rectifying potassium channels and non-selective cation channels. Concurrently we screened transcriptomes of *P. minimum* and nine other dinoflagellate species from "Marine Microbial Eukaryote Transcriptome Sequencing Project" for metazoan ion channel homologues in order to estimate potential diversity of ion channels in dinoflagellates. Transcriptome analysis revealed the presence of many ion channel families: (1) inwardly rectifying potassium channels, (2) voltage-gated potassium channels, (3) calcium-activated potassium channels, (4) cyclic nucleotide-gated channels (EAG and HCN/CNG), (5) TRPV and TRPP channels, (6) two-pore calcium channels TPC, (7) voltage-gated proton channels, (8) CLC channels, (9) Cys-loop receptors, and (10) four-domain voltage-gated channels. Moreover, Local BLAST revealed unusual sequences representing two-domain voltage-gated potassium channels and two-domain cyclic nucleotide-gated channels that had not been described before. Identification of the four-domain voltage-gated channels in the dinoflagellate transcriptomes is of special interest, because these channels are essential to cell excitability and functioning of nervous system in animals. Reconstruction of a maximal-likelihood tree showed that dinoflagellates possess a separate subfamily of the four-domain voltage-gated channels. We suggest that emergence of the four-domain voltage-gated channels occurred at the early stages of evolution of eukaryotes.

This work was supported by RFBR, grant № 13-04-00703-a.

A LINEAGE-DEFINING CYTOSKELETAL STRUCTURE OF PARABASALIAN PARASITES MAKES USE OF PROTEINS THAT RESEMBLE INTERMEDIATE FILAMENT PROTEINS

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The complexity of the eukaryotic cell is supported by an elaborate cytoskeleton. Actin and tubulin are rather conserved and easy to trace throughout eukaryotic evolution, but less so proteins of the intermediate filament (IF) protein family. Canonical IF protein families appear restricted to metazoa, although protists are well known for an extensive number of filamentous structures that support their complex cell architecture that neither involve actin nor tubulin. Trichomonadida are an evolutionary early branching group of excavate parasites that are united by a unique cytoskeletal framework that includes the costa, a stiff backbone that supports the flagellum of the undulating membrane. We determine the proteome of the detergent resistant cytoskeletal backbone of *Tetratrichomonas gallinarum* to investigate the properties of the proteins that built the costa, pelta-axostyle complex and associated structures. Among the 582 proteins identified in each of the three individual MS-MS runs, we found major components of the costa that we also verified through in vivo localisation. Intriguingly, many are united by recognisable features including long central coiled-coil regions, which is reminiscent of eumetazoan IF proteins. The newly identified cytoskeletal proteins cannot be attributed to any known proteins of eumetazoan IF families, albeit they resemble these in terms of predictable structure and apparently also function. It suggest that these protist IF proteins, which form cytoskeletal filaments that do not depend on actin and tubulin, evolved many times independently in eukaryotes.

SPECIFIC COMPOSITION OF THE GENUS PARAMECIUM

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Paramecium (Ciliophora, Oligohymenophorea) is a very suitable subject for the study of protist microevolution, with application of genetic, cytological, and molecular techniques. The genus is composed of several morphological species arranged into subgenera, species in turn consist of cryptic or sibling species, in some morphospecies called syngens (generating together). In fact, they are biological species as reproductively isolated groups. Specific composition of some species has been studied especially carefully, i.e., the *P. aurelia* species complex composed of 14 biological species designated by Sonneborn in 1975, and *P. sonneborni* described by Aufderheide et al. in 1983 and added to the complex as 15th member. Majority of species of the *P. aurelia* complex revealed intra-specific polymorphism (by recent analyses of mitochondrial, ribosomal, nuclear markers, and by previously applied PCR based techniques), interspecific relationships within the complex has also been studied. *P. jenningsi,* closely related species to the *P. aurelia* complex in the subgenus *Paramecium*, is composed of three species, *P. multimicronucleatum* of 3-5 syngens, *P. caudatum* structure is still discussed. *P. bursaria* (subgenus *Chloroparamecium*) is composed of 5 syngens as well as *P. putrinum* (subgenus *Helianter*) and *P. calkinsi* (subspecies *Cypriostomum*) seems composed of 3-5 syngens. Possible correlation of the rate of evolution within particular morphospecies and resulting species composition (number of syngens) with characteristic for them breeding system (inbreeding, outbreeding), and time of species divergence, will be discussed.

IRON SULPHUR CLUSTER ASSEMBLY SYSTEMS IN CRYPTOPHYTA, HAPTOPHYTA, STRAMENOPILA, ALVEOLATA AND RHIZARIA SHARE COMMON FEATURES

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Origin of the secondary plastid in eukaryotes is a highly attractive field of evolutionary biology. Based on Chromalveolate hypothesis secondary plastid was acquired from red algae by common ancestor of Cryptophyta, Haptophyta (CCTH clade), and Stramenopila, Alveolata, Rhizaria (SAR clade). While single origin of the plastid was confirmed using several phylogenetical studies, monophyly of the Chromalveolata is not clear so far. Recent studies suggested close proximity of Cryptophyta to Viridiplantae. Therefore Serial hypothesis arose, where the red plastid is laterally transferred across the lineages mentioned above.

Here we present some basic characteristics of essential Nbp35-like proteins (Nbp35, Cfd1, HCF101, and Ind) in CCTH and SAR. These proteins serve most likely as molecular scaffolds in Iron-sulfur cluster assembly machineries. In eukaryotes, Ind1 is in mitochondria and its presence is associated with building of FeS clusters in respiratory Complex I, Nbp35 and Cfd1 are components of the CIA pathway in cytoplasm, and HCF101 serves in the plastid of plants. We searched for genes of Nbp35-like protein family and predicted cellular localization of the proteins. Our predictions were confirmed in selected representants of Apicomplexa (*Toxoplasma gondii*), Stramenopiles (*Phaeodactylum tricornutum*), Cilliophora (*Tetrahymena thermophila*) by tagged protein expression.

In our investigations we found, that Ind1 gene from mitochondria was lost and replaced by HCF101 gene (named mitHCF101) in all members of SAR and CCTH. Ability of HCF101 to restore function of Complex I in Ind1 knockdown cells was demonstrated previously in yeast. Therefore it is likely that mitHCF101 plays analogical role as Ind1 in their mitochondria. Interestingly, apicomplexans that lost Complex I possess mitHCF101 in cytoplasm whereas Nbp35 is within the mitochondria.

Apart from mitHCF101, another HCF101 gene (chloHCF101) is present in chloroplasts of CCTH and SAR members. Based on phylogenetical analysis, the origin of chloHCF101 is derived from red algae, even that in Chlorarachniophyta members of Rhizaria which are harbouring green plastid. MitHCF101 is monophyletical, but the relationship to chloHCF101 and plants is yet unresolved.

Together all these data suggest that all clades of SAR and CCTH share the distribution and origin of two paralogues of HCF101 as well as absence of Ind1.

DIVERSITY OF CILIATE COMMUNITIES IN A HUMAN-IMPACTED RIVER AT A SPANISH NATIONAL PARK

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Spain is one of the countries with the highest biodiversity in the EU, however the vast majority of the biological inventories carried out in Spanish protected ecosystems have focused on macrobiota. There are no detailed inventories of microorganisms, except for some diatom (periphyton) lists. The present work is part of a 4-year research project, named Microepics, which aims at cataloguing the diversity of protists in areas inside a Spanish National Heritage Park (La Pedriza, Parque Nacional Sierra de Guadarrama) to investigate the disturbance of human and livestock pressure through these microorganisms. La Pedriza is a granite landform ecosystem which offers an ideal location to achieve these objectives because the official restrictions and protection of this natural area must coexist with recreational and economic interests. Moreover, the environment and human health are threatened by overpopulation of mountain goats (*Capra pyrenaica*) which adds complexity and interest to this ecosystem. The goats, whose populations have increased exponentially from 67 individuals introduced in 1990 to more than 3500 at the present time, are known as effective vectors of protist pathogens (e.g *Cryptosporidium* spp. and *Giardia* spp.) and a potential source of pathogenic bacteria (*Salmonella* sp., *Escherichia coli, Enterococcus faecalis,* etc.) via faeces contaminating the water bodies of the Park. As protists are considered one of the most important consumers of bacteria in aquatic and terrestrial ecosystems, we expect to identify bioindicator and bioremediator species to control faecal bacterial populations and protist pathogens.

In this scenario, this work presents preliminary results on the characterization of the ciliated protozoan communities found in three different sites along the Manzanares River as it flows through La Pedriza. The sampling sites were selected considering an increasing risk of human and livestock disturbance, which is expected to produce changes in the taxonomic and functional structure of the ciliate communities. Richness and diversity of species, as well as trophic group characterisation are discussed comparatively for the three sites, and in relation to total and faecal bacterial levels.

*Funded by Project Microepics- MINECO-CGL 2013-40851-P.

EXPLORING THE HEALTH RISK OF PATHOGENIC PROTISTS CONTAMINATION IN RECREATIONAL WATERS OF A PROTECTED AREA.

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The Iberian ibex is a wild ungulate which was reintroduced in the early 90s to the Sierra de Guadarrama. Its population has grown from 67 to more than 3500 individuals reported in 2014. This represents a significant increase from 6.57 ind./km2 to 44.82 ind./km2 in the last field season with the corresponding increase in the risk of the species as a potential source of pathogens (parasites, bacteria) via faeces contaminating the water bodies of the recently declared National Park (Parque Nacional Sierra de Guadarrama). In this park, the Iberian ibex is commonly found in steep rocky areas with granitic weathering pits, where is easy to spot goat droppings. Parasites infecting the National Park goat population include Coccidia (*Eimeria* and *Cryptosporidium*), amoeba (*Entamoeba* spp.) and flagellate (*Giardia* sp.), which are potential agents of zoonotic diseases. Their presence serves as a useful tool for evaluating water quality and determining sanitary risk.

Within the framework of a multidisciplinary 4-year research project carried out inside the National Park, we have initially collected faecal samples from granitic pits at 1.250 m altitude and water samples at three different sites along the Manzanares River coinciding with the areas sampled to characterize ciliate communities. Collected faecal samples were fixed in alcohol, concentrated according to the Ritchie's method and analyzed by optical and fluorescence microscopy. Water samples were concentrated using the technique of inorganic flocculation, followed by fluorogenic viability dying (DAPI and PI) and in vitro direct immuno fluorescent procedure for the simultaneous detection of *Cryptosporidium* oocysts and *Giardia* cysts in fecal material (MERIFLUOR C/G). Our long-term aim is to catalogue the diversity of pathogenic protists in order to further establish potential trophic relationships between ciliates and pathogens.

*Funded by Project Microepics- MINECO-CGL 2013-40851-P.

TARGETING OF C-TAIL ANCHORED PROTEINS INTO HYDROGENOSOMES AND ENDOPLASMIC RETICULUM OF TRICHOMONAS VAGINALIS

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The α-helical C-tail anchored proteins represent a heterogenous group of membrane proteins with a large functional N-terminal domain exposed to the cytosol and a short membrane insertion at their C-terminus. They are components of the outer membrane of organelle such as mitochondria and their relatives, namely hydrogenosomes and mitosomes, as well as they serve as membrane proteins of the endoplasmatic reticulum (ER) or plasma membrane. In general, targeting signals consist of short transmembrane domain (TMD) and positively charged flanking regions. Targeting signals that discriminate the insertion of C-tail anchored proteins between organelles and ER are poorly understood. Proteomic analysis of *Trichomonas* hydrogenosomes revealed the presence of twelve C-tail anchored proteins. TMDs of these proteins are 19-23 amino acid residues in length, somewhat longer than their mitochondrial counterparts. This feature may reflect the thickness and variable composition of the outer membrane present in hydrogenosome and mitochondria. The C-terminal segment that follows TMD is 12-16 amino acid residues in length and contains 2-7 positively charged residues. First, we confirmed the topology of six representative C-tail-anchored proteins in the outer hydrogenosomal membrane by protein protection assay. Further we investigated character of targeting signals, which are responsible for delivery of C-tail anchored proteins into the hydrogenosome or ER. First we replaced C-terminal domain of protein disulfide isomerase (PDI), which is present in outer membrane of ER, with TMD and charged C-terminus of the hydrogenosomal protein TVAG_272350. Expression of this chimeric protein in trichomonads resulted in its delivery to the hydrogenosomal membrane. When PDI was expressed only with C-terminus from the hydrogenosomal protein with native TMD, the chimeric protein was targeted into ER. These data suggest that structure of TMD is critical for specific delivery to hydrogenosomes and ER. To test whether targeting mechanisms is conserved also in highly reduced form of mitochondria, mitosomes, we expressed two selected hydrogenosomal C-tail anchored protein in *Giardia intestinalis.* Both hydrogenosomal proteins were targeted to mitosomes. which indicate that the targeting mechanisms is conserved in this highly reduced form of mitochondria.

THE IMPORTANCE OF HERBIVORY BY PROTISTS IN A NEOTROPICAL FLOODPLAIN SYSTEM

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In tropical environments, there is a predominance of small organisms due to the high temperatures all year round. The prevalence of primary producers of smaller size, such as picophytoplankton and nanophytoplankton, opens the way for the insertion of intermediate trophic levels and suggests a change in the configuration of the food web. This fact raises questions about the preference of food resources by the protists (bacterivory versus herbivory), which can determine the path of the flow of matter and energy within aquatic food webs in tropical environments. Thus, the aim of this study was to evaluate if the carbon flow to the protozoa comes mainly from small primary producers or heterotrophic bacteria in various tropical lakes, assuming the hypothesis that the autotrophic fraction will be more important as a food source in the surface the environments while the heterotrophic fraction will be more consumed in the lower stratum of the water column. We sampled the planktonic organisms in the surface and bottom of 24 lakes in a Neotropical floodplain. We performed a Path Analysis in order to test the models that best describe the relationships between the biomass of food resources (heterotrophic bacteria and various size fractions of phytoplankton), and protists (heterotrophic flagellates and ciliates) in different layers of the column water (surface and bottom). Our results revealed that the contribution of food resources varied between different size classes of protists with a predominance of herbivory regardless of the layer of the water column. These results confirm a recent school of thought that in tropical lakes phytoplankton, especially picophytoplankton, which is abundant throughout the year, provides most of the organic carbon to protists. Herbivory by protists, instead of bacterivory, results in a direct link between the primary producers and protists, increasing the carbon transfer efficiency through the microbial food web and enabling the rapid cycling of nutrients.

KINETICS OF CIRCULATING ANTIBODY RESPONSE TO TRICHOMONAS VAGINALIS: CLINICAL AND DIAGNOSTIC IMPLICATIONS

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Trichomonas vaginalis is a neglected pathogen, despite an ever-growing body of evidence on its impact over public health. The flagellate is the causative agent of the most common non-viral sexually transmitted disease in humans. More than 75 percent of trichomoniasis in men and 50 percent in women are asymptomatic, generating a sub-clinical infection accompanied by chronic local inflammation, that can last for years.

Routine diagnosis of trichomoniasis is based on direct microscopic examination of wet mount preparations and/or on culture-based systems, but both are limited by a low sensitivity; molecular techniques has been proposed in recent years, but they did not enter in the routine trichomonad diagnosis so far.

Indirect immunological techniques represent an effective alternative to direct microbial identification. The clinical importance of serologic confirmation of transmissible diseases is widely accepted, especially during chronic infections; in these cases the parasite load can be below the detection limits of common direct identification methods. In addition, detection of circulating specific immunoglobulins is fundamental for seroepidemiological studies.

Persistence of antibody against pathogens after anti-microbial treatment is a marker of therapy failure or of evolution to chronic infection. Unfortunately, decrease of antibody production following antigen elimination greatly vary among pathogens, and prediction of duration of soluble immunity is often difficult. In order to study the kinetics of IgG class circulating antibodies decrease after the pharmacological treatment of trichomonad infection, we tested a group of patients affected by trichomoniasis before and after treatment with metronidazole, until the complete disappearance of specific antibodies response in sera.

We demonstrated that disappearance of antibody occurs in 1-3 month following infection eradication, while persistence of anti-*Trichomonas vaginalis* antibodies indicates chronic infection, re-infection or treatment failure. Our results can facilitate the correct interpretation of serological data in the diagnosis of sub-clinical trichomoniasis and in the patients' follow up.

RESPONSE OF SOIL MICRO-EUKARYOTES TO CADAVER DECOMPOSITION AS ASSESSED BY HIGH THROUGHPUT SEQUENCING

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Cadaver decomposition is a natural perturbation affecting ecosystems and soil biodiversity. Decomposing cadavers release high amounts of nutrients over a short period of time, which affects the physico-chemical characteristics of the soil, but the extent of these changes remains poorly studied and especially the response of soil micro-eukaryotes including protists is almost unknown. We conducted a field experiment to assess how cadaver decomposition changes the diversity and community composition of soil micro-eukaryotes. We hypothesized that (1) cadavers would cause changes in soil micro-eukaryotic community diversity and structure, (2) that these changes would vary over time, and that (3) both "cadaver lovers" and "cadaver haters" could be identified, responding respectively positively or negatively to cadavers. This research has potential application for the development of new forensic indicators to estimate the Post-Mortem Interval (PMI).

Our field experiment was conducted in an Oak forest and included three treatments: control, fake cadaver (plastic bags filled with soil and covered with a cotton cloth to investigate microclimatic effects without cadaveric fluids, and pig cadavers. To assess the response of soil micro-eukaryotes we extracted total DNA from soils samples and we applied a specific PCR protocol to amplify the hyper-variable V9 region of the small subunit rDNA gene. We sequenced the amplicon by Ultra High sequencing Illumina HiSeq. To select the potential bioindicators we used the Dufrêne-Legendre indicator species analysis (IndVal).

The results show that the pig cadavers significantly changed the micro-eukaryotic community. The IndVal analysis revealed 1147 significant indicator OTUs ($p<0.01$), 225 of which were cadaver "lovers" and 922 were cadaver "haters". Cadaver decomposition caused 1) an overall decrease in soil eukaryotic diversity, 2) clear temporal changes in the community structure and appearance of potential indicator taxa (e.g. *Fonticula, Capsellina*). Significant differences in the structure of soil micro-eukaryotic communities were still visible 3 years after the onset of the experiment, long after the disappearance of any traces of the cadavers except for a few scattered bones. This study illustrates the potential of high throughput sequencing of micro-eukaryotes to develop new tools for PMI estimation and understanding how perturbation influences soil micro-eukaryotic diversity through space and time.

ASSESSING THE RESPONSES OF PEATLAND MICRO-EUKARYOTES TO CLIMATE CHANGE USING NEXT GENERATION SEQUENCING

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Climate change affects ecosystems both in their structure (e.g. communities) and functions (e.g. carbon dynamics). Changes in communities of soil microbes, including eukaryotes may alter soil carbon dynamics, but assessing such an effect requires new approaches such as high throughput sequencing in combination with manipulative experiments. Peatlands store huge amounts of carbon, yet little is known about the diversity of peatland microorganisms and their response to warming and this is especially true for eukaryotic microbes (fungi, protists and micro-metazoa) that play a major role in organic matter decomposition as microbial grazers and osmotrophs.

Our goal was to assess the diversity of Sphagnum peatland eukaryotic microorganisms and their response to environmental change (warming and water table change) using high throughput sequencing of the V9 region of the SSU rDNA gene (Illumina sequencing). We collected Sphagnum samples in an on-going field experiment (www.climpeat.pl) in Poland in which 1) water table and 2) temperature are manipulated by 1) raising and lowering the peat surface and 2) warming the surface passively using open top chambers.

We hypothesized that: (1) the diversity and community structure of micro-eukaryotes would be correlated to ecosystem functioning (e.g. litter decomposition); (2) warming without drought would increase the diversity of primary producers (i.e. different groups of "micro-algae"); (3) drought would decrease the diversity of primary producers, increase the diversity and relative proportion of fungi, and increase the total diversity of micro-eukaryotes.

First data show that patterns of micro-eukaryotic diversity and community structure varied in response to micro-environmental manipulations; drought caused an increase in the number and diversity of micro-algae due to the arrival of predominantly terrestrial taxa (e.g. Trebouxiophyceae) and in the diversity and relative proportion of osmotrophs, including Fungi and Oomycota.

THE KINOME OF THE GIANT CILIATE STENTOR: OVER 2000 KINASES AND NOVEL DOMAIN ARCHITECTURES

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The giant ciliate *Stentor coeruleus* has the ability to fully regenerate after being cut in half, in a way that perfectly preserves cell polarity and structure. This regenerative ability has made it a classical model system for studying regeneration at the cellular level. So far, however, the molecular details behind this incredible phenomenon have remained largely unstudied. Recently, our laboratory has developed a system for RNAi knockdown of *Stentor* genes, and additionally sequenced the *Stentor coeruleus* genome. Interestingly, not only do *Stentor*'s introns appear to possess the smallest average intron length of any organism described to date at 15 bp, but *Stentor* also seems to use the standard genetic code, unlike other ciliates. We wish to understand how the regeneration process is coordinated at the molecular level, so to identify candidates for RNAi knockdown we analyzed the kinome of *Stentor* by looking for protein kinase domains among the predicted protein coding genes. *Stentor* was found to encode more than 2000 kinases, making up 6% of the total protein coding genes. Many of these consist of expansions in mitotic kinase families such as PLKs and NDRs. There are also expansions of families absent in animals and yeast; over 12% of the kinome consists of the calcium-dependent CDPK family, originally identified in plants. We also analyzed additional protein domains found on kinase genes in *Stentor*, revealing a few novel domain architectures. The most notable example is an adenylate kinase fused to a calcium-dependent protein kinase, with a large region in between containing a AAA+ ATPase and other protein domains. RNAi screening of kinase genes is ongoing, and will ultimately reveal which of these kinases help to coordinate the many different precisely timed cellular events required for successful regeneration. In the future, a better understanding of the mechanisms behind single cell regeneration will have important implications for basic biology as a whole, and will reveal how these single cells can establish and maintain their polarity and cortical organization with such a high degree of precision.

ACCUMULATION OF SOME HEAVY METALS IN HYSTEROTHYLACIUM ADUNCUM (NEMATODA, ANISAKIDAE) INFECTING THE COMMON SOLE SOLEA SOLEA (SOLEIDAE) AND ITS ROLE AS A BIOLOGICAL INDICATOR OF POLLUTION FROM MEDITERRANEAN SEA, EGYPT

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Hysterothylacium aduncum is a nematode parasite isolated from the intestine of the common sole *Solea solea* (Soleidae) collected from coasts along Alexandria City at the Mediterranean Sea in Egypt. Light and scanning electron microscopy revealed that this nematode parasite belongs to the family Anisakidae in the genus *Hysterothylacium*. The type species is named *H. aduncum,* based on the presence of three interlocked lips with the interlabium in between, the presence of cephalic papillae, and large numbers of caudal papillae in males. The morphological characteristics of this species was confirmed by molecular analysis of 18S rDNA for these parasites followed by comparison between sequence data for them with those obtained from the Genbank showing that *H. aduncum* is deeply embedded in the genus *Hysterothylacium* with a sequence similarity between 95.5–94.3% with close relationships to other *H. aduncum* specimens and *Hysterothylacium* sp. Concentrations of heavy metals (Zn, Cu, Mn, Cd, Ni, and Pb) accumulated in this parasite species were higher than those in the tissues of host fish with the exception for Zn was found in higher quantity in fish kidney than in the parasite tissues. The objective of this study supported the hypothesis that fish parasites can be regarded as a useful bio-indicator when evaluating the environmental pollution of aquatic ecosystems by heavy metals.

IS THE ALTITUDE IMPORTANT FOR CILIATES FROM TANK BROMELIADS IN MEXICO?

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The formal studies about the ciliate diversity from tank bromeliads in the Neotropics have no more than 15 years and have been developed mainly under a taxonomical perspective but ecological aspects and distributional patterns have not been studied. In Mexico, the only study recorded the presence of 61 species inhabiting in two bromeliad species of the genus *Tillandsia*. The objective of the present work was to record the presence and distribution of *Bromeliophrya brasiliensis, Chilodonella uncinata, Glaucomides bromelicola* and *Leptopharyns bromelicola* in four terrestrial and eight epiphytic bromeliad species with tank morphology of the genus *Aechmea*, *Bromelia* and *Tillandsia* along an altitudinal gradient from de 0 m to the 2210 m in six localities, four of them were natural ecosystems and two were agroecosystems with temperate and tropical flora affinity in Veracruz, Mexico.

We collected 69 samples during the dry season between 2014 and 2015. For each plant we measured the water temperature inside the tank, and for the epiphytic plants the distance above the ground. Ciliate species were identified by using bright field and DIC microscopy for observation in vivo. For taxonomical identification, we also applied silver staining methods. The water inside the tanks presented an average temperature of 18°C and the epiphytic plants were in average at 3 m above the ground. We found that in *Bromelia pinguin*, at 0 m, there was not any of the four species, otherwise other ciliate species inhabited there. From the 400 m to 1334 m, we registered the presence of the four species in *Aechmea* spp. and *Tillandsia* spp. and over the 2000 m we only recorded *Glaucomides bromelicola* inhabiting *Tillandsia gymnobotrya* and *T. macrochlamys*. We conclude that altitude and water temperature from the tank are two major factors that may influence the composition of the bromeliad ciliate community from the Neotropics.

MAPPING THE DIVERSITY OF METOPIDA AND REVEALING NEW MARINE ANAEROBIC CILIATES HOSTING PROKARYOTIC SYMBIONTS

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Remarkably many ciliates live in marine or freshwater anoxic sediments. The ecological importance of ciliates is indisputable, yet understanding of the diversity and their role in anoxic sediments is still very limited. Anaerobiosis has independently arisen in several lineages of ciliates; so far, anaerobes have been found in at least eight ciliate classes. To deepen our knowledge about their diversity, we have cultivated over 100 strains from fresh water, brackish, and marine anoxic sediments worldwide. We determined their SSU rDNA sequences, performed protargol staining techniques, and studied light-microscopic morphology. We used transmission electron microscopy to assess the ultrastructure of some of the strains. In addition, we observed their endosymbiotic methanogens, whose presence, persisting in our cultures for years, has been confirmed by fluorescence microscopy. We have identified over 30 species of Metopida, the free-living anaerobic ciliates of the class Armophorea, including several putative novel species. Importantly, we have discovered a new deep lineage of marine anaerobic ciliates (MURANES). These ciliates undergo a complex life cycle, host prokaryotic endo and ectosymbionts and their hydrogenosomes do not possess cristae. According to the SSU rDNA analysis, they are related to SAL group (Spirotrichea, Armophorea, Litostomatea) and Cariacotrichea, but form a separate lineage, possibly a novel class. Furthermore, we present a new deep lineage of marine anaerobic eukaryovore ciliates, which clusters within Prostomatea, but is not specifically related to the anaerobic Plagiopylidae.

MOLECULAR SYSTEMATICS OF MARINE GREGARINE APICOMPLEXANS FROM PACIFIC TUNICATES: LINKING SURFACE ULTRASTRUCTURE AND MOLECULAR PHYLOGENY

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Eugregarines are apicomplexan parasites that mostly infect the intestines of invertebrates. The high level of morphological variation found within and among species of eugregarines makes it difficult to find consistent and reliable traits that unite even closely related lineages. Based mostly on traits observed with light microscopy, the majority of described eugregarines from marine invertebrates has been classified into a single group, the Lecudinidae. Our understanding of the overall diversity and phylogenetic relationships of lecudinids is very poor, mainly because only a modest amount of exploratory research has been done on the group and very few species of lecudinids have been characterized at the molecular phylogenetic level. In an attempt to better understand the diversity of marine gregarines, we surveyed lecudinids that infect the intestines of Pacific ascidians (i.e., sea squirts) using ultrastructural and molecular phylogenetic approaches; currently, these species fall within one genus, *Lankesteria*. We collected lecudinid gregarines from six ascidian host species, and our data demonstrated that each host was infected by a different species of *Lankesteria*. Visualization of the trophozoites with SEM showed that four of these species were covered with epicytic folds, whereas two of the species were covered with a dense pattern of epicytic knobs. The molecular phylogenetic data suggested that *Lankesteria* species with surface knobs form a clade that is nested within a paraphyletic assemblage of *Lankesteria* species with epicytic folds.

DEVELOPMENT OF A DRUG SELECTION SYSTEM FOR TRANSFECTION OF THE OYSTER PARASITE PERKINSUS MARINUS (ALVEOLATA)

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Plastids in apicomplexan parasites are highly degenerated. The organelles are nevertheless essential for completion of the parasite life cycle. Interestingly, an oyster parasite *Perkinsus marinus*, which has branched at the base of the Dinoflagellata and adapted to parasitism independently of Apicomplexa, also has a DNA-lacking, extremely degenerated plastid. Function analysis of the cryptic organelle is attracting and required to understand the relationship between the organelle degeneration and parasitism. The transgenic technique is a convincing approach for the analyses of proteins of interest and is practicable in *P. marinus*. However, each transfected cell must be isolated from untransfected cells by hand labor using a micromanipulator multiple times to obtain any transfected cell lines. This is because drug selection system has not been established, and here we show that bleomycin is available for selection of transfected *P. marinus* cells. Firstly, we screened antibiotics shown utility in apicomplexan parasites and determined that bleomycin is the strongest inhibitor (IC50 = 1.64 μM). Contrary to expectation, dihydrofolate reductase inhibitors WR99210 and pyrimethamine, which are generally used in *Plasmodium* spp. and *Toxoplasma gondii*, did not inhibit *P. marinus* growth efficiently. Then, a bleomycin resistance gene Sh-ble was fused downstream of gfp or mCherry genes, and transfected to the parasite. After two-month culture with bleomycin, GFP or mCherry signals were observed in cytoplasmic region of almost all parasite cells. To apply the system to organelle proteins, we inserted a self-cleaving 2A peptide sequence between gfp and Sh-ble gene, enabling the mature Sh-ble to be localized in cytoplasm regardless of the localization of proteins fused with GFP. Genes for alternative oxidase and 1-deoxy-D-xylulose 5-phosphate reductoisomerase, which have targeting sequence to mitochondria and plastids, respectively, were fused upstream of gfp, and transfected to the parasite; GFP-positive cells were successfully selected by bleomycin and the fusion proteins were localized on mitochondria and plastids, respectively. These results show that our drug selection system is available for not only cytoplasmic but also organellar proteins and provides new opportunities for functional analyses of the organelles in the parasite.

A DRAFT GENOME OF THE ANAEROBIC FLAGELLATE CARPEDIEMONAS MEMBRANIFERA, A FREE-LIVING RELATIVE OF METAMONAD PARASITES

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Carpediemonas membranifera is a free living flagellated metamonad related to well-known diplomonad parasites such as *Giardia intestinalis*. We sequenced the genome of *C. membranifera* to elucidate the evolutionary transitions to anaerobiosis and parasitism within metamonada. The draft genome sequence was prepared with genomic (Illumina and 454 paired end reads and 454 single end reads) and transcriptomic (RNA-seq paired end) information. Our assembly is 22.4 Mb long (54.1 GC%, N50: 19.3 kb) with 11328 predicted protein-coding genes. Forty one percent of predicted genes have introns, with 55% containing only a single intron. We will study in depth the frequency, size and characteristics of all introns. For all predicted genes, structural elements (signal peptides and transmembrane domains) were used as queries for searches against the KOG53, Interpro, PFAM, Prosite, TIGR, and KEGG54 databases to identify domains, assign putative functions and predict metabolic pathways. We are currently studying the structure and annotate candidate mitochondrion-related organelle proteins, cell surface or secreted proteins involved in host tissue adhesion, immune evasion, pathogenicity, nutrient acquisition, metabolite transport and environmental sensing, DNA repair proteins and mRNA degradation, among others. All genes involved in pathways of interest will be manually curated. Predictions will be displayed in the web-accessible GenomeView editor. Additionally, we will study the role of gene transfer and duplication in the emergence of anaerobiosis and parasitism within Metamonada.

LAND-USE AND CLIMATE FACTORS DRIVE SOIL CILIATE DIVERSITY

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Land-use intensification is one of the most eroding processes for biodiversity with likely feedbacks on ecosystem functioning. However, most studies focus on the spatial variation in the diversity and composition of soil bacterial communities, ignoring the soil eukaryotic microbes and thus, making it difficult to assess overall land-use effects. Protists, as the main components of micro-eukaryotes, are major players in providing ecosystem services, being responsible for approximately 70% of soil animal respiration. Ciliates colonize and inhabit virtually all environments and thus, are one of the most successful groups of protists on Earth and are a key functional group within the soil microbial loop. Changes in soil ciliate diversity have been suggested as bioindicator of environmental stress. Furthermore, ciliates are among the most studied group of soil protists, due to their high copy numbers of short SSU sequences that ease amplification, making the existent database a robust starting point for soil protist diversity studies. Despite this, little is known about overall diversity of soil protists, including ciliates, and effects of land-use and climate factors on the soil ciliate diversity.

In this study we investigate soil ciliate diversity in soils sampled across European geographical locations and climatic zones, covering different vegetation types and land-uses. An 18S amplicon-based approach was applied to study soil ciliates, demonstrating that their diversity differs significantly between geographical locations. Likewise, vegetation types and land-use also influenced the overall ciliate community. Hence, agricultural management can have large effects on soil ciliate communities, with soil water availability as an important climate determinant for the distribution of soil ciliates. Our data suggest that soil ciliate communities exhibited compositional shifts that followed changes in land-use and soil management. This suggests the great importance of ciliates in soil food-webs and their interaction with plants and soil microbes. These findings contribute to future research for bio-indicators of soil quality and sustainable land-use.

SEARCHING FOR GENES INVOLVED IN MATING TYPE DETERMINATION IN SELECTED SPECIES OF PARAMECIUM AURELIA COMPLEX

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In each species of the *Paramecium aurelia* complex, two mating types are distinguished: O (Odd) and E (Even). The O and E types are homologous in all species, but they are determined by one of three different systems of inheritance: Mendelian, random (caryonidal) or maternal (synclonal) (Sonneborn 1975). Studies concerning mating type determination in *P. tetraurelia* showed a requirement for three genes for expression of mating type E (mtA, mtB, and mtC) (Singh et al. 2014). The mtA gene encodes an E-specific transmembrane protein involved in agglutination with O cells, while mtB and mtC genes encode transcription factors required for mtA expression. In *P. tetraurelia*, mating type O is determined during macronuclear development by excision of the mtA promoter as an IES; the rearrangement is regulated by the scnRNA pathway, explaining the maternal inheritance of mating types (Singh et al. 2014).

We have started a survey of selected species from each of the 3 systems to test whether mating type E is characterized by expression of mtA orthologs in all species, and to determine whether this always requires mtB and mtC orthologs. The final aim is to identify the mating-type determination mechanism in each species and to determine how the evolution of mating type systems proceeded.

The results obtained so far are consistent with the idea that mtA expression is always associated with mating type E, but the mechanisms of mating-type determination appear to vary widely among *P. aurelia* species

Work was supported partially by grant from the National Science Center No. 2013/09/N/NZ8/03198 and COST action BM1102.

DIVERSITY OF THE DIATOM GENUS FRUSTULIA IN NORTHERN EUROPE

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Diatoms are one of the most diversified groups of primary producers among protists. These microorganisms typically possess silica frustule and as such are hugely involved in carbon and silicon circulation in nature. Diatoms are traditionally used as an ecological indicator of water pollution. All in all correct species identification of diatoms is necessary not only for biomonitoring, but also for deeper understanding its biogeography and ecology.

Taxonomy of diatoms is traditionally based on the morphology of silica frustules. Nevertheless recent studies revealed, that this approach significantly underestimates real species diversity. Purpose of this study was to test the utility of fine-grained morphological species concept in diatoms with the help of molecular markers.

This study used molecular-assisted alpha taxonomy approach for identification of *Frustulia* species. There were used molecular markers LSU D1-D2 and rbcL-3P and morphometric methods. Freshwater samples were taken from twenty-nine oligotrophic localities in northern Europe. In total, was sequenced over four hundred strains and examined over one thousand pictures of *Frustulia* frustules representing morphological variability of both strains and natural populations. Moreover extra part of our dataset was morphologically determined by seven specialists.

Based on the molecular results three lineages were unambiguously determined to species (*F. gaertnerae, F. krammerii, F. septentrionalis*), one lineage resembles species reported only from the southern hemisphere (*F. maoriana*), and a complex of two lineages with overlapping morphology has uncertain phylogenetic position (*F. crassinervia-saxonica*). Morphometric results comparing morphology of frustules from diatom keys with the strains established with molecular markers were mostly in agreement. However, independent identification of strains and natural populations by specialists has shown many mismatches.

Determination problems comes from predominance of *Frustulia crassinervia-saxonica* complex in our samples, which has very variable morphological features. However, if you are aware of this, other species can be distinguished well, as shown by our morphometric analyses. With geometric and traditional morphometrics, we chose characters that allow identification of *Frustulia* species apart from *F. crassinervia-saxonica* complex. An approach similar to ours will help improve the ecological studies and stabilize the taxonomy of diatoms.

METABARCODING OF SOIL EUKARYOTES – MULTIPLE APPLICATIONS FOR BIODIVERSITY ASSESSMENT TO APPLIED ECOLOGICAL RESEARCH

Christophe Seppey (Université de Neuchâtel), Enrique Lara (Université de Neuchâtel), David Singer (Université de Neuchâtel), Ildikò Szelecz (Université de Neuchâtel), Bertrand Fournier (Université de Montpellier), Emanuela Samaritani (Université de Neuchâtel), Edward Mitchell (Université de Neuchâtel).

High-throughput sequencing metabarcoding (HTS-M) is a powerful approach to study the biodiversity of environmental samples. This approach now makes it possible to analyze the nearly entire taxonomic composition by retrieving up to millions of sequences, overcoming also cultural biases and morphological misidentifications.

Our focus is on soil micro-eukaryotes using the V9 region of the 18S ribosomal RNA gene. This genetic marker is short yet taxonomically informative, common to all eukaryotes and thus well adapted to HTS-M.

We first grouped V9 sequences by their similarity into OTUs using clustering algorithms (Swarm, Dbc454) and assigned the OTUs to meaningful taxonomy using the PR2 database.

We will illustrate this approach with several on-going ecological studies conducted in peatlands, floodplains and forensic experiments and covering either all eukaryotic diversity or specific taxa (i.e. euglyphid testate amoebae).

BIODIVERSITY OF PROTISTS AND PROKARYOTES OF TWO PLAYA-LAKES FROM CENTRAL SPAIN

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Microbial mats developed in two shallow natural playa-lakes from Central Spain were studied from a geological and biological perspective. They differ from each other in some biogeochemistry aspects although some similarities can also be found. These ecosystems are unique since they showed distinctive environmental conditions: the "North lake" is highly alkaline (pH ranges from 8 to 12) and the "South lake" is saline and reach values up to 75 µS·cm-1. Besides, carbonates, silicates and halides of magnesium and sodium (with few sulphates) dominate the sediment composition of the "North lake" bed whilst sulphates and silicates with low percentage of halides and carbonates are characteristics of the "South lake". According to the Piper-Hill-Langelier water classification, the northern lake is categorized into SO42--(Cl-)-Mg2+-(Na+)-(Ca2+) water type while the southern lake is Cl--(SO42-)-(HCO3-)-Na+. Protist populations together with prokaryotes communities were identified to describe the linkage between microbial activities and abiotic factors. "In vivo" observations reveal that, commonly, protists are more abundant in the mat surface, whilst bacteria grow in complex biofilms and appear embedded within EPS. The protists community includes diatoms, flagellates, ciliates and amoeba groups although the biodiversity of these eukaryotic microorganisms is low (greater in the "North lake"). Diatoms are abundant in both lakes and this prevalence is shared with Euglenids in the "North lake". These eukaryotic microorganisms interact with abundant photosynthetic oxygenic cyanobacteria (*Oscillatoria* was representative), purple sulfur bacteria (i.e. *Chromatium, Thiocystis*) and filamentous bacteria accumulating sulfur granules within their cells (*Beggiatoa* among other genera). Composition of prokaryotes in samples was also assessed by FISH. Results confirmed and completed previous data showing that samples from both lakes were dominated by the same bacteria groups of Alphaproteobacteria and Gammaproteobacteria. To the present, Archaea were not found in the samples analyzed.

GENOME ANNOTATION OF ACRASIS KONA

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Acrasids are single-celled amoebae that can undergo aggregative multicellularity in response to adverse environmental conditions, similar to the well-studied dictyostelid social amoebas. However, acrasids are unrelated to dictyostelids (supergroup Amoebozoa), being instead the only multicellular lineage in the eukaryotic supergroup Excavata. This makes *Acrasis* an interesting model system to study parallel evolution of social behavior in microbes as well as to explore the diversity of eukaryotes in general. We have sequenced the genome and transcriptome of *Acrasis kona* and are currently preparing transcriptomes from the four main stages of its life cycle. In initial work, we assembled the complete *A. kona* mitochondrial genome (mtDNA) and find that it is missing 14 protein genes present in the mtDNA of its closest sequenced relative, *Naegleria gruberii*. We further identified 11 of these protein genes in *A. kona* nuclear DNA and find that they carry mitochondrial important signals (transit peptides, Fu et al. 2014). We are now using RNAseq data and the *N. gruberii* genome in an annotation pipeline to create a fully annotated *A. kona* nuclear assembly. The results will be used to investigate parallel evolution of simple multicellularity, early steps in the evolution of eukaryotes and to aid in resolution of the eukaryote tree of life by breaking up some of the longer deep branches.

TAXONOMY, MORPHOLOGY AND PHYLOGENY OF A NEW OLIGOTRICH CILIATE-STROMBIDIUM HONGKONGENSIS N. SP. (PROTOZOA: CILIOPHORA) FROM CLEAR WATER BAY, HONG KONG

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The morphology and infraciliature of one new marine oligotrich ciliate, *Strombidium hongkongensis* n. sp., isolated from a bloom of *Noctiluca scuntillans* near Port Shelter, Hong Kong, was studied from live and protargol-stained specimens and the sequence of the small subunit rDNA. *S. hongkongensis* is different from its congeners by the combination of the following characters: cell usually heart-shaped, slightly flattened dorsoventrally; cell size mostly 20-35 × 20-30 μm in vivo; deep and prominent buccal cavity extending obliquely to about 1/2 of cell length; prominent apical protrusion; the adoral zone of membranelles divided in to 17-19 anterior membranelles and 4 ventral membranelles; one ball - shaped macronucleus; the girdle kinety form a closed loop and locate on the posterior of the cell; ventral kinety absent. The sequence of the SSU rDNA of *S. hongkongensis* was approximately 2% different from *S. paracalkinsi* (GenBank Accession No. KJ737432), the closest species in the SSU rDNA sequence.

USE OF PROTOZOA FOR ASSESSING WATER QUALITY IN A MID-SUBTROPICAL URBAN WETLAND ECOSYSTEM, SOUTHERN CHINA

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Multivariate bioassessment based on community data has many advantages to assess environmental quality status in aquatic ecosystems. The protozoan microfauna and their use in evaluating water quality status was studied in a mid-subtropical urban (XiXi) wetland system southern China, during the period of June 2013-May 2014. Samples were collected every month at six sampling stations within different pollution/eutrophication levels. A total of 85 protozoan species were recorded, including 67 ciliates, 10 flagellates, 8 sarcodines. A clear variation on spatial scale in protozoan community structures were represented at the six stations. Multivariate approaches revealed that the variations in the community structure were significantly related to the changes of environmental variables, especially nutrients ammonia-N (NH4-N), nitrate-N (NO3-N) and total phosphorus (TP), alone or in combination with water temperature (T) and dissolve oxygen (DO) and Four dominant species (*Euglena acus, Cucurbitella mespiliformis, Codonella acutula* and *Hemiophrys punctata*) were significantly correlated with nutrients. Otherwise, the species richness, diversity and evenness indices represented significant correlations with the nutrients. The results demonstrated that the community-based bioassessment using protozoa may be used as a feasible protocol for determining the water quality status and human disturbance in mid-subtropical urban wetland system.

SECONDARY STRUCTURE OF ITS TRANSCRIPTS IN SPIROSTOMATID CILIATES (CILIOPHORA, HETETRICHEA): IMPLICATIONS FOR STRUCTURAL EVOLUTION AND PHYLOGENETIC RECONSTRUCTION

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Spirostomum ciliates are common fresh water species and widely used as model organisms in environmental studies as well as symbioses between ciliates and human pathogenic bacteria. Molecular phylogenetic analyses have barely been performed to understand the evolutionary relationships among spirostomatid ciliates. Phylogenetic relationships within this genus remain under debate, and molecular discrimination between morphospecies is often difficult because of the highly conservative characteristics of SSU rRNA gene. Sometimes, fast evolving internal transcribed spacer (ITS) regions can be effective tools for resolving phylogenetic relationships among species level. In this study, the nucleotide characteristics of ITS 1 and ITS 2 of 50 individuals of seven morphospecies of *Spirostomum* were analyzed. We also used the secondary structure information of ITS 2 novely to reveal the evolutionary relationships within this genus.

VITAL SPECIES OF FLAMELLA (AMOEBOZOA: VARIOSEA) ISOLATED FROM ANCIENT ARCTIC PERMAFROST SEDIMENTS

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Permafrost occupies about a quarter of the Earths land surface. It has been shown that microorganisms trapped in permafrost sediments in Arctic and Antarctic regions can survive during long period of time. Permafrost is not only a bacterial depository, it also contains viable archaea, cyanobacteria, green algae, yeasts, actino- and micromycetes, viable spore of mosses and seeds of higher plants. Viable protozoans – ciliates, flagellate and heliozoan were found in permafrost samples of different age and origin.

In the present study six vital strains of naked amoebae belonging to the genus *Flamella* (Amoebozoa, Variosea) were isolated from permafrost sediments sampled in Russian Arctic region. Two of them are from late Pleistocene permafrost in North-East of Siberia, and four - from Holocene and late Pleistocene in North-West of Siberia. Light- and electron-microscopic study and molecular phylogeny show that these isolates represent two new species. We named them *Flamella pleistocenica* n.sp. and *Flamella beringiania* n.sp. Both species are cyst-forming. This is a remarkable case of high resistance of protozoan cysts, allowing them to survive and recover amoebae population after a very long, geologically significant period of rest. This study directly shows for the first time that amoeba cysts can be conserved not only for years and decades but for many thousands years and then recover, contributing to the formation of active microbial community. Phylogenetic analysis shows that the genus *Flamella* is a robust and potentially species-rich group of Variosea.

Supported with RFBR grants 15-04-05267 (LS) and SPSU project 1.38.251.2014 (AS).

INVESTIGATING THE DIVERSITY AND EVOLUTION OF NEOPARAMOEBA SPECIES AND THEIR KINETOPLASTID ENDOSYMBIONTS

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Members of the genus *Neoparamoeba* (Amoebozoa) are well known as single-celled parasites of various fish and invertebrates in marine and estuary environments around the world. Because of their involvement in Amoebic Gill Disease (AGD) in various fishes, paramoebiasis in lobster and wasting disease in green sea urchins, they are of economic and ecological interest. These amoebae possess a distinctive perinuclear body historically known as the parasome. The parasome is in fact a eukaryotic endosymbiont belonging to Kinetoplastida that is closely related to *Icthyobodo*, a well-known ectoparasite of various fish species. Despite ongoing interest in the study of *Neoparamoeba*, the pathobiology of the organism and the nature of the endosymbiotic relationship between *Neoparamoeba* spp. and its *Perkinsiella*-like-organism (PLO) is poorly understood. The Archibald lab is using genomic, molecular and phylogenetic approaches in an attempt to better understand the evolution of *Neoparamoeba* spp. and its PLO endosymbiont as well as the nature of their association. Here I present molecular sequence data and light microscopy observations from novel isolates from Hawai`i, USA. The phylogenetic characterization of these new isolates, the evolutionary relationships between *Neoparamoeba* species and coevolution of the host and endosymbiont is discussed in light of comprehensive phylogenetic trees of 18S ribosomal DNA sequences.

MICROPOLAR - DIVERSITY AND DYNAMICS OF MICROBIAL EUKARYOTES IN THE ARCTIC

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The interaction between the biosphere, the atmosphere and the hydrosphere is mediated by microorganisms being the main drivers of biogeochemical cycles in the ocean and the main producers and consumers of inorganic nutrients, organic carbon and CO2. Microbial communities, including phytoplankton, heterotrophic protists, bacteria, archaea and virus are by far the most abundant, and taxonomic and genetically diverse group of organisms in marine pelagic ecosystems. Biological activity, biomass, production and remineralization in these systems are essentially microbial; and these microorganisms, at the base of the marine food web, also delimit the production at trophic levels of economic interest (crustaceans, fish, and mammals). Despite their abundance and likely importance in polar ecosystems, little is known about the composition of polar microbial communities through the year. In the MicroPolar project, we address the overarching questions "Who are they?", "What do they do? ", "How do they interact?" and "How do they respond to environmental change?".

We here present high throughput sequencing data of microbial eukaryotes from environmental samples taken during five sampling cruises in the Arctic.

Sampling cruises to North or West of Svalbard were carried out five times during 2014, in January, March, May, August and November. During each cruise, samples were collected at 3-6 stations, at 1 m, deep chlorophyll maximum, 500 m and 1000 m depths. Fifty L of seawater was size fractionated into the 200-50, 50-10, 10-3 and 3-0.45 micron size fractions. DNA was extracted and the following markers were amplified and sequenced with Illumina MiSeq: the V4 region of 18S rDNA, the D1-D2 loop of 28S rDNA, and the V9 region of plastid 16S rDNA.

To identify the protists, and determine the taxonomic composition, the sequence data will be compared to existing and in house reference databases (e.g. Protist Ribosomal Reference Database, PhytoRef). Distribution of the different taxa in time and space (geographically and by depth) will be investigated. The protist community composition and distribution will be compared to data from bacteria and virus samples, to assess possible interactions between these groups.

3-DIMENTIONAL ANALYSIS OF DINOFLAGELLATE NUCLEUS BY ELECTRON MICROSCOPY

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The dinoflagellate is a member of eukaryotic organism that belongs to a diverged protist group, Alveolata. Because the nucleus of dinoflagellates possesses several unusual features being not observed in other eukaryotes, the nucleus in the dinoflagellates is especially called "dinokaryon". In this study, we investigated the morphology and the localization of chromosomes in the dinokaryon of the early-branching dinoflagellate *Oxyrrhis marina*, using electron microscopic techniques. *O. marina* cells were cryofixed at G1 phase and freeze-substituted in acetone with OsO4 for electron microscopic observation. A whole dinokaryon was reconstructed three-dimensionally from 56 serial thin sections. The reconstructed 3D-model demonstrated that the volume of the dinokaryon is approximately 40 μm3 and contains 399 chromosomes and a single nucleolus. All of the chromosomes were highly condensed with diverse volumes (between 0.32 and 3.68 × 10-2 μm3). More than half of the chromosomes were attached to the inner surface of the nuclear membrane. Two long and narrow chromosomes were found to be encircling the nucleolus, from which nucleofilaments were branched and intruded into the nucleolus to form tunnel-like protrusions. The arrangement of the nucleofilaments in *O. marina* was compared with that in a typical dinoflagellate *Heterocapsa circularisquama* by electron tomographic analysis. The tomographic slices demonstrated that chromosomes of *O. marina* has a homogeneous appearance without any specific structure, while those of *H. circularisquama* has a distinct typical cholesteric liquid crystal appearance.

ANAEROBIC CIA PATHWAY IN TRICHOMONAS VAGINALIS

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The key pathways of iron-sulfur (FeS) cluster assembly are extensively investigated in model organisms such as *Saccharomyces cerevisiae*. However, their character is not fully elucidated in anaerobic protists such as a human pathogen *Trichomonas vaginalis*. Although both the hydrogenosomal and the Cytosolic Iron-sulfur Assembly (CIA) pathways in trichomonads are similar to the corresponding yeast machineries, they are different in two characters: (i) most of the key protein components in *T. vaginalis* are present in multiple copies, while (ii) some components conserved in most organisms are absent. The CIA components that are apparently not present in *T. vaginalis* include Tah18, Dre2, MMS19, and Grx3/4. Absence of Dre2 and Tah18 seems to be a general trend of anaerobic protists. However, we identified two paralogs for Cia1, Nbp35, and Cfd1 proteins, while Nar1 and Cia2 (Mip18) are present in a single copy. Cell localization studies of tagged CIA components confirmed cytosolic localization for all of them. Interestingly more detail investigation reveal also the association of Cfd1-A and Cia1-B with outer hydrogenosomal membrane. Moreover, we co-imunnoprecipitated several CIA components to investigate their partner proteins for better understanding of their cellular roles.

COMPARATIVE PROTEOMICS OF PERISYMBIONT AND DIGESTIVE VACUOLES IN PARAMECIUM BURSARIA

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The green ciliate *Paramecium bursaria* (supergroup: Alveolata) is a microorganism that can accommodate several hundred endosymbiotic *Chlorella* cells in its cytoplasm. The host and the symbiotic *Chlorella* can be cultured separately, and the endosymbiotic relationship can be re-established simply by co-culturing the two organisms; therefore, *P. bursaria* is a useful model of algal endosymbiosis. Symbiotic algal cells are enclosed by a single membrane (perisymbiont vacuole (PV) membrane) that may serve as a conduit to exchange various substances between the two partner organisms. The PV membrane is originated from the digestive vacuole, and may also be functioning as a barrier to prevent digestion of the symbionts in the hosts cytoplasm. Thus the presence of the PV membrane is regarded as a key element in their successful mutual endosymbiosis. Combining proteome and de novo transcriptome sequencing analyses is a powerful tool of protein identification in non-model organisms. Here, we employed this approach to identify proteins from the PV membranes isolated from monoxenically-cultured *P. bursaria* (strain Kb-1), and compared the protein constituents of the PV membrane with those of the digestive vacuole membrane.

INTESTINAL EPITHELIAL CELL-PARASITE CROSS-TALK DURING GIARDIASIS

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The non-invasive protozoan parasite *Giardia intestinalis* is a major cause of diarrhea worldwide. Humans are infected by *Giardia* parasites from two genotypes or assemblages (A and B) but it is unclear if parasites of different assemblages induce different symptoms. The mechanisms of disease remain poorly defined but no major intestinal tissue destruction and inflammation is induced. To better understand the crosstalk between *G. intestinalis* and human intestinal epithelial cells, and especially the initial response in human cells, we studied gene expression in human intestinal epithelial cells and the parasites during interaction in vitro.

RNA sequencing was performed upon in vitro interaction of human intestinal epithelial cells (Caco2) and parasites. We used the *G. intestinalis* isolate WB (assemblage A) as well as the assemblage B isolate GS in order to detect assemblage-specific differences. Results were complemented with specific experiments using proteomics, RT-PCR, Western Blots and ELISA to verify the results from RNA sequencing.

The two *Giardia* isolates lead to highly correlated response in human intestinal epithelial cells after 1.5 hours, dropping at later time points of 3 and 4.5 hours. Gene network analysis revealed that *Giardia*-infection leads to the immediate activation of chemokines (CCL2, CCL20, CXCL1, CXCL2, CXCL3) and cytokines (IL8) on the RNA level but the level of secreted cytokines is low. Further, regulatory proteins of apoptosis and proliferation as well as cell adhesion molecules were induced after 1.5 hours of host-parasite interaction. Most of the early induced genes were down-regulated on transcript-level before 3 hours. Data analysis suggested that this was due to RNA decay of AU-rich element-containing transcripts. In the parasites the expression of high-cysteine rich membrane proteins and genes related to protection against oxidative stress was induced. Several proteins are specifically secreted during interaction with the host cells.

Interactions between *Giardia* trophozoites and host intestinal epithelial cells induce specific gene expression changes in both cell types. These gene expression changes can partly explain the low levels of inflammation and the disease mechanism during giardiasis.

EVOLUTIONARY SIGNIFICANCE OF FREE-LIVING PREAXOSTYLA

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Preaxostyla (Excavata: Metamonada) is one of the least studied eukaryotic lineages. All members of Preaxostyla are anaerobic and are divided into free-living, morphologically relatively uniform genus *Trimastix* (3 species), and endobiotic, morphologically diverse oxymonads (more than 100 species living mostly in termites). In order to examine the diversity of free-living Preaxostyla more deeply, we isolated and cultured 40 fresh-water and two marine strains morphologically consistent with *Trimastix*, and determined their SSU rDNA sequences. Results of phylogenetic analyses showed that the strains are extensively diversified. Two marine strains form either a clade or two paraphyletic basal lineages of the whole Preaxostyla. The fresh-water strains constitute several lineages that form a robust clade with oxymonads. Although the precise phylogenetic position of the oxymonads is not completely resolved, it seems that they are closely related to a novel free-living clade represented by four strains.

The strains were examined also by means of light microscopy. Morphology of both marine and several fresh-water strains roughly corresponds to *Trimastix marina* suggesting that this species is polyphyletic. Most freshwater strains are similar to *T. pyriformis*, but are phylogenetically far too diverse to represent a single species. Three strains forming a clade within the *T. pyriformis* complex possess tiny cells in comparison with the other strains.

Our data convincingly show that the phylogenetic diversity of free-living Preaxostyla is more extensive that of the endobiotic oxymonads, despite of the relatively uniform morphology. Preaxostyla could serve as a good example of several evolutional events (reduction of mitochondria, diversity of fresh-water free-living protists, miniaturization of cells).

COMPARATIVE ANALYSIS OF CHLORARACHNIOPHYTE MITOCHONDRIAL GENOMES; EVOLUTIONARY INSIGHTS FROM GENOME ARCHITECTURE AND ENDOSYMBIOTIC GENE TRANSFER

Goro Tanifuji (**University of Tsukuba**), **John Archibald** (**Dalhousie University**), **Tetsuo Hashimoto** (**University of Tsukuba**).

Chlorarachniophyte algae maintain nucleomorphs, relic nuclei derived from eukaryotic secondary endosymbionts. These unusual organelles are found in the chlorarachniophytes and cryptophytes, and are intriguing given that other secondary algae do not retain a nucleomorph. In order to gain insight into genome evolution in endosymbiogenesis, comparative genome analyses of nuclear, nucleomorph and plastid genomes of nucleomorph-bearing organisms have been carried out. However, comparative genomic data on the chlorarachniophyte mitochondrial genome (mtDNA) are currently lacking. We have sequenced the complete mtDNA of *Lotharella oceanica* and compared it to that of the 'model' chlorarachniophyte, *Bigelowiella natans.* The *L. oceanica* mtDNA is 36.7 kbp in size (similar to that of B. natans) and contains 35 protein genes, three rRNAs and 24 transfer RNAs. Overall G + C content is 49.27 %, and the gene density was found to be 0.95 genes per kbp. No introns were found. The linear structure of the genome was verified using Southern hybridization analysis. GUG (Valine) and UUG (Leucine) appear to be initiation codons in the *L. oceanica* mtDNA, in addition to AUG (Methionine). These initiation codon usage patterns are similar to those in bacteria (i.e., AUG, GUG, UUG plus AUA), but are not found in *B. natans*. Interestingly, rpl16, rps4 and atp8 genes are missing in *L. oceanica* mtDNA, despite being present in *B. natans* mtDNA. We searched for, and found, mitochondrial rpl16 and rps4 genes with spliceosomal introns in the *L. oceanica* nuclear genome, suggesting recent endosymbiotic gene transfer. Despite being of similar size and coding capacity, the level of synteny between *L. oceanica* and *B. natans* mtDNA is low, suggesting frequent recombination. Overall, these results suggest that chlorarachniophyte mitochondrial genomes are more evolutionarily dynamic than their plastid counterparts.

APUSOMONAD ENVIRONMENTAL SURVEYS ESTABLISH NEW CLADES IN BOTH MARINE AND FRESH WATER ENVIRONMENTS

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Apusomonadida is a eukaryotic group of gliding biflagellates that prey on bacteria. Their diversity and biology remains fairly unknown, with only 10 species morphologically and molecularly described and few environmental sequences available on public databases. The clearly monophyletic clade of apusomonads comprises 5 known lineages, although the relationships between them are not clearly established due to the limited resolution of the available 18S rRNA at such deep phylogenetic level. Phylogenomic studies including data from one available genome and 3 transcriptomes place apusomonads as sister group to Opisthokonta, a clade containing two complex multicellular lineages (animals and fungi). Apusomonadida is also considered one of the deepest amorphean/opimodan lineages together with other incertae sedis lineages such as ancyromonads, mantamonads or collodictyonids. Accordingly, apusomonads are fundamental to understand the early evolution of the domain Eucarya, and the origins of multicellularity in Opisthokonta.

In order to fulfill such expectations, it is crucial to increase the taxonomic sampling of apusomonads. Environmental studies of protist diversity using eukaryote-specific 18S rRNA primers and Sanger and/or high-throughput sequencing approaches generally fail to find apusomonads. This may be explained by their lower abundance in natural environments, but also by the fact that some benthic biotopes remain poorly explored as compared to plankton. Here we present an environmental molecular survey based on the amplification, cloning and Sanger sequencing of 18S rRNA genes using one apusomonad-specific primer. 18S rRNA genes were amplified from a variety of benthic environments collected worldwide from marine (coastal, deep-sea, hydrothermal vents and cold-seeps) as well as fresh water settings (peat bogs, brooks and ponds). Almost full-length 18S rRNA genes for about 50 new apusomonad OTUs have been determined. Phylogenetic analysis reveals that some of these OTUs are scattered among the 5 known apusomonad clades, others cluster with previously published environmental sequences, and some of them form robust new clades. The results show apusomonads in both fresh water and marine benthic environments, generally associated with low oxygen concentrations. Additionally, many of the identified OTUs are present in both marine and fresh water samples, suggesting that members of this deep-branching lineage easily cross such ecological barriers.

SYSTEMATICS AND EVOLUTION OF THE CELL COAT IN AMOEBAE OF THE GENUS KOROTNEVELLA (AMOEBOZOA, DISCOSEA)

Ilya Udalov (**Saint-Petersburg State University**).

Naked lobose amoebae of a genus *Korotnevella* are characterized by a presence of a layer of scales in their cell coat. A structure of these scales is considered to be species-specific. Thus *Korotnevella* is one of a few naked amoebae genera, which species can be easily distinguished on the electron microscopy level. At the present time three marine (*K. monacantholepis, K. hemistylolepis, K. nivo*) and three freshwater species (*K. stella, K. bulla, K. discophora*) are known. Sequences of 18S rDNA gene are available for three *Korotnevella* species only (*K. stella, K. monacantholepis* and *K. hemistylolepis*). All these species have scales with similar structure, thus available phylogenetic data do not allow conclusions on the evolution of scales within this group of amoebae.

We obtained a number of new sequences of *Korotnevella* species with different scale type, and this essentially clarified this situation. One of the studied species falls into *Korotnevella* diagnosis by its morphology: it has typical for *Korotnevella* locomotive form and scales in its cell coat. At the same time its 18S rRNA gene sequence forms a robust clade with that of *Pseudoparamoeba pagei. Pseudoparamoeba* differs from *Korotnevella* in presence of hexagonal glycostyles instead of scales in the cell coat and its 18S rRNA gene sequence branches separately from those of *Korotnevella* species. Thus this new finding shows that only morphological approach is not enough to establish the boundaries between paramoebid genera and the diagnosis of *Korotnevella* needs to be revised. Also new data show that the presence of scales in the cell coat is probably the primitive state for a clade which includes *Pseudoparamoeba, Korotnevella, Paramoeba* and *Neoparamoeba* species, within which loss of scales occurred several times.

Supported by RSF grant 14-04-00474.

pH PREFERENCES IN A DIATOM SPECIES COMPLEX

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Ecology of diatom is based on observational studies that correlate occurrence of taxa with environmental variables. Unfortunately, species identification based on morphology is problematic due to widespread cryptic diversity in diatoms. Number of cryptic species recovered by molecular methods in past 20 years elicited doubts, whether small genetic differences among cryptic species are translated into functional diversity. To address this question, we experimentally determined preferences of 15 species belonging to *Eunotia bilunaris/flexuosa* species complex toward pH, which is one of the most important factors structuring diatom communities in freshwater lentic habitats. 44 strains were grown in 9 different pH levels ranging from 3 to 9. Growth of strains was measured by PAM fluorometry and modeled as a function of pH. All calculated parameters - lower and upper pH limit, optimal pH - differed significantly between both strains and species. Moreover, all parameters were significantly correlated with the pH of the source habitat. In conclusion, our results showed presence of both intra- and interspecific variation in pH preference. At least some species in this complex differ in their fundamental pH niche and this difference has a bearing on their distribution in nature. Clearly, recognizing species diversity in *Eunotia bilunaris/flexuosa* species complex would improve environmental reconstructions.

IDENTIFICATION AND SUBCELLULAR LOCALIZATION OF A PUTATIVE SODIUM-CALCIUM EXCHANGER OF ENTAMOEBA HISTOLYTICA.

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In *Entamoeba histolytica*, the protozoan parasite responsible of human amoebiasis, calcium has an important role on signaling of different cellular processes, including development and pathogenesis. However, few is known about the proteins that are involved in calcium regulation. Sodium calcium exchangers (NCX) are proteins that play an important role in calcium homeostasis by catalyzing the active efflux of this ion by using the energy stored in the electrochemical gradient of sodium. These proteins allow sodium to flow down its gradient across the plasma membrane in exchange for the counter transport of calcium ions. Here, we identified a gene that possibly encodes a plasma membrane NCX of *E. histolytica*. By RT-PCR assays we found that this gene is expressed in basal conditions. We also detected that the expression of this gene changes when trophozoites are grown with high or low calcium. Specific antibodies generated against this protein located this putative NCX in the plasma membrane, supporting the hypothesis that it corresponds to the exchanger responsible for calcium efflux in *E. histolytica*.

THE PROTEIN PHEROMONE FAMILY OF E. PETZI, A PSYCHROPHILIC AND EARLY BRANCHING EUPLOTES SPECIES

Adriana Vallesi (University of Camerino, Italy), Claudio Alimenti (University of Camerino, Italy), Bill Pedrini (Paul Scherrer Institute, Switzerland), Pierangelo Luporini (University of Camerino, Italy).

Euplotes species are valuable for the study of the structural and functional biology of water-borne protein pheromones that cells constitutively synthesize and use in intra-specific chemical communication. We have recently devoted particular attention to the pheromone family of the "cold-loving" (psychrophilic) species *E. petzi* which dwells in the freezing Antarctic and Arctic coastal sea waters, and forms, together with *E. sinicus,* the earliest branch of the *Euplotes* phylogenetic tree. From cultures of genetically distinct strains, we have isolated and sequenced four *E. petzi* pheromones. With respect to the known pheromones from *E. raikovi, E. octocarinatus, E. nobilii* and *E. crassus,* the *E. petzi* pheromones are smaller (32 amino acids) and richer in Cys residues (eight) located in strictly conserved positions. These residues are predicted to form four intra-chain disulfide bridges, which suggests a compact globular fold of the molecules. However, the NMR solution structure determined for one of the *E. petzi* pheromones challenges this hypothesis. The structure consists of one more extended eight-residue alpha-helix and one smaller four-residue helix, and shows large polypeptide segments devoid of regular secondary structures. Pheromones from other *Euplotes* species which live in temperate waters and branch later than *E. petzi* in the *Euplotes* phylogenetic tree are known to be characterized by a three-helix fold and unstructured regions of comparatively limited dimensions. In the light of this knowledge, we can thus draw two distinct conclusions from our findings. The first, of phylogenetic nature, is that the structural evolution of the *Euplotes* pheromones involves an increase in size and complexity. This is in line with the smaller and simpler organization that also the macronuclear *E. petzi* pheromone genes show with respect to their homologues in other Euplotes species. The second conclusion is that the extended unstructured regions of *E. petzi* pheromones are likely correlated with an increased flexibility of the molecular backbone and, hence, reflect a common feature of protein cold-adaptation. In this regard, further insights will be obtained by ongoing experiments which aim to assess the unfolding and refolding properties of *E. petzi* pheromones when exposed to increased temperatures and variations of other environmental parameters.

INVESTIGATION OF THE EUGLENA GRACILIS TRANSCRIPTOME AND PLASTID PROTEOME WITH A FOCUS ON PLASTID MEMBRANES EVOLUTIONARY HISTORY AND PROTEIN-TARGETING

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Euglenids are a group of flagellates known for their diversity of nutritional modes. The ancestral and most widespread mode of nutrition among euglenids is heterotrophy (bacteriovory, eukaryovory and primary osmotrophy). However, one monophyletic clade inside this group, the euglenophytes, acquired a green secondary plastid and uses photosynthesis as a main energy source. This plastid is surrounded by three membranes whose origin is not yet resolved, and its ancestor is closely related to prasinophyte alga *Pyramimonas*. The establishment of a new organelle was accompanied by lateral gene transfer from nuclear and plastid genomes of the endosymbiont to the host nucleus. Paralelly, a system for translocation of nuclear-encoded proteins to plastid had to arise. It is expected that the outermost membrane of the euglenid plastid is derived from the host endomembrane system and the translocation across it involves N-terminal signal peptide and resembles the beginning of a secretory pathway. However, membrane recognition and fusion mechanism remains unknown. Protein translocation across the two innermost membranes is expected to be provided by TOC and TIC complexes which were described and studied in detail in plant chloroplast and identified on gene level in various organisms with independent secondary and higher plastids. In our search for possible translocases in transcriptomes of four euglenophytes, we found in silico evidence for multiple thylakoid sorting pathways, two subunits of TIC, but no TOC subunit or any other outer envelope protein. We propose that the TIC complex might be reduced in the number of subunits while TOC complex might be very divergent or even completely missing. We are elaborating our results by immunofluorescence localisation of selected proteins and by analyzing whole plastid proteome as well as the proteome of narrowed set of transmembrane proteins from isolated *Euglena gracilis* plastids using mass spectrometry.

PHYLOGENETIC SPECIES DELIMITATION IN THE EUNOTIA BILUNARIS/FLEXUOSA SPECIES COMPLEX (BACILLARIOPHYTA)

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There is mounting evidence based on molecular phylogenies and their congruence with sexual compatibility and valve morphology that there is a large (pseudo)cryptic species diversity in diatoms, as in other microalgae. This implies that a huge taxonomic effort will be necessary to define species limits based on data sources other than only valve morphology. A DNA barcoding database can be developed to identify these species and even indicate the presence of yet unknown species diversity, but the possibility to do so may depend on the (variability of) the barcode marker(s) that is chosen. Automated DNA-based species delimitation methods are suitable for a first rapid, large-scale and objective assessment of species limits. However, their performance and marker-dependence should be evaluated using model taxa in which species limits are well-investigated. Here, we first applied three of these methods, statistical parsimony network analysis, the PTP model and the GMYC model approach, to assess species boundaries in a diatom species complex, *Eunotia bilunaris/flexuosa*, based on four molecular markers (cox1, rbcL, D1-D3 LSU and V4 SSU rDNA) and their congruence with valve morphology. This revealed the presence of 17 clear-cut species and a final one with an extraordinary morphological and genetic diversity, which consists of a mixture of heterothallic and apomictic strains. Next, we use the species complex to evaluate (1) the suitability of each of these four molecular markers, each proposed as DNA barcode marker in diatoms but differing widely in variability, for diatom species discovery and (2) the taxonomic resolution of Operational Taxonomic Units outlined in environmental next-generation sequencing datasets based on V4 SSU or partial rbcL.

CILIATES COMMUNITY IN THE ASSESSMENT OF IMPACTS ON NEOTROPICAL STREAMS

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Human population growth and the consequent increase in the use and occupation of the landscapes are some of the main factors altering the structure and functioning of the aquatic ecosystems, contributing to several ecological consequences, such as biotic homogenization and consequently decrease of the biodiversity. This environmental stress leads to a demanding of rapid, reliable, and cost-effective methods to assess both the environmental and ecological qualities. Bioassessment studies using the ciliated protist as tools are mostly based on the identification at the lowest taxonomic level as possible, usually species. Nevertheless, this taxonomic resolution requires extremely qualified professionals and long-term, high cost techniques, which hampers the use of these organisms, especially by environmental agencies that commonly perform large scale, short-term studies. In this context, this study investigated the possibility of using ciliated protists as bioindicators to detect and assess the impacts caused by urbanization in tropical streams. Moreover, we tested the potential use of coarser levels of taxonomic resolution (taxonomic sufficiency) in order to evaluate the possibility to implement the use of protist ciliates in biomonitoring programs, based on higher taxonomic levels in different numerical resolutions. This study was conducted in 10 first-order tropical streams located in both urban and rural areas, during two distinct hydrological periods (rainy and dry periods). We recorded 143 species of protist ciliates belonging to 30 orders, 69 families and 88 genera. Variation in the ciliates abundance and species composition was detected specially in a spatial scale, evidencing that ciliates are good indicators of rural and urban streams. Regarding the taxonomic sufficiency analysis, a second-stage MDS revealed a high similarity between the matrices of Genus and Family. In contrast, the Order matrix was divergent from the remaining taxonomic categories, especially from Species matrix, during both the dry and the rainy seasons. Although our results support the use of taxonomic sufficiency as a tool for inclusion of ciliated protist in biomonitoring programs of streams, due to the necessity of quickly technical works, specialized taxonomists are fundamental for the scientific research in the diverse areas of biology and ecology.

DIVERGENT PATTERNS OF COMMON AND RARE TAXA OF PLANKTONIC CILIATES AND THE INFLUENCE OF FLOOD EVENTS IN NEOTROPICAL FLOODPLAINS

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After much discussion about the cosmopolitan nature of microbes, the great issue nowadays is to identify at which spatial extent microorganisms may display biogeographic patterns, and if temporal variation is important in altering those patters. Planktonic ciliates were sampled from shallow lakes of four Neotropical floodplains in the high and low water period, along with several abiotic and biotic variables potentially affecting the ciliate community. Species were classified as common or rare. We hypothesized that common species would be more associated with environmental variables and rare species with spatial variables. We also expected a temporal variation on the contribution of the spatial and environmental components at the small spatial scale, and the prevalence of dispersal limitation at the largest spatial extent (among-floodplains). We found that common species were more associated with the environmental gradients and rare species were more likely to conform to neutral processes, however, this pattern seem to change depending on the temporal and spatial scales considered. Environmental gradients (species sorting) were more important in the high waters for both common and rare species, while in low waters common species continued to be mainly governed by the environmental conditions, but rare species were more associated with the spatial component, suggesting dispersal limitation, likely because of differences in dispersal ability and ecological tolerance. We also found that common and rare species were related to different limnological (both biotic and abiotic) factors, suggesting different ecological niches. At the largest spatial extents, both common and rare species showed biogeographic patterns.

STRUCTURE AND DYNAMIC OF PLANKTONIC CILIATES COMMUNITY ALONG THE ONLY REMAINING DAM-FREE STRETCH OF A GREAT TROPICAL RIVER

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The damming of great rivers produces expressive changes in the natural processes of ecosystems, with a significant amount of variations in the water characteristics, a strong decrease in the flood pulse amplitude, as well a gradual reduction of nutrients load and a continuous increase in the water transparency. The aim of this study was to investigate the spatial and temporal patterns of abundance and species composition of ciliates community, in the last undammed stretch of Upper Paraná River, in Brazil. In order to reach this result, four field campaigns were performed for a year. Plankton samples were collected from 10 transects through this stretch of the river (230 Km), near the banks and on the center, as well on 7 of its tributaries. 92 ciliates species were identified, among which the peritrichs were the most abundant while the oligotrichids was the more specious group. We recorded a remarkable increase in abundance and species richness along the river, especially in the higher water period. Moreover, in this period we found an increase in the beta-diversity along the river, which consists in a remarkable distinction among the low, middle and high stretch of the river. As a consequence of the aspects previously presented, continuous changes in species composition evidenced a great influence of precipitation and tributaries in the maintenance of higher regional diversity of the studied stretch. Therefore our results strongly suggest the requirement for conservation actions with the purpose to maintain those tributaries undammed, in order to avoid biotic homogenization processes and the consequent reduction of aquatic biodiversity in this important tropical ecosystem.

PROTISTAN NANOFAUNA DISTRIBUTION IN THE MESOSCALE: SPECIES RICHNESS IN 150 GRASSLAND SOIL SAMPLES

Paul Venter (Universität zu Köln), Frank Nitsche (Universität zu Köln), Peter Heger(Universität zu Köln), Anne Domonell (Universität zu Köln), Hartmut Arndt (Universität zu Köln).

Very little if any information exists on the taxa-area relationship of the small size class (1-100µm) of heterotrophic nanoflagellates and ciliates. This study is a first attempt to get a comprehensive comparative data set on soil protist species richness, community structure, and taxa-area relationship. By using high through-put 454 sequencing and a single set of eukaryotic specific primers, we aim to explain how land use intensity (LUI) may influence species richness in the mesoscale (1 – 1000km). Whole consortium genomic DNA was extracted from 150 geo-referenced grassland plots representing climatic, geographical and topographical ranges typical for the middle of Europe. The V4 region of the small subunit (SSU) ribosomal RNA (rRNA) gene was sequenced. Blast results from the curated protist ribosomal reference (PR2) database indicated ribotype identity up to eight taxonomic levels, and a comparatively high species richness in the region at 97% pairwise identity (PI). OTUs were identified at ≥97% PI and ≥99.7% PI, respectively. The majority of OTUs occurred very seldom. Some ribotypes for apicomplexans (gregarines) occurred ubiquitous across all exploratories, whereas other OTUs only occurred at specific sites. Land use intensity (LUI), in particular mineral fertilization, caused significant selective pressure. Low LUI samples were more species rich and represented by diverse taxonomic groups compared to high LUI.

MITOCHONDRIAL PYRUVATE CARRIER IN TRYPANOSOMA BRUCEI

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Pyruvate is the final product of glycolysis that is further metabolized in various pathways, of which the major one is pyruvate oxidation in mitochondria yielding substantial number of ATP molecules. Alternatively, it can be excreted out of the cell as metabolic waste product. Due to its physicochemical properties, pyruvate requires a membrane transporter to reach mitochondrial matrix. The mitochondrial pyruvate carrier (MPC) was recently identified to be composed of MPC protein family members (Mpc1 and Mpc2 in mammals, and in addition Mpc3 in yeast). Here, we characterized pyruvate import into mitochondrion of a kinetoplastid parasite *Trypanosoma brucei*, the causative agent of sleeping sickness. We identified Mpc1 and Mpc2 homologs in the genome of *T. brucei* with attributes of MPC protein family and we confirmed that both proteins are present in mitochondrial membrane of the parasite. Investigations of mpc1/mpc2 gene knock-out cells including measurement of pyruvate uptake by isolated mitochondria, and determination of metabolic end products by high pressure liquid chromatography as well as by nuclear magnetic resonance spectroscopy provided evidence that *T. brucei* Mpc1/2 proteins facilitate mitochondrial pyruvate transport. Interestingly, MPC is expressed not only in procyclic trypanosomes with fully activated mitochondria but also in bloodstream trypanosomes in which most of pyruvate is excreted. Moreover, MPC seems to be essential for bloodstream parasite grown under nutrient limitation in vitro and in infectivity of mice.

ORAL TRICHOMONADS IN CATS AND DOGS

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Trichomonads are anaerobic flagellated protists that are either parasites or commensals. They frequently inhabit digestive, respiratory, and urogenital tracts of vertebrates, including domestic cats and dogs. In these hosts, four trichomonad species has been described: *Tetratrichomonas canistomae* and *Tetratrichomonas felistomae* that are commensals of the host oral cavity; *Pentatrichomonas hominis,* a commensal of intestinal tract that could be found in dogs and cats but also in other mammals including humans; and pathogenic *Tritrichomonas foetus* that causes, in addition to cattle infection, feline intestinal trichomonosis. Although, trichomonads in dogs and cats are probably of cosmopolitan distribution we have no information about their presence in Czech Republic.

The aim of this study was to investigate trichomonads present in the oral cavity of dogs and cats and to get preliminary epidemiological data. Cultivation and nested PCR were used to determine the presence of trichomonads in dogs and cats. Sequencing and phylogenetic analysis based on ITS1-5.8rRNA-ITS2 gene sequence was used to identify species of isolated trichomonads. A cross-sectional study was conducted involving cats and dogs from different populations. Host management information was assessed through a questionnaire. Odds ratios (OR) with 95% confidence intervals and P values were calculated by stepwise logistic regression to estimate the magnitude of association between demographic information and the trichomonad infection.

Our investigations revealed a presence of a new *Trichomonas* species from the mouth of dogs and cats, which we suggest to be named *Trichomonas brixi.* Unexpectedly, we also found another trichomonad in mouth of dogs and cats *Trichomonas tenax,* which is known as a commensal from the mouth of human. None of isolated samples belong to previously described Tetra*trichomonas canistomae* and *Tetratrichomonas felistomae.* The prevalence of all oral trichomonads (without species differentiation) was 45,2 % (57/126) in dogs and 19,3 % (26/135) in cats. The prevalence of *Trichomonas brixi* from the mouth of dogs and cats were 30,6 % (34/111) and 6,6% (8/122), respectively and of T*richomonas tenax* were 8,1 % (9/111) in dogs and 4,1 % (5/122) in cats. Our study distinguished species of oral trichomonads in dogs and cats in the Czech Republic.

SYNCHRONIZED AND ER-ASSOCIATED DIVISION OF GIARDIA INTESTINALIS MITOSOMES

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Mitosomes are the smallest evolutionary forms of mitochondria and are adapted to anaerobic environments. This adaptation manifests as the absence not only of the mitochondrial genome but also of the vast majority of the mitochondrial proteome, including the components of the mitochondrial division machinery. Here, we studied the dynamics of *Giardia intestinalis* mitosomes during interphase and mitosis and during differentiation into the cyst stage. The organelles were followed throughout the cell cycle by live-cell and immunofluorescence microscopy. We found that mitosomal division is restricted to mitosis, when both central and peripheral organelles divide in a unique and synchronized manner. Surprisingly, despite the absence of the responsible components, the division involves the association of mitosomes with the endoplasmic reticulum, a relationship commonly seen during the division of mammalian and fungal mitochondria. Currently we test the presence of several protein candidates in the contact sites between ER and mitosomes and the involvement of the only dynamin homologue in giardia during mitosomal division. Given that mitochondria undergo fusion events as well as division during the cell cycle, we test in vitro fusion of mitosomes which has not been observed so far. Our preliminary data demonstrate that mitosomal dynamics include a combination of both conserved and distinct species-specific traits.

STUDY ABOUT THE TOXICITY EFFECTS OF THE HG2+ AND CD2+ ON STENTOR COERULEUS

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The biological toxicity of heavy metal ions is hot spots of toxicology field. Most of the reports were about the related impact on multicellular higher animals under the half lethal concentration. This work took *Stentor coeruleus* as the research object, testing the minimum repression concentration of the two kinds of heavy metal ions, mercury (Hg2+) ion and cadmium ion (Cd2+) on *Stentor coeruleus* asexual binary fission rate (time/day). Then we observed the changes of TEM ultrastructures of *Stentor coeruleus* under the stress of the concentration of various heavy metal ions. The results showed that: 1) Mercury ion and cadmium ion can impact asexual binary fission speed of *Stentor coeruleus*. The minimum inhibition concentration range respectively is 0.0090 ~ 0.0125 mg/L, and 0.0150 ~ 0.0150 mg/L; 2) Mercury and cadmium ions made the nucleus shape seriously distort, fold, double membrane structure of the nuclear became fuzzy, the number of nucleolus decreased. Folded crest structure in the mitochondria and the mitochondrial outer membrane became not clear. These results suggest that different agents can cause the change of ultrastructure of single-celled organisms under the minimum inhibition concentration. That might explore the mechanism of the biological toxicity of heavy metal ion.

The work was supported by the National Science Foundation of China (NO.31471950, 30970311, 31101613).

NEW TAXA REFRESH THE PHYLOGENY AND CLASSIFICATION OF PLEUROSTOMATID CILIATES (PROTOZOA, CILIOPHORA, LITOSTOMATEA)

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Our knowledge of the diversity of pleurostomatid ciliates has increased rapidly in recent years with the discovery or rediscovery of numerous new and poorly known taxa. Consequently there is a pressing need to revise the systematics of this group using both morphological and molecular data. In the present work, a new genus, *Protolitonotus* gen. n., and two new species, *Protolitonotus magnus* sp. n. and *P. longus* sp. n., from southern China were studied using live observation and sliver staining methods. In addition, the small-subunit DNA of five species representing three genera, *Acineria, Kentrophyllum* and *Protolitonotus* gen. n., was sequenced for the first time. The phylogenetic relationships of pleurostomatid ciliates were reassessed based on new and previously published molecular and morphological data. We conclude that: (1) *Protolitonotus* gen. n. is a sister clade to all other pleurostomatids and thus represents an independent lineage and a separate family, Protolitonotidae fam. n., which is defined by the presence of a semi-suture formed by somatic kineties on the right side of the body; (2) the families Litonotidae and Kentrophyllidae are both monophyletic, whereas Amphileptidae is non-monophyletic; (3) the genera *Loxophyllum* and *Kentrophyllum* are both monophyletic, whereas *Litonotus* is non-monophyletic. Based on these findings, the classification of the order Pleurostomatida is revised and a key to its families and genera is supplied.

ADAPTATION TO A FREE-LIVING LIFESTYLE VIA GENE ACQUISITIONS IN THE DIPLOMONAD TREPOMONAS SP. PC1

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Diplomonads is a group of unicellular protist that includes mostly intestinal parasites or commensals of various animals. However, there are also diplomonads found in other oxygen-poor environments such as sediments of lakes and oceans. All these free-living diplomonads are found nested within host-associated diplomonads in the phylogenetic tree, which have led to the proposal they could be secondarily free-living. There are genome sequences from two parasitic diplomonads available: *G. intestinalis* and *S. salmonicida*. Genomic studies on free-living diplomonad could give insights into the evolutionary transitions between the different lifestyles within diplomonads. Here we present a transcriptome study of *Trepomonas* sp. PC1, a diplomonad isolated from marine sediment and cultured with mixed bacteria.

We annotated 7995 fragments of genes from the *Trepomonas* sp. PC1 transcriptome data. Contaminating sequences were identified as not having the alternative genetic code used by diplomonads and removed. In the *Trepomonas* data we identified genes responsible for a more complex membrane trafficking system, in agreement with a more potent phagocytosis. The transcriptome included a gene encoding squalene-tetrahymanol cyclase (STC). This enzyme is synthesizing tetrahymanol, a molecule required for phagocytosis in free-living anaerobic eukaryotes. The analysis of the metabolic genes revealed a number of proteins involved in degradation of the bacterial membrane and cell wall, as well as an extended set of enzymes involved in carbohydrate degradation and nucleotide metabolism. Phylogenetic analyses showed that the vast majority of these differences in metabolic capacity between the free-living *Trepomonas* and the host-associated diplomonads are due to recent acquisitions of bacterial genes via gene transfer. Noteworthy, one of the acquired proteins encodes a ribonucleotide reductase which makes *Trepomonas* independent of scavenging of deoxyribonucleosides.

These findings indicate that genes important for a metabolism associated with a free-living lifestyle originated via lateral gene transfer in the *Trepomonas* genome. This suggests that the ancestor of diplomonads already was dependent on a host and that *Trepomonas* has adapted to a free-living lifestyle secondarily.

RESPONSE OF BENTHIC PROTIST COMMUNITIES TO MACROALGAL AND GIANT JELLYFISH BLOOMS: REGIME SHIFT IN SEAFLOOR ECOSYSTEMS

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The annual green macroalgal *Ulva prolifera* bloom from May through July since 2008 as well as the giant jellyfish *Nemopilema nomurai* bloom from June through September has been a frequent event in the Yellow Sea. However, their impacts on the benthos and ecosystem have never been evaluated. We investigated the distribution and changes of bacteria, cyanobacteria, diatoms, phototrophic (PNFs) and heterotrophic nanoflagellates (HNFs) and ciliates in sediments from the Yellow Sea during and after the macroalgal and giant jellyfish blooms. The macroalgal bloom in the coastal region of the southern Yellow Sea resulted in a distinct decrease in microphytobenthos (cyanobacteria, PNFs and diatoms) and an increase in microbenthic heterotrophs (bacteria, HNFs and ciliates). The increased ciliate biomass was attributed to the increase of HNFs. The occurrence and decomposition of the giant jellyfish induced a significant increase in the HNFs and PNFs, whose biomass increased by approximately 59% and 50%, respectively, and a slight decrease in the biomass of bacteria likely due to the increased predation pressure of HNFs. Benthic ciliates also increased and carnivorous ciliates constituted the primary feeding type in terms of biomass and species richness, followed by bacterivores, algivores and omnivores. The increasing dominance of carnivorous ciliates is likely a response to the increase of predominant HNFs. The data indicate that the bloom of green macroalgae together with the giant jellyfish induced regime shifts in the structure of the benthic community from larger benthos towards smaller sizes and in benthic metabolism from autotrophic to heterotrophic in the Yellow Sea. Such environments may favour the occurrence of the obligatorily rapacious giant jellyfish *Nemopilema nomurai.*

Supported by the Major State Basic Research Development Program (Grant No.: 2011CB403604) and the Natural Science Foundation of China (41476144).

TAXONOMY AND PHYLOGENY OF KARYORELICTEAN CILIATES

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The class Karyorelictea is widely believed to represent the nature of the ancestral ciliate lineage. Due to their "primitive" nondividing diploid macronuclei, karyorelicteans are important experimental materials in the many research fields. However, research on morphology and fauna of karyorelicteans has been neglected for a long time because this group of ciliates is uncultivable and too fragile to be fixed. This work focused on taxonomy and phylogeny of karyorelicteans collected from intertidal zone of east China. As a result, 26 species were studied, in which there was one new family (Wilbertomorphidae Xu et al., 2013), two new genera (*Wilbertomorpha* Xu et al., 2013 and *Apotrachelocerca* Xu et al., 2011), 10 novel species (*Wilbertomorpha colpoda* Xu et al., 2013, *Remanella macrostoma* Xu et al., 2013, *R. achroma* Xu et al., 2013, *R. sinica* Xu et al., 2012, *Apocryptopharynx discoidalis* spec. nov., *Cryptopharynx minuta* spec. nov., *Geleia sinica* Xu et al., 2011, *Tracheloraphis huangi* Xu et al., 2011, *T. dragescoi* Xu et al., 2014, *Trachelocerca chinensis* Xu et al., 2014), and three new combinations. Meanwhile, phylogeny of three families within Karyorelictea was studied: 1) Phylogenetic trees inferred from SSU rRNA gene sequence support the establishment of Wilbertomorphidae, and reveal that it forms sister group with Geleiidae. Based on the combination of morphological and molecular data, Wilbertomorphidae is placed incertae sedis within Karyorelictea. 2) Phylogenetic trees including 11 new SSU rRNA gene sequences of loxodids indicate that *Remanella* is paraphyletic with *Loxodes* species nesting within it, which supports the hypothesis that freshwater genus *Loxodes* was evolved from its marine congeners *Remanella*. 3) Trachelocercidae is monophyletic and Apotrachelocerca arenicola occupies the basal position in phylogenetic trees, which suggests the compound circumoral kinety may be the ancestral character state, whereas the simple, uninterrupted circumoral kinety in Trachelocerca is a derived character state.

GENETIC DIVERSITY AND CLASSIFICATION OF PATHOGENIC BOVINE EIMERIA SPECIES FROM CENTRAL ANATOLIA REGION OF TURKEY BASED ON 18S RRNA, ITS-1 AND MT-COI GENES

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Coccidiosis is one of the most economically important disease of cattle caused by *Eimeria* species and widespread throughout the world. More than 20 species of *Eimeria* have been described in cattle and 13 of these have been reported from Europe including Turkey. Of these species *E. alabamensis, E. auburnensis, E. bovis, E. ellipsoidalis* and *E. zuernii* are recognized as pathogenic. In this study, 18S rRNA, ribosomal ITS-1 and mt-COI gene regions of pathogenic *Eimeria* species along with some other non-pathogen ones isolated from cattle in Central Anatolia Region of Turkey were sequenced and phylogenetic analyzes were performed in order to explore the genetic diversity among the isolates belong to different *Eimeria* species. Genomic DNA was extracted from the single sporulated oocysts which were identified into the species level by using morphological characteristics. The target gene regions were amplified with the predesigned genus specific primer pairs and the obtained amplicons were gel purified, ligated into the cloning vector, and transformed into the *E. coli* cells and plasmids were obtained. The plasmids including the target gene sequences were sequenced with the vector specific primer pairs. According to the phylogenetic analyses of the 18S rRNA, ITS-1 and mt-COI gene regions, intraspecific homology indexes of over 99.3%, 71.9% and 99.0% were determined among the sequences from the same species, respectively. The mean interspecific genetic diversity among the bovine *Eimeria* species was designated as 1.9%, 49.4%, 13.7% within the same gene regions above, respectively. The phylogenetic constructions of all target gene regions exhibited an agreement to each other with relatively high bootstrap values. There were four clusters of bovine Eimeria species. The first major cluster contains *E. auburnensis, E. cylindrica, E. wyomingensis* and *E. canadensis,* while the second contains *E. bovis, E. ellipsoidalis, E. zuernii* and *E. illionensis.* The third cluster includes *E. subspherica, E. bukidnonensis and E. alabamensis* and the fourth cluster which is more distant than the others contains *E. brasiliensis.*

This study provides first data on mt-COI barcoding of bovine *Eimeria* species and has been supported by Scientific and Technological Research Council of Turkey with the project no 113O597.

A NOVEL BIO-MONITORING SYSTEM WITH THE HELIOZOON RAPHIDIOPHRYS CONTRACTILIS FOR CONTINUOUSLY DETECTING TOXIC SUBSTANCES IN WATER

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An improved model of biological monitoring system for detecting toxicants in water has been developed by using the heliozoon *Raphidiophrys contractilis* as an indicator organism. In this system, adhesiveness of the heliozoons to the substratum was used as a measure of health of the organism. A flow-through type chamber was designed for toxicity testing, in which cells that had been damaged by harmful materials were flushed away by the water flow. The number of remaining heliozoons was continuously monitored by a computerized automatic system with a digital camera. The test results revealed that this novel monitoring system has high durability and efficiency in comparison with other bio-monitoring systems, enabling us to make a quicker and easier detection of toxic substances for a period up to one month without any maintenance. This system has a high sensitivity for heavy metals such as mercury, arsenic, lead and cadmium contained in either waste or drinking water, while less sensitive for organic toxicants. Due to high sensitivity (ex. >10-9 M for Hg2+ and >10-8 M for As3+), fast response time (<20 min) ans small size (30 x 23 x 16 cm), this system has distinct advantages over other conventional biomonitoring systems using fish and crustaceans.

ULTRASTRUCTURE AND PHYLOGENETIC POSITION OF A NOVEL DEEP-BRANCHING KINETOPLASTID

Naoji Yubuki (Departments of Botany and Zoology, University of British Columbia, Canada), Ken-ichiro Ishida (Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan), Brian Leander (Departments of Botany and Zoology, University of British Columbia, Canada).

Kinetoplastids comprise one of three major lineages within the Eulgenozoa and include bodonids, trypanosomatids and prokinetoplastids. A shared feature of kinetoplastids is a unique mitochondrial inclusion called a "kinetoplast", which is a condensed mass of DNA (i.e., kDNA). Bodonids are mainly free-living flagellates that play important roles in aquatic ecosystems; for instance, many members of *Bodo* are common, cosmopolitan and abundant consumers of bacteria and detritus. Trypanosomatids, by contrast, are obligate parasites of animals including humans. *Trypanosoma brucei*, for example, is among the best-studied kinetoplastids because it is the causative agent of African sleeping sickness. Prokinetoplastids represent early diverging lineages within kinetoplastids in phylogenetic trees inferred from molecular data; there are two known representatives of parasitic prokinetoplastids, *Ichtyobodo* and *Parkinsela*. *Ichtyobodo* is a biflagellate, ectoparasite, which infects a wide range of marine and freshwater fish. *Parkinsela* is a non-flagellated organism that lives in the cytoplasm of certain amoeba infecting the gills of fish. Although many lineages of kinetoplastids are parasitic, the most recent ancestor of kinetoplastids is inferred to be free-living, like the vast majority of other euglenozoans (euglenids, diplonemids and symbiontids).

We discovered a novel free-living kinetoplastid from beach sand in the Republic of Palau and successfully established it as a culture strain. We characterized the new isolate at the ultrastructural and molecular phylogenetic levels. The cells are 8 µm long and have two subapical flagella with typical euglenozoan paraxonemal rods. The phylogenetic analysis of small suunit (SSU) rRNA gene sequence demonstrated that this isolate is the earliest diverging branch of kinetoplastids, earlier than all of the prokinetoplastid lineages. The mitochondria have conspicuous kinetoplasts with discoidal mitochondrial cristae. The mitochondria genomes of kinetoplastids consist of so-called maxicircles and minicercles that undergo a complex and distinctive process of RNA editing prior to translation. Our new isolate may provide important evolutionary insights into the architecture and organization of this novel mitochondrial genome.

RESOLVING AN ONGOING DEBATE WHETHER SUBCLASS PERITRICHIA (CILIOPHORA, OLIGOHYMENOPHOREA) IS MONOPHYLETIC BASED ON A MULTI-LOCUS ANALYSIS

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Consensus about many relationships based on 18S rDNA inferences has been reached throughout the ciliate tree of life, but there is still much debate whether the subclass Peritrichia – composed of the Sessilida and Mobilida – is monophyletic. To further assess the inter- and intra-subclass relationships of this group, we characterized 57 sequences for three linked loci (18S rDNA, ITS-5.8S rDNA, 28S rDNA) from 20 reprehensive species of five subclasses in the Oligohymenophorea. Phylogenetic trees reveal the following: (1) the combined three-locus tree provides more resolution in nodes than in the 18S rDNA topologies; (2) the subclass Peritrichia and its two orders Sessilida and Mobilida are monophyletic; (3) the subclass Peniculia branches basally in the Oligohymenophorea; (4) the subclass Scuticociliatia is closed related to Astomatia and Apostomatia; (5) within Mobilida, *Trichodinella* nests within *Trichodina, Urceolaria* branches basally, and *Trichodina* is non-monophyletic. Further, approximately unbiased tests reveal that the monophyly of Peritrichia could not be rejected. Additionally, in an effort to provide a better resolution of evolutionary relationships, the secondary structures of ITS2 transcripts are predicted, and the results support the monophyly of Peritrichia.

This work is supported by National Natural Science Foundation of China (Project No. 41406171 and 41476144).

TINTINNIDS (CILIOPHORA, SPIROTRICHEA, CHOREOTRICHIDA) FROM CHINA SEAS: PHYLOGENIES BASED ON THREE RDNA LOCI

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The great potential of molecular classification for speciose tintinnid ciliates depends on a comprehensive database and suitable molecular markers. A total of 25 morphospecies (28 isolates) of tintinnid ciliates were sampled from surface waters of the Yellow Sea and East China Sea. Using molecular analyses for single cells, 61 sequences were obtained for three rDNA loci (the 18S, the ITS1-5.8S-ITS2, and the D1-D5 region of the 28S). The 18S rDNA for the genus *Leprotintinnus*, the ITS regions for the families Ptychocylididae, and the 28S rDNA for the families Stenosemellidae, Dictyocystidae, and the subclass Oligotrichia are first reported in this study. Phylogenetic analyses based on the three loci indicate that the genus *Leprotintinnus* is closely related to *Tintinnopsis radix* independent from the algorithm used. The basal position of the genus *Tintinnidium* in the order Tintinnida is supported by both the 18S and the ITS phylogenies. Nine subclades of *Tintinnopsis* species (shortened as TIPS) are strongly supported by the comprehensive 18S trees. The monophyly of the clades TIPS I, TIPS III-1, TIPSVI, TIPS VII, TIPS VIII, and TIPS III-3 are supported by at least two gene regions, which confirm the stability of this topology and enables the usage of these ITS and 28S regions as barcoding markers in molecular ecological studies.

CANDIDATE PROTOZOA BARCODE GENES IN ECOLOGICAL RESEARCH

Yan Zhao (Research Center for Eco-Environment Sciences, Chinese Academy of Sciences).

Protozoa comprise many highly diverse eukaryote lineages inhabiting all biotopes and playing crucial roles in regulating microbial food webs, therefore they are at the core of ecosystem developing. Species identification is, nevertheless, a hard task due to subtle morphological differences and tiny sizes. The suite of DNA barcode markers now applied to specific taxonomic groups of plant and animal organisms are proving invaluable for understanding species boundaries, community ecology, functional trait evolution, trophic interactions, and the conservation of biodiversity. The application of next-generation sequencing (NGS) technology will greatly expand the versatility of DNA barcodes across the Tree of Life, habitats, and geographies as new methodologies are explored and developed. Compared to numerous DNA-barcoding methods carried out in plants and animals ecological studies, few studies have been performed on evaluating DNA barcoding genes for protists. We evaluate the performance of seven nuclear and mitochondrial loci for the species-rich taxa. Genetic distance analysis is used to assess the identification efficiency of different candidate genes. Morphological features and ecological characteristics (salinity) are integrated into genetic results, in an attempt to identify ciliate species with character-based barcode analysis. Our studies revealed that ITS2 region and the hypervariable D1-D2 region of LSU rDNA are promising candidates for next-generation sequencing and species delineation in ecology study, and the mitochondrial *cox 1* gene with the best resolution for population level analyses should be providing more genetic information of key species evolution and variation in adapting different environments.

POSSIBLE PHYLOGENETIC POSITION OF THE PARASITIC DINOFLAGELLATE SYLTODINIUM LISTII DREBES (DINOPHYCEAE, GYMNODINIALES)

Carmen Zinssmeister (Senckenberg am Meer, German Centre for Marine Biodiversity Research (DZMB), Wilhelmshaven, Germany), Mona Hoppenrath (Senckenberg am Meer, German Centre for Marine Biodiversity Research (DZMB), Wilhelmshaven, Germany).

At least ten percent of all dinoflagellates have developed a parasitic life style, infecting a wide range of hosts such as other protists up to vertebrates. *Syltodinium listii* as well as *Gyrodinium undulans* are parasitizing on copepod and rotifers eggs. Morphologically both species seem to be identical and can only be distinguished through their seasonal occurrence, which is summer for *S. listii* and wintertime for *G. undulans.* The second is predominantly parasitizing on diatoms. Although, these parasitic dinoflagellates have been documented within several studies, sequencing and molecular phylogenetic analyses of *S. listii* didn´t take place yet.

For this study *S.listii* cells have been isolated from plankton net samples from the inner harbor of Wilhelmshaven, Germany during summertime. A culture has been established, supplied with fresh copepod eggs every two days. By these the life cycle has been documented with an inverted light microscope equipped with a camera. Also some cells have been isolated for molecular analysis.

The molecular phylogeny inferred from ribosomal DNA (ITS1, 5.8S, ITS2 and partial LSU) will be presented. Molecular studies have shown before, that a dinoflagellate determined as cf. *G. undulans* belongs to Gymnodiniales (*Gymnodinium* s.s. clade). Our sequence of *S. listii* has been compared with cf. *G. undulans* and our results are that they are identical.

This leads to the question, if we are dealing with only one instead of two species. For verification both *S. listii* and *G. undulans* have to be cultivated and molecularly analyzed from their type locality in North Sea or nearby. Nevertheless, if we are dealing with one or two species, a taxonomic revision of the parasitic genus will be necessary.

MORPHOLOGICAL TAXA DELIMITATION OF DINOPHYSALES (DINOPHYCEAE), WITH EMPHASIS ON DINOPHYSIS AND PHALACROMA.

Carmen Zinssmeister (Senckenberg am Meer, German Centre for Marine Biodiversity Research (DZMB), Wilhelmshaven, Germany), Mona Hoppenrath (Senckenberg am Meer, German Centre for Marine Biodiversity Research (DZMB), Wilhelmshaven, Germany).

Dinophysales are thecate dinoflagellates, which comprise phototrophic as well as heterotrophic and some toxin producing species. The majority of this group attracts attention through their morphological diversity, exhibiting extraordinary appendages at cingular as well as sulcal lists and the cell body. Nevertheless, the taxon shows a particular tabulation with a constant amount of plates, and a laterally flattened cell body.

Molecular phylogenetic analyses have shown discrepancies to morphology based classification. Especially the largest genera, *Dinophysis* and *Phalacroma*, seem to be polyphyletic. So far it becomes apparent, that the suitability of the character traits used for taxonomic work are not suitable and a revision is needed.

For a detailed morphological (re)investigation taxa have been collected from different regions/oceans. Intraspecific variations and character traits enabling an unambiguous differentiation between *Dinophysis* and *Phalacroma* and possibly new genera will be discussed. A special focus will also be on *Phalacroma rotundata,* a species with character traits in between the two genera, which has therefore a taxonomic doubtful position.

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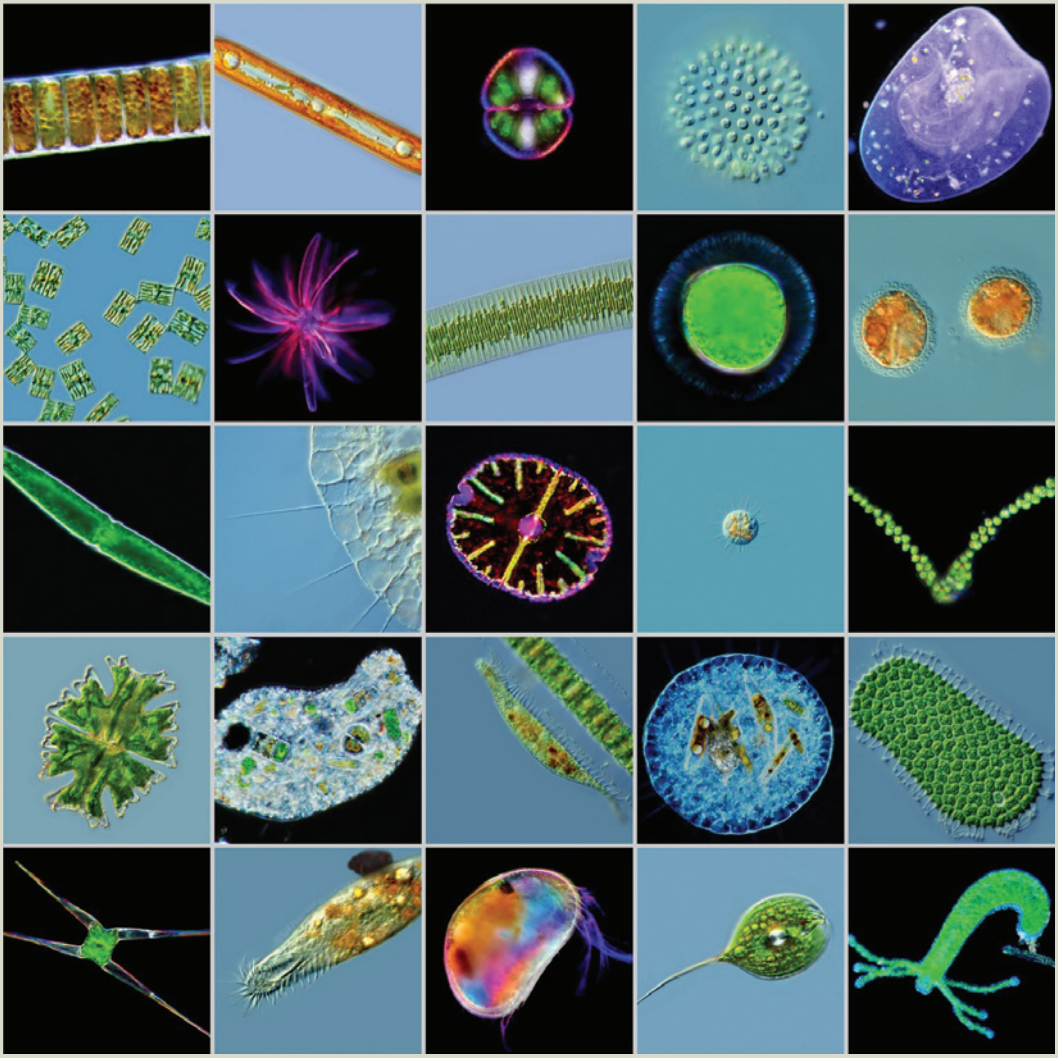
ACKNOWLEDGMENTS-CREDITS

Proyecto Agua-Water Project, Dr. Antonio Guillen (Logroño, Spain).
Dr. Juan C. Gutiérrez (Univ. Complutense de Madrid, Spain)
Dr. Klaus Haussman (Berlin, Germany)



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