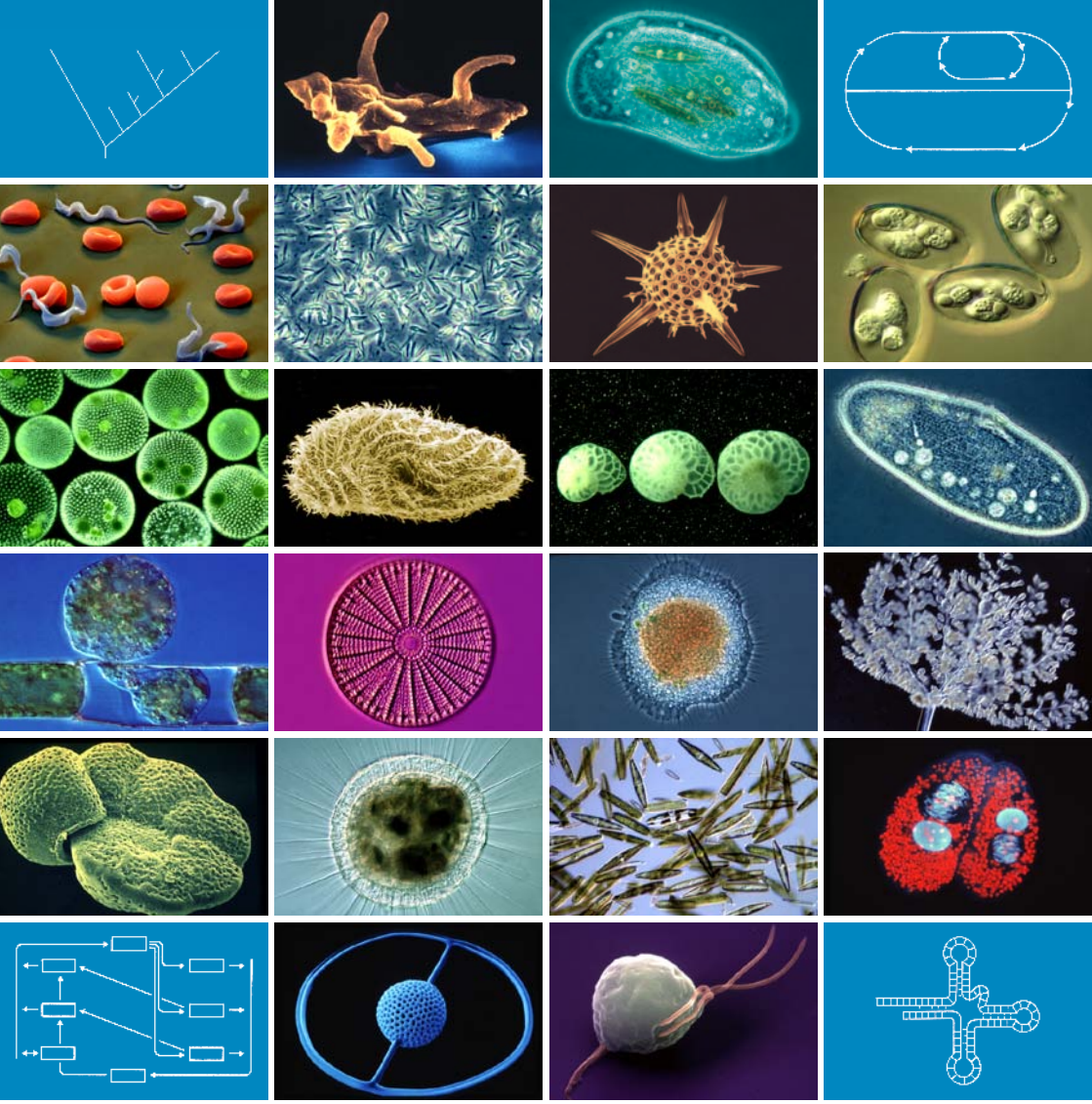
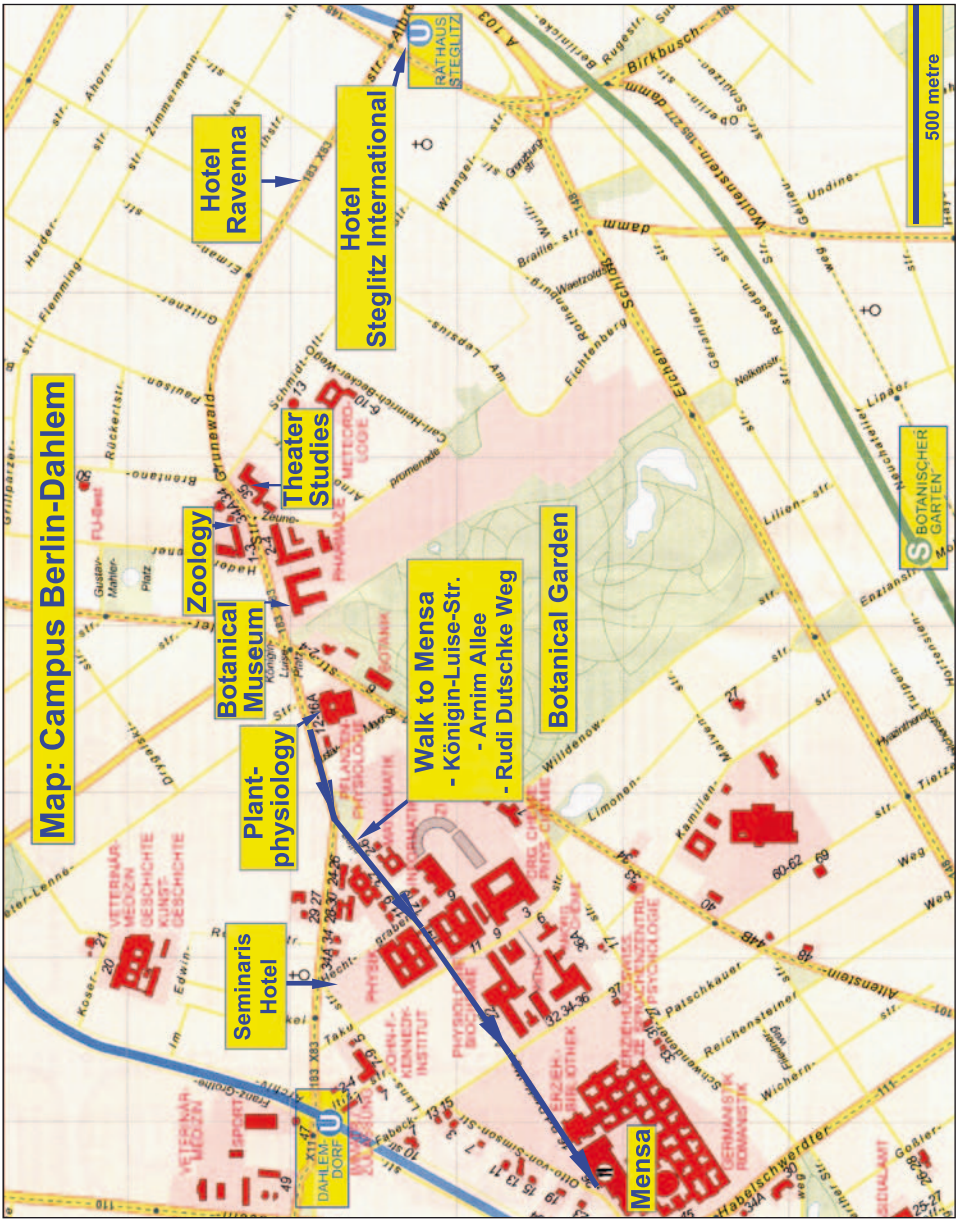


VI EUROPEAN CONGRESS OF PROTISTOLOGY

Special focus: Concepts in Protistology

25 – 29 July 2011 // Berlin, Germany
www.ecop2011.org

FINAL PROGRAMME and ABSTRACTS



The VI ECOP Congress takes place in four different buildings of the Free University of Berlin:

Institute of Biology/ Plant Physiology
Königin-Luise-Str. 12–16
14195 Berlin (Dahlem)
Auditorium Maximum
Lecture Hall 2

Institute of Biology/ Zoology
Königin-Luise-Str. 1–3
14195 Berlin (Dahlem)
Auditorium Maximum

Botanical Museum
Königin-Luise-Str. 6–8
1495 Berlin (Dahlem)
Auditorium Maximum

Institute of Theater Studies
Grunewaldstr. 35
12165 Berlin (Steglitz)
Auditorium Maximum

German Society of Protozoology (DGP)
www.protozoologie.de

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Institute of Biology / Zoology
WG Protozoology
Königin-Luise-Str. 1 – 3
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Co-Organisers

Dr. Regine Jahn
Botanic Garden and
Botanical Museum Berlin
Germany

Prof. Dr. Richard Lucius
Humboldt University of Berlin
Germany

Prof. Dr. Ulrich Szewzyk
Technical University of Berlin
Germany

Dear Colleagues,

in the year 2011 Protistologists from Europe and from all over the world meet in Berlin on the occasion of the European Congress of Protistology (ECOP). It is the sixth such conference in a long line, beginning with the congress in Reading (England) in 1992, then in Clermont-Ferrand (France) in 1995, in Helsingør (Denmark) in 1999, in San Benedetto del Tronto (Italy) in 2003 and in St. Petersburg (Russia) in 2007. In this historical place we succeeded – after many years of discussions – in establishing the Federation of European Protistological Societies (FEPS) as an umbrella organization for the national protistological societies in Europe.

The German Society of Protozoology (DGP) is proud to be the host of the VI ECOP, which takes place in Berlin. We gather together to report about the newest scientific achievements in the many and diverse fields of protistology. It is our hope that it will be an occasion of vivid and fruitful scientific discussions, finally leading into further cooperation across borders.

Besides the more conventional and expected fields and topics such as structural and molecular biology, systematics and evolution, symbiosis and parasitism, ecology and biodiversity, one important focus of the congress will be on the origin of the concept of protistology, its current status worldwide and its future crossing of – or better ignoring of – the artificial, traditional borders between botany and zoology. And where could be a better place for such an event than the centre of Europe? For here is where scientists like Dujardin, Schwann, Schleiden, Purkyně, Cohn, Mohl, Schultze, and Haeckel initiated the fruitful discussion of "what a cell has to be called". And it was Germany where the first and oldest journal in the field appeared, where in the year 1902 Fritz Schaudinn founded the famous Archiv für Protistenkunde, a publication organ which still exists, now under the name Protist. And it was Berlin where the European Journal of Protistology was established as the successor of the famous French Protistologica in 1997. No doubt, the centre of Europe was the cradle of protistology.

Besides these scientifically exciting facets of the congress, we should keep in mind that Berlin is a place with a long history, being – except for one well-known interruption – the capital of Germany. You can find numerous buildings and institutions of historical dimensions and significance. At the same time, it is a modern, inspiring location with a wide variety of things to see and do.

You are now in this city. Experience it with all your senses!

Protistologically yours,

Klaus Hausmann

Klaus Hausmann
President of the 6th ECOP

Congress Venue
Institute of Biology/Plant Physiology
Königin-Luise-Str. 12 – 16
14195 Berlin, Germany
www.fu-berlin.de

Lecture Halls
The VI ECOP Congress takes place in four different buildings of the Free University of Berlin:

► **Institute of Biology/Plant Physiology**
Königin-Luise-Str. 12 – 16
14195 Berlin (Dahlem)
Auditorium Maximum
Lecture Hall 2

► **Institute of Biology/Zoology**
Königin-Luise-Str. 1 – 3
14195 Berlin (Dahlem)
Auditorium Maximum

► **Botanical Museum**
Königin-Luise-Str. 6 – 8
1495 Berlin (Dahlem)
Auditorium Maximum

► **Institute of Theater Studies**
Grunewaldstr. 35
12165 Berlin (Steglitz)
Auditorium Maximum

Congress Registration Counter
All congress material is available at the Congress Counter. The congress counter will be located in the lobby of the
Institute of Biology/Plant Physiology
Königin-Luise-Str. 12 – 16
14195 Berlin
Phone: +49 (0) 30 838 53104

Opening hours

Sunday,	24 July 2011 ► 14.30 – 18.30 h
Monday	25 July 2011 ► 08.00 – 18.00 h
Tuesday	26 July 2011 ► 08.30 – 18.00 h
Wednesday	27 July 2011 ► 08.30 – 16.00 h
Thursday	28 July 2011 ► 08.30 – 18.00 h
Friday	29 July 2011 ► 08.30 – 13.30 h

Name Badges
Participants are kindly requested to wear their name badge at all times during the congress including the Welcome Reception and Opening Ceremony.

The badge serves as the ticket for the Botanic Garden and the Botanical Museum.

The colours of the name badges have the following significance:

Delegate:	Yellow
Accompanying Person:	Purple
Day Passes:	Blue
Staff:	Green

Congress Language
The official language of the VI European Congress of Protistology is English.

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

Abstract Book
All abstracts are published in this booklet as well as online on the congress website at www.ecop2011.org

Programme Changes
The organisers 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
Institute of Biology/Plant Physiology
Königin-Luise-Str. 12 – 16
room no. 034 (ground floor)
The WLAN-key is: c5pzwdbw

Wireless network access during the conference
Conference participants should connect to the wireless network with the SSID (Service Set Identifier) "conference" and open an arbitrary web page. Instead of the web page called, a form will appear, in which the user can enter the key provided by the conference organizer or host. Access to the wireless network will then be granted, and the user will be automatically forwarded to the web page that was originally called.
Note: For technical reasons the connection to the wireless network may be interrupted at midnight. If the key is still valid for the following day, the user must re-enter the key in order to continue using the wireless network.
Attention: Connections to the wireless network "conference" are not encrypted and can be eavesdropped. To ensure confidentiality and encryption, please use appropriate protocols (https, ssh, VPN).

Congress Website
Further and updated information will be available on the internet at www.ecop2011.org.

Speakers' Centre
Speakers are kindly asked to submit their contributions on the day before their presentation in the lobby of the Plant Physiology on Sunday from 15.00 to 17.00 h and from Monday through Friday between 8.00 and 9.00 h or between 12.45 and 13.45 h (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 meeting rooms.

Mobile Phones
Participants are kindly requested to keep their mobile phones turned off while attending the scientific sessions in the meeting rooms.

Poster Exhibition
Posters are displayed from Monday, 25 July, 2011 until Friday, 29 July 2011 in the lobby of the
Institute of Biology/Plant Physiology
Königin-Luise-Str. 12 – 16
14195 Berlin

Poster Sessions
Monday, 25 July 2011 ► 17.00 – 18.00 h
Tuesday, 26 July 2011 ► 17.00 – 18.00 h
Thursday, 27 July 2011 ► 17.00 – 18.00 h

During these session times, it is the responsibility of the presenter to ensure that at least one of the authors is present.

Set-up and dismantling times for posters
► Set-up:
Sunday, 24 July, from 15.00 h to Monday, 25 July 2011, 9.00 h
► Dismantling:
Friday, 29 July 2011 between 12.00 and 15.00 h

Posters which have not been removed by 15.00 h on Friday will be disposed of.
Material to put up the poster is available at the poster service desk in the poster area.

Best Poster
The three best posters will be awarded the 'Best Poster Prize'.
1st price ► EUR 500
2nd price ► EUR 300
3rd price ► EUR 200
Furthermore, the three awardees will receive a free subscription of the European Journal of Protistology for one year.
Kindly supported by Elsevier (Jena, Germany)

The award ceremony will take place during the congress dinner on Thursday, 28 July 2011 at the Seminaris Campus Hotel.

Coffee Breaks
Coffee and tea will be served to all registered delegates during the coffee breaks from Monday to Friday. Coffee bar stations are located at each congress location. In the afternoon however the coffee will be served only at the Plant Physiology.

Lunch Breaks
From Monday through Thursday, lunch is included in the registration fee for delegates. Accompanying persons can purchase a ticket for EUR 7.50 per lunch at the registration counter.
Lunch will be served during the lunch breaks between 12.30 and 14.00 h at the cafeteria at
Mensa FU II,
Otto-von-Simson-Str. 26
14195 Berlin

How to get to the cafeteria:
When you exit Plant Physiology, please turn left down Königin-Luise-Straße. Turn the second left into Arnimallee. After approx. 500 m cross Fabeckstraße and continue straight on into Rudi-Dutschke-Weg. After about 250 m you will see the entrance to the cafeteria.

Congress Office
CPO HANSER® SERVICE
CPO HANSER SERVICE GmbH
Paulsborner Str. 44
14193 Berlin, Germany
Phone: +49 – 30 – 300 669-0
Fax: +49 – 30 – 305 73 91
Email: ecop2011@cpo-hanser.de

Welcome Reception
Sunday, 24 July 2011 from 17.30 to 18.30 h

The VI ECOP will begin with a Welcome Reception in the lobby of the
Institute of Biology/Plant Physiology
Königin-Luise-Str. 12 – 16
14195 Berlin
The Welcome Reception is free of charge for registered delegates and accompanying persons.

Congress Dinner
Thursday, 28 July 2011 at 19.00 h

The Congress Organisers would like to invite you to join for the Congress Dinner which will take place at the
Seminaris Campus Hotel Berlin
Science & Conference Center
Takustraße 39
14195 Berlin
The cost contribution per person is EUR 30.00. If you would like to take part in this event, please buy your ticket at the registration counter at the latest by Tuesday, 26 July 2011.

Botanic Garden and Botanical Museum Berlin-Dahlem

All registered delegates and accompanying persons can visit the Botanic Garden and the Botanical Museum free of charge. Please present your name badge at the entrance.

The Botanic Garden Berlin-Dahlem invites visitors to inform themselves about the fascinating world of plants. Outdoors, the gardeners maintain a large phytogeographical area containing a diversity of plants from Europe, Asia, and North America.

A spacious greenhouse complex, including the Great Tropical House, the Victoria House, and the Mediterranean House, accommodates plants of the tropics and subtropics.

The institution in Berlin-Dahlem has a long tradition as one of Germany's leading botanical research facilities. Investigation is predominantly focused on plant biodiversity and conservation, systematic botany and phytogeography. The museum houses the largest botanical library in Germany with about 275,000 volumes, including the library of the Institute of Biology of the Free University of Berlin.

You are also invited to enjoy the displays of the Botanical Museum: Unique in Central Europe, the museum features the anatomy and morphology of protists, algae, fungi, mosses, ferns and higher plants, and offers information on botanical history worldwide, on special medicinal plants, and on agricultural crops. Changing special exhibitions deal with various botanical subjects of particular interest.

- Entrance Botanic Garden
  Königin-Luise-Platz, Berlin
  Opening hours: daily 9 – 21 h

- Entrance Botanical Museum
  Königin-Luise-Str. 6 – 8, Berlin
  Opening hours: daily 10 – 18 h

German Bundestag at the river Spree

The new Berlin presents itself to visitors as a cosmopolitan city. The Brandenburg Gate, once a symbol of the divided Germany, has become the emblem of the German capital. Today, Berlin is situated in the heart of a continent moving towards unification, and plays an important role in the European integration process.

Berlin's cultural scene offers unlimited opportunities for leisure activities. The Museumsinsel (Museum Island) with its world-famous museums was inscribed on UNESCO's World Heritage list in 1999. Kurfürstendamm, Friedrichstraße and Potsdamer Platz are ideal places for extensive shopping trips.

Potsdamer Platz

Old Museum on Museum Island

Berlin is one of the prime locations for science or research facilities and the media. As a Competence Centre, Berlin has relied on the fields of technology, biotechnology, medical and environmental technology. Globally operating firms draw benefit from the large number of institutions resident in Berlin and the knowledge available here.

Berlin is also a green city. Numerous woods, lakes, parks and gardens offer recreation for everyone. In addition, the historical city of Potsdam in the neighbouring Land of Brandenburg, with its historic palaces and gardens and numerous highlights of historical architecture, invites visitors to stay for a while.

Berlin offers a variety of public transportation means which enable visitors to get from one place to the other very quickly.

Each delegate as well as the accompanying persons can use the public transportation with their congress badge in combination with their passport.

The bus stop closest to the congress venue is Königin-Luise-Platz/Botanischer Garten.

How to get from the airport to the congress venue
Berlin has two airports, Berlin Tegel which is located in the north-west of the city and Berlin Schönefeld which is in the south-east.

From Airport Tegel
Duration: approx. 50 minutes
Bus:
- ► Airport Tegel to Olivaer Platz
- ► bus no. 109 towards S+U Zoologischer Garten
- ► get off at Olivaer Platz
- ► bus no. 101 towards Sachtlebenstraße
- ► get off at Königin-Luise-Platz/Botanischer Garten

From Airport Schönefeld
Duration: approx. 60 minutes
Bus/S-Bahn/Bus:
- ► Airport Schönefeld to S Südkreuz Bhf.
- ► bus no. SXF 1 towards S Südkreuz Bhf.
- ► get off at S Südkreuz Bhf.
- ► S-Bahn no. S25 towards S Teltow Stadt
- ► get off at S Lankwitz
- ► at S Lankwitz
- ► bus no. X83 towards Königin-Luise-Str./Clayallee
- ► get off at Königin-Luise-Platz/Botanischer Garten

How to get to the city center
From the congress venue at Königin-Luise-Straße you can easily reach Kurfürstendamm and Unter den Linden/Friedrichstraße.

To Kurfürstendamm
Duration: approx. 20 minutes
Bus/Underground
- ► bus stop Königin-Luise-Platz/Botanischer Garten
- ► bus no. 101 towards U Turmstraße
- ► get off at U Breitenbachplatz
- ► change in the U 3 towards Nollendorfplatz
- ► get off at Wittenbergplatz

To Unter den Linden/Friedrichstraße
Duration: approx. 35 minutes
- ► bus stop Königin-Luise-Platz/ Botanischer Garten
- ► bus no. X 83 towards Nahmitzer Damm
- ► get off at S+U Rathaus Steglitz
- ► take S 1 towards Oranienburg Bhf
- ► get off at S+U Friedrichstr. Bhf
- ► take U 6 towards Alt-Mariendorf
- ► get off at Französische Straße

For detailed information on the Berlin public transportation system, please visit the website of the municipal public transport company, BVG at:
www.bvg.de

Time	Sunday, 24 July	Monday, 25 July	Tuesday, 26 July
8.00		Poster Set-up	
9.00		**Opening Ceremony**	PL III: **Patrick Keeling, CA** Comparative genomics of protists
10.00		**Award Ceremony**	WS SE
11.00		*Coffee Break*	*Coffee Break*
		PL I: **Lynn Margulis, USA** Eukaryosis: Protist origins from bacterial communities	WS SE
12.00			
		Lunch	*Lunch*
13.00			
14.00		PL II: **Peter Morin, USA** Protists as model organisms in search of ecosystem functioning	PL IV: **John Boothroyd, USA** *Toxoplasma gondii:* Molecular determinants of pathenogenicity
15.00	Poster Set-up	WS SE	WS SE
16.00		*Coffee Break*	*Coffee Break*
17.00		PS I	PS II
	Welcome Reception		
18.00		Annual Meeting of the DGP Annual Meeting of the DGPF	FEPS Meeting
19.00			

PL → Plenary Lecture // WS → Workshops // SE → Sessions // PS → Poster Session

Time	Wednesday, 27 July	Thursday, 28 July	Friday, 29 July
8.00			
9.00	**Concepts in Protistology Part I** **Jens Boenigk, DE** Keynote Lecture	PL V: **Aaron Turkewitz, USA** Intracellular trafficking in ciliates	PL VII: **Masahiro Fujishima, JP** Endosymbiosis in *Paramecium*
10.00	*Coffee Break*	WS SE	WS SE
	Concepts in Protistology Part II		
11.00		*Coffee Break*	*Coffee Break*
		WS SE	WS SE
12.00			
	Lunch	*Lunch*	
13.00			Farewell
14.00	**Symposium in Honour of Prof. Dr. Miklós Müller Part I**	PL VI: **Linda Sperling, FR** *Paramecium* genome	
15.00		WS SE	
	Coffee Break		
16.00	**Part II**		
		Coffee Break	
17.00		PS III	
18.00			
19.00		Congress Dinner at Seminaris Campus Hotel Berlin	

PL → Plenary Lecture // WS → Workshops // SE → Sessions // PS → Poster Session

In the programme the presenter of the contribution is the first author except for those where the presenter is underlined.

▸ Sunday, 24 July 2011

17.30 – 18.30 h Plant Physiology Lobby

Welcome Reception

▸ Monday, 25 July 2011

09.00 – 10.00 h Plant Physiology Auditorium Maximum

Opening Ceremony
Chair: Klaus Hausmann, Berlin, Germany

Manfred and Christina Kage, Weissenstein, Germany
Protist meditation

Thomas Weisse, Mondsee, Austria
Welcome by President of DGP

Klaus Hausmann, Berlin, Germany
Welcome by President of VI ECOP

Jens-Peter Fürste, Berlin, Germany
Virtual historical walk

10.00 – 11.00 h Plant Physiology Auditorium Maximum

Award Ceremony
Chair: Thomas Weisse, Mondsee, Austria

Helmut Plattner, Konstanz, Germany
Eduard-Reichenow-Medal Laudatio

Janine Beisson, Gif-sur-Yvette, France
Laureate Eduard-Reichenow-Medal

Wilhelm Foissner, Salzburg, Austria
Wilhelm und Ilse Foissner-Stiftung Laudatio

Peter Vd'ačný, Bratislava, Slovakia
Laureate Wilhelm und Ilse Foissner-Stiftung

11.00 – 11.30 h Coffee break

11.30 – 12.15 h Plant Physiology Auditorium Maximum

Plenary Lecture I
Chair: Renate Radek, Berlin, Germany

Lynn Margulis, Amherst, USA
Eukaryosis: Protist origins from bacterial communities

12.30 – 14.00 h Lunch

14.00 – 14.45 h Plant Physiology Auditorium Maximum

Plenary Lecture II
Chair: Stephen Wickham, Salzburg, Austria

Peter Morin, New Brunswick, USA
Protists as model organisms in studies of community ecology and ecosystem functioning

15.00 – 16.30 h Plant Physiology Auditorium Maximum

Workshop: Using the diversity of protists to educate students and the public about evolution
Chairs: Avelina Espinosa, Bristol, USA
Janet Keithly, Albany, USA

Guillermo Paz-y-Miño C., North Dartmouth, USA
The impact of creationism and Intelligent Design (ID) in the public's perception of evolution: Strategies adapted to regional, national or international idiosyncrasies

Avelina Espinosa, Bristol, USA
Archamoebae (Amoebozoa) as case examples to illustrate diverse protistan evolutionary history

Janet Keithly, Albany, USA
Anaerobic protists with mitochondrion-related organelles as evidence of evolution

Samuel Bowser, Albany, USA,
Jefrey L. Travis, Andrea Habura
Foraminiferan protists as pontiffs of evolution

15.00 – 16.30 h Plant Physiology
Lecture Hall 2

Workshop: Protist genomics: The next generation
Chair: Joel Dacks, Edmonton, Canada

Joel Dacks, Edmonton, Canada
Emily Herman, Alex Greninger, Govinda Vishvesvara, Francine Marciano-Cabral, Charles Chiu
Initial insights from the Naegleria fowleri genome initiative

Jane Carlton, New York, USA
From malaria pathogens to sexually transmitted bugs: Comparative genomics of parasites

Xiaoyu Zhang, San Marcos, USA
Chrystal Snroepfer, Ahmed Hadaegh, Betsy Read
Dissecting biomineralization by mining the transcriptome of two closely related coccolithophorids, Emiliania huxleyi and Isochrysis galbana

15.00 – 16.30 h Botanical Museum

Session: Ecology I: Ciliate communities
Chairs: Bettina Sonntag, Mondsee, Austria
Alan Warren, London, UK

Young-Ok Kim, Geoje, Republic of Korea
Kyoung-Soon Shin, Eun-Jin Yang, Jae-Hoon Noh
Tintinnid species as biological sensors for monitoring the Kuroshio Extension in Korean coastal waters

Barbara Kammerlander, Innsbruck, Austria
Ruben Sommaruga, Bettina Sonntag
Ciliate assemblages and their vertical distribution in two alpine lakes of contrasting transparency

Lucia Safi, Porto Alegre, Brazil
Laura Utz, Nelson Fontoura, Henrique Severo, Ana Carolina Rodrigues
Peritrich community succession in a subtropical lake in southern Brazil

Irena Telesh, St. Petersburg, Russia
Ekaterina Mironova, Sergei Skarlato
Planktonic ciliates in the Baltic Sea: Species diversity, community structure and seasonal succession

Weibo Song, Qingdao, China
Alan Warren, Khaled Al-Rasheid, Xiaofeng Lin
Diversity of free-living marine ciliates in Chinese coastal waters, with notes on new taxa from the South China Sea

Alan Warren, London, UK
Xiaozhong Hu, Mingzhuang Zhu, Weibo Song
Documenting the marine free-living ciliate diversity of northern China

15.00 – 16.30 h Zoology

Session: Phylogeny I: Ciliated protists I
Chairs: Michaela Strueder-Kypke, Guelph, Canada
Xiaozhong Hu, London, UK

Wilhelm Foissner, Salzburg, Austria
Thorsten Stoeck, Sabine Agatha,
Micah Dunthorn
Intraclass evolution and classification of the Colpodea (Ciliophora)

Yuanjun Zhao, Chongqing, China
Fahui Tang, Alan Warren
Phylogenetic analyses of trichodinids (Ciliophora, Oligohymenophora, Mobilia) inferred from 18S rRNA gene sequence data

Denis Lynn, Vancouver, Canada
Chitchai Chantangsi, Sonja Rueckert, Anna Prokopowicz, Michaela Strueder-Kypke, Brian Leander
A new genus and species of apostome ciliate infecting the hyperiid amphipod Themisto libellula in the Canadian Beaufort Sea (Arctic Ocean)

Chen Shao, Xi'an, China
Weibo Song, Helmut Berger
A new species of Hemigastrostyla and notes on the non-monophyly of some oxytrichid genera (Ciliophora, Hypotricha)

16.30 – 17.00 h Coffee break

Poster Session I
(for details please see pages 27 – 32)

17.00 – 18.00 h Plant Physiology Lobby

Annual Meeting of the DGP

18.00 – 19.00 h Plant Physiology Lecture Hall 2

Annual Meeting of the DGPF

19.00 – 1930 h Plant Physiology Lecture Hall 2

► Tuesday, 26 July 2011

09.00 – 09.45 h Plant Physiology Auditorium Maximum

Plenary Lecture III
Chair: Alastair Simpson, Halifax, Canada

Patrick Keeling, Vancouver, Canada
Comparative genomics of protists

10.00 – 12.30 h Plant Physiology Auditorium Maximum

Workshop: Species taxonomy of protists: Morphological and molecular perspectives
Chairs: Wilhelm Foissner, Salzburg, Austria
Peter Vd'ačný, Bratislava, Slovakia

Wilhelm Foissner, Salzburg, Austria
Species taxonomy of protists: Morphological and molecular perspectives

David Bass, London, UK
Species taxonomy of protists: morphological and molecular perspectives from flagellates

Ralf Meisterfeld, Aachen, Germany
Species taxonomy of protists: Morphological and molecular perspectives in testate amoebae

Peter Vd'ačný, Bratislava, Slovak Republic
Species taxonomy of protists: Morphological and molecular perspectives in ciliates

10.00 – 12.30 h Plant Physiology Lecture Hall 2

Workshop: Pathogen-host interactions of apicomplexan parasites
Chairs: Richard Lucius, Berlin, Germany
Nishith Gupta, Berlin, Germany

Richard Lucius, Berlin, Germany
Jörg Stange, Matthew Hepworth, Anja Kühl, Sebastian Rausch
IFN-γ and its receptor are essential for the suppression of pathological Th17 responses during infections with an intracellular intestinal parasite

Richard Lucius, Berlin, Germany
Manuela Schmid, Nishith Gupta
The apicomplexan parasite Eimeria falciformis co-opts host indolamine-2, 3-dioxygenase for its life cycle progression

Hanna Lucia Worliczek, Vienna, Austria
Bärbel Ruttkowski, Lukas Schwarz, Débora Wernitznig, Anja Joachim
An in vitro model of neonatal porcine coccidiosis: Isospora suis in an epithelial cell culture system

Richard Lucius, Berlin, Germany
Vera Sampels, Isabelle Dietrich, Lilach Sheiner, Boris Striepen, Thomas Pomorski, Isabelle Coppens, Nishith Gupta
Membrane biogenesis in Toxoplasma gondii: De novo synthesis versus selective scavenging of major phospholipids by the parasite

Richard Lucius, Berlin, Germany
Martin Blume, Marion Hliscs, Diana Rodriguez-Contreras, Marco Sanchez, Scott Landfear, Tobias Feige, Dominique Soldati-Favre, Kai Matuschewski, Nishith Gupta
A tale of twins: One addicted to sugared candy and the other not

10.00 – 12.30 h Botanical Museum

Workshop: Termite flagellates I
Chairs: Renate Radek, Berlin, Germany
Andreas Brune, Marburg, Germany

Moriya Ohkuma, Wako, Japan
Roles of symbiotic flagellated protists in the gut of termites in efficient utilization of cellulose

Vladimír Hampl, Prague, Czech Republic
Phylogeny and classification of parabasalids: Situation in 2011

Kevin Carpenter, Livermore, USA
Peter Weber, Patrick Keeling, Jennifer Pett-Ridge, Lee Davisson, Michael Haverty, Moriya Ohkuma
Unraveling protist-bacterial symbioses in the termite gut: Inferences from structural and experimental studies with scanning and transmission electron microscopy and NanoSIMS

Franck Dedeine, Tours, France
Evolutionary dynamics of the protist communities living in the hindgut of lower termites

10.00 – 11.00 h Zoology

Session: Ecology II: Nutrition
Chairs: Thomas Posch, Kilchberg, Switzerland
Anja Scherwass, Cologne, Germany

Andre Schieffer, Cologne, Germany
Mar Monsonis Nomdedeu, Christine Willen, Hartmut Arndt
Ciliate predation mediated phenotypic response and chaotic coexistence in an experimental microbial food web

Stephen Wickham, Salzburg, Austria
Monika Claessens
Explaining a paradox: Response of ciliates and microbes to nutrient addition in a highly oligotrophic system

Miroslav Macek, Tlalnepantla, Mexico
Antonio Picazo, Antonio Camacho
Why does picocyanobacteria-feeding Spirostomum teres accompany zoochlorella-bearing Pelagothrix plancticola in the oxycline of a meromictic lake?

10.00 – 11.00 h Theater Studies

Session: Parasitology I: Flagellates
Chairs: Lori Peacock, Langford, UK
Heather Esson, Vienna, Austria

Lori Peacock, Langford, UK
Vanessa Ferris, Reuben Sharma, Jack Sunter, Mick Bailey, Mark Carrington, Wendy Gibson
Identification of the meiotic life cycle stage of Trypanosoma brucei in the tsetse fly

Wendy Gibson, Bristol, UK
Lori Peacock, Vanessa Ferris, Mick Bailey
Transfer of the human infectivity trait by sexual reproduction in Trypanosoma brucei

Kristyna Markova, Prague, Czech Republic
Magdalena Uzlikova, Eva Nohynkova
Giardia intestinalis and regulation of mitotic progression

Pavla Smejkalova, Prague, Czech Republic
Klara Petrzelkova, David Modry, Ivan Čepička
Diversity and host specificity of intestinal trichomonads of non-human primates

11.00 – 11.30 h Coffee break

11.30 – 12.30 h Zoology

Session: Molecular Biology I: Extraordinary organelles
Chairs: Roberto Docampo, Athens, USA
Jude Przyborski, Marburg, Germany

Vojtech Vacek, Prague, Czech Republic
Jan Fousek, Jitka Hlavackova, Jan Pyrih, Jakub Ridl, Cestmir Vlcek, Vladimír Hampl
Searching for mitochondria of Monocercomonoides

Michelle Leger, Halifax, Canada
Laura A. Hug, Andrew J. Roger
A hydrogenosome in the free-living excavate Andalucia incarcerata: Common themes in mitochondrial reduction in anaerobic eukaryotes

David Leitsch, Vienna, Austria
Daniel Kolarich, Michael Duchêne
NADP-dependent alcohol dehydrogenase I from Trichomonas vaginalis: An unusual bifunctional enzyme

Jude Przyborski, Marburg, Germany
Protein transport to the apicoplast of apicomplexan parasites

11.30 – 12.30 h Theater Studies

Session: Phylogeny II: Ciliated protists II
Chairs: Sabine Agatha, Salzburg, Austria
Weibo Song, Qingdao, People's Republic of China

Michaela Strueder-Kypke, Guelph, Canada
Sabine Agatha, Denis H. Lynn
Polyphyly and paraphyly within the order tintinnida: How can we solve the dilemma?

Letizia Modeo, Pisa, Italy
Roberto J. P. Dias, Noemi Fernandes, Gabriele Tomei, Vittorio Boscaro, Franco Verni, Inacio da Silva-Neto, Giulio Petroni
Phylogenetic systematics of the genus Condylostoma (Ciliophora, Heterotrichea) by means of a multidisciplinary analytical study of some marine and brackish morphospecies

Xiaofeng Lin, Guangzhou, China
Zhenzhen Yi, Weibo Song
Classification and phylogeny of pleurostomatid ciliates (Ciliophora, Protozoa)

12.30 – 14.00 h Lunch break

14.00 – 14.45 h Plant Physiology Auditorium Maximum

Plenary Lecture IV
Chair: Richard Lucius, Berlin, Germany

John Boothroyd, Stanford, USA
Viennese Waltz or Berlin Rave: How Toxoplasma injects polymorphic proteins that determine the character of its dance with the host

15.00 – 16.30 h Plant Physiology Auditorium Maximum

Workshop: Trafficking
Chair: Helmut Plattner, Konstanz, Germany

Angelika Noegel, Cologne, Germany
Nosa Napoleon Omosigho, Tanja Rihyai
The professional phagocyte Dictyostelium discoideum

Markus Meissner, Glasgow, UK
Systematic analysis of protein trafficking in the apicomplexan parasite Toxoplasma gondii

Mark Field, Cambridge, UK
Ka-Fai Leung
Ubiquitin and turnover of the trypanosome surface

15.00 – 16.30 h Botanical Museum

Workshop: Termite flagellates II
Chairs: Renate Radek, Berlin, Germany
Betsey Dyer, Norton, USA

Jürgen Strassert, Marburg, Germany
Renate Radek, Andreas Brune
Diversity and localization of bacterial symbionts associated with Trichonympha flagellates in lower termites

Renate Radek, Berlin, Germany
Stephanie Tamschick
Colonization and structure of the hindgut wall of lower termites

Betsey Dyer, Norton, USA
Re-inventing the wheel: Overcoming low Reynolds numbers convergently, in the termite hindgut

Gillian Gile, Halifax, Canada
Phylogenetic position of Lophomonas and implications for character evolution in Parabasalia

Ivan Čepička, Prague, Czech Republic
Vit Ceza, Frantisek Stahlavsky
Extensive molecular and morphological diversity of free-living trichomonads

15.00 – 16.30 h Plant Physiology Lecture Hall 2

Session: Parasitology II: Apicomplexans
Chairs: Maria Cristina Angelici, Rome, Italy
Sonja Rueckert, Shimoda, Japan

Sonja Rueckert, Shimoda, Japan
Petra Villette, Brian Leander
Species boundaries in gregarine apicomplexan parasites: A case study. Comparison of morphometric and molecular variability in Lecudina cf. tuzetae (Eugregarinorida, Lecudinidae)

Maria Cristina Angelici, Rome, Italy
Pietro di Pinto, Cristina Giuliani, Antonella Vimercati, Michelina Pugliese, Valentina Terio, Elisabetta Monteduro, Angela di Pinto, Giuseppina Marilia Tantillo
Biodiversity as indicator of environmental risk factors in molecular epidemiology studies: The case of Toxoplasma gondii

Sarah Reiff, Athens, USA
Lilach Sheiner, Shipra Vaishnava, Boris Striepen
Genetic dissection of the maintenance and replication of the apicoplast genome in Toxoplasma gondii

Farida Ghalmi, Algiers, Algeria
Bernard China, Bertrand Losson
Neospora caninum is associated to abortion in Algerian cattle

15.00 – 16.30 h Zoology

Session: Molecular Biology II: Molecular diversity
Chairs: Fernando Dini, Pisa, Italy
Frank Nitsche, Cologne, Germany

Anne Domonell, Cologne, Germany
Frank Nitsche, Hartmut Arndt
Comparative study of cercomonad community structure at different soil sites by high-throughput parallel tag sequencing

Lucie Bittner, Roscoff, France
Elianne S. Egge, Sarah Romac, Ian Probert, Stéphane Audic, Bente Edvardsen, Colomban de Vargas, The BioMarKs Consortium
A pluralistic approach to interpreting protistan diversity using Next Generation Sequencing data: The case of Haptophyta

Michael MacGillivary, Sackville, Canada
Irena Kaczmarska
Molecular assessment of natural and ship ballast derived Paralia (Bacillariophyta) populations reveals worldwide cryptic diversity

Laura Mather, Sackville, Canada
France Muise, Irena Kaczmarska
Morphological and genetic comparison of the diatom Asterionellopsis glacialis in relation to biogeography

Alexandra Stock, Kaiserslautern, Germany
Hans-Werner Breiner, Maria Pachiadaki, Virginia Edgcomb, Violetta La Cono, Michail Yakimov, Thorsten Stoeck
Exploring micro-eukaryotic life in the deep hypersaline anoxic lake Thetis

Sabine Filker, Kaiserslautern, Germany
Thorsten Stoeck
Environmental metatranscriptome analysis of a hypersaline anoxic deep-sea basin (Urania, Mediterranean)

15.00 – 16.30 h Theater Studies

Session: Phylogeny III: Ameboid and flagellated protists
Chairs: Alexander Kudryavtsev, Geneva, Switzerland
Alastair Simpson, Halifax, Canada

Alastair Simpson, Halifax, Canada
Jong-Soo Park, Byung-Cheol Cho
The diversity and evolution of obligately halophilic protozoa

Jan Weinert, Cologne, Germany
The diversity of naked amoebae in soil along a gradient of forest management intensities

Alexander Kudryavtsev, Geneva, Switzerland
Jan Pawlowski, Klaus Hausmann
Expanding our knowledge of Amoebozoa: Atlantic and Pacific deep-sea amoebae from two expeditions – phylogenetic and taxonomic implications

Matthew Brown, Halifax, Canada
Jeffrey Silberman, Frederick Spiegel,
Martin Kolisko, Andrew Roger
Evolutionary history of aggregative multicellularity: Insights from phylogenomics of Guttulinopsis

Vasily Zlatogursky, St. Petersburg, Russia
Biodiversity, taxonomy and evolution of "Heliozoa"

Áron Keve Kiss, Vác, Hungary
Éva Ács, Keve Tihamér Kiss, Hartmut Arndt
Transmitted light super-resolution nanoscopy: The real-time ultrastructure of living protists

16.30 – 17.00 h Coffee break

Poster Session II
(for details please see pages 27 – 32)

17.00 – 18.00 h Plant Physiology Lobby

FEPS Meeting

18.00 – 19.00 h Plant Physiology Lecture Hall 2

▶ Wednesday, 27 July 2011

09.00 – 10.00 h Plant Physiology Auditorium Maximum

Focus: Concepts in Protistology I
Chair: Jens Boenigk, Essen, Germany

Jens Boenigk, Essen, Germany
Concepts in protistology: Protist classification and diversity in the crossfire of evolutionary differentiation and historic misconceptions

Marc Ereshefsky, Calgary, Canada
The conceptual basis of species

10.00 – 10.30 h Coffee break

10.30 – 12.30 h Plant Physiology Auditorium Maximum

Focus: Concepts in Protistology II
Chair: Jens Boenigk, Essen, Germany

Kerstin Hoef-Emden, Cologne, Germany
Species concepts

James Mallet, London, UK
Speciation is easy: It's all around us!

David Bass, London, UK
Conceptual progress in protistology: Conclusions and ways forward

10.30 – 12.30 h Plant Physiology Lecture Hall 2

Workshop: Inter- and intracellular signalling
Chairs: Helmut Plattner, Konstanz, Germany
Angelika Noegel, Cologne, Germany

Martin Simon, Kaiserslautern, Germany
Simone Marker, Miriam Cheaib, Ulrike Guenzler, Timo Oppermann, Alexandra Müller, Eva Schloter, Eric Meyer
Epigenetic regulation of the surface protein coat of Paramecium tetraurelia

Pierangelo Luporini, Camerino, Italy
Adriana Vallesi, Claudio Alimenti
Pheromone-mediated cell-cell signaling in Euplotes

Kildare Miranda, Rio de Janeiro, Brazil
Wanderley de Souza, Roberto Docampo
Signaling pathways involved in the dynamic control of the contractile vacuole complex and regulatory volume decrease in trypanosomatid parasites

Helmut Plattner, Konstanz, Germany
High diversification of intracellular calcium-release channels in Paramecium cells

10.30 – 12.30 h Botanical Museum

Workshop: Evolutionary aspects of energy metabolism in protists
Chair: Aurelio Serrano, Sevilla, Spain

Fabio Facchinelli, Düsseldorf, Germany
Functional and comparative analysis of the plastid proteome of the glaucophyte Cyanophora paradoxa and the red alga Cyanidioschyzon merolae

Michael Ginger, Lancaster, UK
Robert Brown, Peter Collingridge
Flagellar energy metabolism in protists and a possible loss of flagellar glycolysis during trypanosomatid evolution

Roberto Docampo, Athens, USA
Noelia Lander, Guozhong Huang, Zhu-Hong Li, Silvia N.J. Moreno
The role of acidocalcisomes in the stress response of Trypanosoma cruzi and T. brucei

Aurelio Serrano, Sevilla, Spain
Gloria Serrano Bueno, Agustín Hernández, José R. Pérez Castiñeira
Evolutionary dynamics of inorganic pyrophosphatases in photosynthetic protists: Endosymbiotic replacements, lateral gene transfers and domain fusions

12.30 – 14.00 h Lunch break

14.00 – 17.00 h Theater Studies

Miklós Müller Symposium
Chair: Klaus Hausmann, Berlin, Germany

Samuel Bowser, Albany, USA
Welcome

Klaus Hausmann, Berlin, Germany
Laudatio

15.30 – 16.00 h Coffee break

Andreas Brune, Marburg, Germany
Honorary Lecture: Bacterial symbionts of termite gut flagellates: A tripartite symbiosis

Jan Tachezy, Czech Republic
Honorary Lecture: The hydrogenosome: Biogenesis, function and evolution

▸ Thursday, 28 July 2011

09.00 – 09.45 h Plant Physiology
Auditorium Maximum

Plenary Lecture V
Chair: Helmut Plattner, Konstanz, Germany

Aaron Turkewitz, Chicago, USA
Membrane traffic in ciliates

10.00 – 12.30 h Plant Physiology
Auditorium Maximum

Workshop: Spatial patterns of protistan biodiversity I
Chairs: Thorsten Stoeck, Kaiserslautern, Germany
Micah Dunthorn, Kaiserslautern, Germany

Alban Ramette, Bremen, Germany
Deciphering the effects of space and of environmental heterogeneity on the variation of microbial communities at the global scale

John Dolan, Villefranche-sur-Mer, France
Similarity in widely separated communities of planktonic protists (tintinnid ciliates)

Bettina Sonntag, Mondsee, Austria
Ciliate biodiversity patterns in alpine and subalpine lakes

Wilhelm Foissner, Salzburg, Austria
How resting cysts, spatial constraints, time and endemics structure protist communities

10.00 – 12.30 h Plant Physiology
Lecture Hall 2

Workshop: Biodiversity, phylogeny and species problem among Amoebozoa I
Chairs: Alexey Smirnov, St. Petersburg, Russia
Jan Pawlowski, Geneva, Switzerland

Alexey Smirnov, St. Petersburg, Russia
Lobose amoebae: Diversity, phylogeny and species problem

Anna Maria Fiore-Donno, Greifswald, Germany
M. Meyer, Y. Novozhilov, Martin Schnittler
Species concept in Myxogastria (Amoebozoa): Morphology, mating and molecules

Eliška Ptácková, Prague, Czech Republic
Ivan Čepička
The enigmatic amoeboflagellate Rhizomastix is a member of Archamoebae and a close relative of the genus Entamoeba

Ludmila Chistyakova, St. Petersburg, Russia
Alex Kostygov, Alexander Frolov
Diversity of Pelomyxa species (Archamoebae, Pelobiontida)

10.00 – 11.00 h Botanical Museum

Session: Parasitology III: Spore forming parasites
Chairs: Fathy Abdel-Ghaffar, Giza, Egypt
Elena Nassonova, St. Petersburg, Russia

Fathy Abdel-Ghaffar, Giza, Egypt
Heinz Mehlhorn, Abdel-Rahman Bashtar, Saleh Al-Quraishy, Khaled A.S. Al-Rasheid, Kareem Morsy
Myxosporidia infecting Nile fishes in Egypt, light and electron microscopic study

Elena Nassonova, St. Petersburg, Russia
Alexander Gorbunov, Anton Naumov, Irma Issi, Alexey Smirnov
Molecular phylogeny of Bertramia asperospora, a protozoan rotifer parasite with obscure taxonomic position: Shuffling cards in favor of the ichthyosporeans

Scott Campbell, Exeter, UK
Darren Soanes, Bryony Williams
Dissecting the microsporidian secretome: An interface between host and parasite

Kristina Hamilton, Exeter, UK
Bryony Williams
Environmental diversity of an intracellular parasite group, the Microsporidia

10.00 – 11.00 h Zoology

Session: Molecular Biology III: Molecular properties of cell constituents
Chairs: Maria Rautian, St. Petersburg, Russia
Paola Ricciolini, Pisa, Italy

Elena Sabaneyeva, St. Petersburg, Russia
Konstantin A. Benken, V.V. Kosheverova
Can actin be involved in contractile vacuole functioning in Paramecium caudatum?

Sven Gould, Düsseldorf, Germany
Geoff McFadden, Ross Waller
Charged repeat motifs: Common characteristic of eukaryotic cytoskeleton proteins

Maria Rautian, St. Petersburg, Russia
Properties of species structure in ciliates as a consequence of nuclear organization properties

Paola Ricciolini, Pisa, Italy
Fernando Dini, Graziano di Giuseppe
High-level interspecific diversity of Euplotes revealed by assessment of mitochondrial cox1 gene for ciliate DNA barcoding

10.00 – 11.00 h Theater Studies

Session: Special Topics I: Associations with algae
Chairs: Angelika Preisfeld, Wuppertal, Germany
Michael Schweikert, Stuttgart, Germany

Mark Freeman, Kuala Lumpur, Malaysia
Ales Horak, Matthias Eydal, Patrick Keeling
X-cell parasites of Atlantic cod are basal dinoflagellates

Sebastian Hess, Cologne, Germany
Nicole Sausen, Michael Melkonian
Protistan parasites of freshwater algae

Jon Bråte, Oslo, Norway
Anders Krabberød, Jane Dolven, Randi Ose, Tom Kristensen, Kjell Bjørklund, Kamran Shalchian-Tabrizi
Radiolaria revealed as a reservoir for marine alveolates

David Lloyd, Cardiff, UK
Ultradian clocks (with periods of about one hour) in protists constitute a synchronizing time base and coherence for organized complexities

11.00 – 11.30 h Coffee break

11.30 – 12.30 h Botanical Museum

Session: Phylogeny IV: Flagellated protists I
Chairs: Hartmut Arndt, Cologne, Germany
Vladimír Hampl, Prague, Czech Republic

Naoji Yubuki, Vancouver, Canada
Brian Leander
Comparative ultrastructure of Fornicata excavate, Kipferlia bialata

Tomáš Pánek, Prague, Czech Republic
Vladimír Hampl, Eliska Ptackova, Jeffrey D. Silberman, Naoji Yubuki, Brian S. Leander, Ivan Cepicka
The evolution, diversity and biogeography of anaerobic Heterolobosea (Excavata)

Jana Szabová, Prague, Czech Republic
Jan Fousek, Jakub Rídl, Cestmír Vicek, Vladimír Hampl
The evolution of MAT and MATX genes in euglenids

Anna Karnkowska-Ishikawa, Warsaw, Poland
Donovan Watza, Matthew Bennett, Bozena Zakrys, Richard E. Triemer
Evolutionary relationships of green euglenoids inferred from taxon-rich analyses of 5 genes

11.30 – 12.30 h Zoology

Session: Ecology III: Spatial distribution of protists
Chairs: Thomas Weisse, Mondsee, Austria
Stephen Wickham, Salzburg, Austria

James Mallet, London, UK
Problemy evolutsii i ekologii: Gause's r-K formulation of competition and the apparent discord between ecology and evolution

Felix Weber, Rostock, Germany
Javier del Campo, Claudia Wylezich, Ramon Massana, Klaus Jürgens
Taxonomic novelty of heterotrophic protists revealed by unamended brackish water incubations from the Baltic Sea

Esra Elif Aydin Dede, Ankara, Turkey
Won Je Lee
Free-living heterotrophic flagellates from intertidal sediments of Saros Bay, Aegean Sea (Turkey)

11.30 – 12.30 h Theater Studies

Session: Special Topics II: Parasites
Chairs: Wendy Gibson, Bristol, UK
Julia Walochnik, Vienna, Austria

Mirjana Drinic, Vienna, Austria
Florian Astelbauer, Adriane Raninger, David Leitsch, Walther Wernsdorfer, Brigitte Brem, Andreas Obwaller, Julia Walochnik, Harald Greger, Michael Duchêne
In vitro activity of methylgerambullin from Glycosmis mauritiana against Entamoeba histolytica and Giardia intestinalis

Maria Vanessa Gomez Ramirez, Bogotá, Colombia
Moises Wasserman
Detection of two spliceosomal proteins in the divergent eukaryote Giardia intestinalis

Olimpia Coppellotti, Padova, Italy
Clara Fabris, Marina Soncin, Laura Guidolin
Photosensitized inactivation of protozoa in the medical and environmental control of waterborne diseases

Sigrid Neuhauser, Innsbruck, Austria
David Bass, Simon Bulman, Martin Kirchmair
Phylogeny and biodiversity of phytomyxea („Plasmodiophorids")

12.30 – 14.00 h Lunch break

14.00 – 14.45 h Plant Physiology Auditorium Maximum

Plenary Lecture VI
Chair: Helmut Plattner, Konstanz, Germany

Linda Sperling, Gif-sur-Yvette, France
Paramecium genome organization and evolution

15.00 – 16.30 h Plant Physiology Auditorium Maximum

Workshop: Spatial patterns of protistan biodiversity II
Chairs: Thorsten Stoeck, Kaiserslautern, Germany
Micah Dunthorn, Kaiserslautern, Germany

Mona Hoppenrath, Wilhelmshaven, Germany
Biodiversity patterns in dinoflagellates

Ramon Massana, Barcelona, Spain
Raquel Rodríguez-Martínez
Genetic structure and biogeography of an abundant, widespread, and uncultured marine protist group, the MAST-4

Jan Pawlowski, Geneva, Switzerland
Franck Lejzerowicz, Béatrice Lecroq
Global distribution of deep-sea protists: Insights from Next Generation Sequencing

Virginia Edgcomb, Woods Hole, USA
William Orsi, Hans-Werner Breiner, Michail Yakimov, Thorsten Stoeck
Spatial distribution of kinetoplastid flagellates in hypersaline anoxic deep-sea basins in the Eastern Mediterranean

15.00 – 16.30 h Plant Physiology Lecture Hall 2

Workshop: Forams and Radiolaria: Getting together I
Chair: Jan Pawlowski, Geneva, Switzerland

Roberto Sierra, Geneva, Switzerland
Jan Pawlowski
Evolution of Rhizaria: comparing transcriptomes of Foraminifera and Acantharea

Cedric Berney, London, UK
David Bass
Diversity and phylogeny of Endomyxa, the possible ancestors of Foraminifera and Radiolaria

Samuel Bowser, Albany, USA
Andrea Habura, Jeffery L. Travis
Compare and contrast: Pseudopodia of Foraminifera, Radiolaria and Gromia

Martin Brasier, Oxford, UK
Jonathan Antcliffe
A Protistan Explosion coincident with the Cambrian Explosion of animal life: In search of Precambrian rhizarians and their relatives

15.00 – 16.30 h Botanical Museum

Workshop: Control mechanisms of establishment of the secondary symbiosis and kleptoplastids
Chairs: Yuuki Kodama, Kochi, Japan
Matthew Johnson, Woods Hole, USA

Yuuki Kodama, Kochi, Japan
Masahiro Fujishima
Control mechanisms of establishment of the endosymbiosis between Paramecium bursaria and symbiotic Chlorella sp.

Ryo Hoshina, Kusatsu, Japan
Algal symbionts of Paramecium bursaria: Origins and diversification

Matthew Johnson, Woods Hole, USA
Chris Brown, Holly Moeller
Karyoklepty and the reduced endosymbiont of Mesodinium rubrum: A tertiary plastid in the making?

Myung-Gil Park, Gwangju, Republic of Korea
Miran Kim, Seung-Won Nam, Woong-Ghi Shin, D. Wayne Coats
On the way to gaining plastids in the marine dinoflagellate genus Dinophysis?

15.00 – 16.30 h Zoology

Session: Ecology IV: Biogeography and diversity of protists
Chairs: Thomas Berendonk, Germany
Irena Telesh, St. Petersburg, Russia

Manfred Wanner, Cottbus, Germany
Daniel Puppe, Michael Elmer, Michael Sommer
Testate amoebae spatiotemporal dynamics of biogenic silica pools and their relevance for desilication

Thomas Weisse, Mondsee, Austria
Ulrike Scheffel, Peter Stadler
Temperature dependent resistance to starvation of the freshwater ciliate Meseres corlissi Petz and Foissner, 1992 (Ciliophora, Spirotrichea)

Sabine Agatha, Salzburg, Austria
Diversity and biogeography of oligotrichid and aloricate choreotrichid ciliates (Protista, Ciliophora, Spirotricha, Oligotrichea) in marine and brackish sea water

Sergei Skarlato, St. Petersburg, Russia
Irena Telesh, Hendrik Schubert
Novel biodiversity pattern: A protistan species maximum in the horohalinicum of the Baltic Sea

Ute Risse-Buhl, Bad Saarow, Germany
Denise M. Akob, Martina Herrmann, Patricia Geesink, Natalia Pizani, Wilfried Schönborn, Kirsten Küsel
Polyphasic characterization of protist diversity in the subsurface of a Thuringian aquifer

15.00 – 16.30 h Theater Studies

Session: Special Topics III: Harmful bacteria
Chairs: Sergei Fokin, Pisa, Italy
Hans-Dieter Görtz, Stuttgart, Germany

Júlia Katalin Török, Budapest, Hungary
Orsolya Molnár, Zsuzsanna Bartha, Csaba Kiss, Károly Márialigeti
Amoeba-resisting bacteria in testate amoebae: Insights from the long term investigation of an Arcella strain and fresh environmental samples

Claudia Vannini, Pisa, Italy
Filippo Ferrantini, Martina Schrallhammer, Alessandro Ristori, Giovanna Rosati, Franco Verni, Giulio Petroni
Symbiosis evolution: Establishment, replacements and re-replacements in the association between Polynucleobacter-like bacteria and the ciliate Euplotes

Sergei Fokin, Pisa, Italy
Vittorio Boscaro, Mickael Schweikert, Giulio Petroni
New Holospora-like bacterium from the Paramecium genus

Martina Schrallhammer, Dresden, Germany
Valerio Vitali, Thomas U. Berendonk, Alexey Potehkin, Hans-Dieter Görtz, Giulio Petroni
First molecular description of the Paramecium parasite Holospora caryophila

Maria Rautian, St. Petersburg, Russia
Natalia Wackerov-Kousova, Natalia Lebedeva, Inna Skoblo
Intranuclear symbionts of paramecia: Genus Holospora revisited

Giulio Petroni, Pisa, Italy
Martina Schrallhammer, Claudia Vannini, Filippo Ferrantini, Vittorio Boscaro, Alessandro Ristori, Valerio Vitali, Michael Schweikert, Hans-Dieter Görtz, Sergei Fokin, Franco Verni
Trojan Ciliates: Selected examples of potentially pathogenic bacterial endocytobionts of ciliates

16.30 – 17.00 h Coffee break

Poster Session III
(for details please see pages 27 – 32)

17.00 – 18.00 h Plant Physiology Lobby

Congress Dinner, Poster Award Ceremony

from 19.00 h Seminaris Campus Hotel
Takustraße 39 · 14195 Berlin

▶ Friday, 29 July 2011

09.00 – 09.45 h Plant Physiology Auditorium Maximum

Plenary Lecture VII
Chair: Hans-Dieter Görtz, Stuttgart, Germany

Masahiro Fujishima, Yamaguchi, Japan
Endosymbiosis in *Paramecium*

10.00 – 12.30 h Plant Physiology Auditorium Maximum

Workshop: Forams and Radiolaria: Getting together II
Chair: Jan Pawlowski, Geneva, Switzerland

Johan Decelle, Roscoff, France
Noritoshi Suzuki, Frederic Mahé, Colomban de Vargas, Fabrice Not
Molecular phylogenetics and evolutionary history of planktonic Acantharia (Radiolaria)

Jon Bråte, Oslo, Norway
Anders Krabberød, Jane Dolven, Randi Ose, Dag Klaveness, Tom Kristensen, Kjell Bjørklund, Kamran Shalchian-Tabrizi
18S + 28S rDNA phylogeny divides Radiolaria into Polycystina and Spasmaria and supports the Retaria hypothesis

Loic Pillet, Geneva, Switzerland
Jan Pawlowski
Molecular characterization of kleptoplastidy in Foraminifera

Ivan Kuznetsov, St. Petersburg, Russia
Protelphidium cf. niveum (Lafrenz) from the White Sea: Towards a better view on systematics of primitive elphidiids

10.00 – 12.30 h Plant Physiology Lecture Hall 2

Workshop: Biodiversity, phylogeny and species problem among Amoebozoa II
Chair: Alexey Smirnov, St. Petersburg, Russia

Alexander Kudryavtsev, Geneva, Switzerland
Jan Pawlowski
Genetic structure of amoebozoan morphospecies: A case study on freshwater Cochliopodium spp.

Anna Glotova, St. Petersburg, Russia
Biogeography of amoebae: A case study of seven naked lobose amoebae species isolated from North-American habitats

10.00 – 11.00 h Botanical Museum

Session: Phylogeny V: Flagellated protists II
Chairs: Matthew Brown, Halifax, Canada
Ivan Čepička, Prague, Czech Republic

Hartmut Arndt, Cologne, Germany
Áron Kiss, Cecile Reed, Melanie Müller, Nicole Nopper, Claudia Wylezich, Frank Nitsche
Phylogeny and ecology of bicosoecids

Frank Nitsche, Cologne, Germany
Hartmut Arndt
Is there a phylogenetic separation of marine and freshwater choanoflagellates?

Áron Keve Kiss, Vác, Hungary
Marian Brabender, Anne Domonell, Frank Nitsche, Hartmut Arndt
Phylogenetical, morphological and ultrastructural diversity of novel soil cercomonad species and genera: Few correspondences are found between ultrastructure and 18S rDNA phylogeny

Josephine Scoble, Oxford, UK
Tom Cavalier-Smith
Remarkable genetic and silica-scale diversity in the colourless chrysomonad, Paraphysomonas: Taxonomic and evolutionary implications

11.00 – 11.30 h Coffee break

11.30 – 12.30 h Plant Physiology
Auditorium Maximum

Workshop: Art in Protistology
Chair: Klaus Hausmann, Berlin, Germany

Johan Decelle, Roscoff, France
Colomban de Vargas
Rhizaria: The simultaneous rise of protistology and Art Nouveau

Ivan Kuznetsov, St. Petersburg, Russia
Foraminifera of the White Sea: Expressing biodiversity through zoological drawings

Cyril Obadia, Geneva, Switzerland
Mikro-Dialoge

Patrick Keeling, Vancouver, Canada
Protist themed jewelry

Kevin Carpenter, Livermore, USA
Three protist meditations

Samuel Bowser, Albany, USA
The InterfaCE of Art and Science

11.30 – 12.30 h Botanical Museum

Session: Parasitology IV: Cyst formation
Chairs: Wilhelm Foissner, Salzburg, Austria
David Lloyd, Cardiff, UK

Alexei Kostygov, St. Petersburg, Russia
Alexander Frolov
Cyst-forming trypanosomatids and general issues in systematics of Trypanosomatidae family

Maria Siegesmund, Exeter, UK
Mark van der Giezen
Differential expression of Entamoeba invadens dynamin related proteins in encystation and excystation

Julia Walochnik, Vienna, Austria
Martina Köhsler, David Leitsch, Martina Marchetti-Deschmann, Andrea Deutsch, Günther Allmaier, Michael Duchêne
Encystation in Acanthamoeba: Dynamics on the protein level

Catrin Williams, Cardiff, UK
Miguel A. Aon, Nigel Yarlett, Joanne Cable, David Williams, David Lloyd
Effects of inhibitors on the antioxidant system of Spironucleus vortens

13.00 – 13.30 h Plant Physiology
Auditorium Maximum

Farewell Session

POSTER SESSIONS

Poster sessions are held on Monday, Tuesday, and Thursday, from 17.00 to 18.00 h in the lobby of the Plant Physiology. During these session times, it is the responsibility of the presenter to ensure that at least one of the authors is present.
Only the affiliation of the first author is given.

Posters: Ecology
Chair: Jürgen Strassert, Berlin, Germany

01 *Jael Winkels, Cologne, Germany*
Hartmut Arndt
Direct and indirect competition among heterotrophic nanoflagellates

02 *Thomas Posch, Kilchberg, Switzerland*
Bettina Eugster
Feeding of ciliates on toxic filamentous cyanobacteria in Lake Zurich (Switzerland)

03 *Blanca Pérez-Uz, Madrid, Spain*
Sara Gago, Lucía Arregui, Susana Serrano
Bacterivory and feeding behaviour of protists from wastewater treatment plants (WWTP)

04 *Krzysztof Wiackowski, Kraków, Poland*
Justyna Iwaniec
Simultaneous effect of two predators on prey abundance: Laboratory-microcosm studies with protists

05 *Katharina Sklorz, Cologne, Germany*
Andre Schieffer, Christine Willen, Mar Monsonis Nomdedeu, Hartmut Arndt
Colony formation in a freshwater bacterial strain (Acinetobacter johnsonii) as a result of predation pressure: The role of micro-evolution and phenotypic plasticity

06 *Anja Scherwass, Cologne, Germany*
Johann Cesarz, Hartmut Arndt
Bacterial 'response' to intensive protozoan grazing

07 *Kristina Joachim, Cologne, Germany*
Hartmut Arndt, Frank Nitsche
Investigation on cryo-conservation of free-living heterotrophic flagellates

08 *Andrea Amaroli, Genova, Italy*
Bruno Bianco, Maria Giovanna Chessa
Dictyostelium discoideum: A bioethical model for the study of the effects of Extremely Low-Frequency Electromagnetic Fields (ELF-EMF)

09 *Marwa Shumo, Cologne, Germany*
Hartmut Arndt
Influence of pressure on deep-sea heterotrophic flagellates

10 *Geoffrey Ongondo, Innsbruck, Austria*
Andrew Wamalwa Yasindi, Bettina Sonntag, Jens Boenigk, Michael Schagerl, Steve Omondi
Ciliated protist assemblage in two shallow saline-alkaline lakes in Kenya

11 *Claudia Wylezich, Rostock, Germany*
Klaus Jürgens
Protist diversity in suboxic and sulfidic waters of the Black Sea

12 *Abela-Posada Andrea, México, México*
Chávez-Avila Sandra, Mariño-Pérez Ricardo, Rosaura Mayén Estrada
Mexican arthropod-ciliated protozoa system: An overview

13 *Carsten Dietrich, Marburg, Germany*
Andreas Brune
Fermentation products of termite gut flagellates and the effect of oxygen

14 *Daniela Pöll, Innsbruck, Austria*
Bettina Sonntag
Do heterotrophic freshwater ciliates acquire UV sunscreen compounds from algal food?

15 *Adalgisa Fernanda Cabral, Maringá, Brazil*
Paulo Roberto Bressan Buosi, Fábio Amodeo Lansac-Tôha, Laura Roberta Pinto Utz, Luiz Felipe Machado Velho
First record of Epistylis procumbens Zacharias, 1897 (Ciliophora, Peritrichia) as epibiont on planktonic copepods in a neotropical floodplain lake

16 *Bettina Eugster, Kilchberg, Switzerland*
Ester Eckert, Jakob Pernthaler, Thomas Posch
Exploring the species richness of ciliated protists in Lake Zurich (Switzerland)

17 *Luiz Felipe Velho, Maringá, Brazil*
Paulo Roberto Bressan Buosi, Adalgisa Fernanda Cabral, Claudia Costa Bonecker, Fábio Amodeo Lansac-Tôha
Plankton ciliates community (Protozoa: Ciliophora) in the Upper Paraná River floodplain, Brazil: Patterns of composition, species richness and abundance

18 *Luiz Felipe Velho, Maringá, Brazil*
Fábio Amodeo Lansac-Tôha, Geziele Mucio Alves, Deise de Morais Costa
Abundance and size structure of testate amoebae community (Arcellinida and Euglyphida) in different biotopes from a floodplain lake

19 *Daniel Puppe, Cottbus, Germany*
Otto Ehrmann, Michael Sommer, Manfred Wanner
The significance of testate amoebae as a biogenic Si pool in soils

20 *Shahla Monir, Cologne, Germany*
Sandra Lagauzère, Frank Nitsche, Hartmut Arndt
Benthic heterotrophic flagellates of the River Rhine: Community structure and seasonal dynamics

21 *Thomas Weisse, Mondsee, Austria*
Ulrike Scheffel, Peter Stadler, Wilhelm Foissner
Life cycle and ecology of Bromeliothrix metopoides Foissner, 2010, an enigmatic ciliate (Protista, Colpodea) from tank bromeliads (Bromeliaceae)

22 *Paulo Roberto Bressan Buosi, Maringá, Brazil*
Adalgisa Fernanda Cabral, Fábio Amodeo Lansac-Tôha, Laura Roberta Pinto Utz, Luiz Felipe Machado Velho
Ciliate community composition in phytotelms of the bromeliad Aechmea distichantha Lem. from riparian vegetation of the Upper Paraná River, Brazil

23 *Rosaura Mayén Estrada, México City, Mexico*
Carlos Durán-Ramírez, Lourdes Flores-Flores, Itzel Sigala-Regalado
Ciliated protozoa from caves, tank bromeliads and springs from Central Mexico: An ecological approximation

24 *Krzysztof Rychert, Slupsk, Poland*
Katarzyna Spich, Kinga Laskus, Michalina Paczkowska, Magdalena Wielgat-Rychert
Protozoan communities at two stations located in the coastal zone of the Southern Baltic Sea

25 *Oriol Canals, Barcelona, Spain*
Humbert Salvadó, Maria Auset, Cristina Hernández, Carlos Rodríguez, Jorge Malfeito
Protozoa as bioindicators in a Shortcut Biological Nitrogen Removal process treating high ammonium loaded wastewater

26 *Humbert Salvadó Cabré, Barcelona, Spain*
Oriol Canals, Pere Aguiló, Lucia Arregui, Blanca Perez-Uz, Susana Serrano
Changes in the structure of protozoa community in a wastewater treatment plant with advanced biological nutrient removal system

27 *Katarína Nemjová, Prague, Czech Republic*
Pavel Škaloud, Anna Novotna, Frederik Leliaert
Genetic differentiation and population structure in desmids (Desmidiales, Zygnematophyceae, Streptophyta)

28 *Cecile Lepere, Coventry, UK*
Dave Scanlan, Laure Guillou, Martin Ostrowski
The role of parasites in the regulation of small phytoplankton communities in the open ocean

29 *Sigrid Neuhauser, Innsbruck, Austria*
Rebecca Brenner, Thomas Liener, Martin Kirchmair
Biodiversity and abundance of Phytomyxea (plasmodiophorids) at the Rotmoosferner glacier forefront

30 *Edvard Glücksman, Oxford, UK*
David Bass, Thomas Cavalier-Smith
Diversity and ecology of Apusozoa (Protozoa): A mysterious phylum of free-living zooflagellates

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Posters: Molecular Biology
Chair: Jürgen Strassert, Berlin, Germany

31 *Lubos Voleman, Prague, Czech Republic*
Pavlina Vondrackova, Pavel Dolezal
Mitosomal fusion induced by SNARE protein in Giardia intestinalis

32 *Lenka Horvathova, Prague, Czech Republic*
Pavel Dolezal, Jan Tachezy
Iron-inducible transcription in Tritrichomonas foetus

33 *Pavlina Vondrackova, Prague, Czech Republic*
Jan Tachezy, Pavel Dolezal
Putative peptidase in the mitosomes of Giardia intestinalis

34 *Ivana Fixova, Prague, Czech Republic*
Eva Martincova, Pavel Dolezal
Identification of protein transport channel in the mitosomes of Giardia intestinalis

35 *Sven Gould, Düsseldorf, Germany*
Verena Zimorski, William Martin, Peter Major, Xavier Pereira Bras
Internal targeting motifs are common in hydrogenosomal proteins of Trichomonas vaginalis and found in multiple domains

36 *Tomás Poklépovich Caride, Caba, Argentina*
Mariela Tomazic, Mauro Rinaldi, Alejandro Nusblat, Clara Nudel
Heterologous expression of C5(6) sterol desaturase from Tetrahymena thermophila restores ergosterol biosynthesis in Saccharomyces cerevisiae ERG3 knockout strain

37 *Adriana Vallesi, Camerino, Italy*
Francesca Ricci, Pierangelo Luporini
Structure and expression of Euplotes pheromone genes

38 *Ulrike Günzler, Kaiserslautern, Germany*
Timo Oppermann, Helmut Schmidt, Martin Simon
The surface antigen multigene family of Paramecium tetraurelia

39 *Sascha Krenek, Dresden, Germany*
Corinna Burkart, Paul Czechowski, Theresa Treuner, Thomas U. Berendonk
Where have all the microsatellites gone? A two-method survey in Paramecium caudatum unveils only weak amounts of short tandem repeats

40 *Tommy Harding, Halifax, Canada*
Alastair G.B. Simpson, Andrew J. Roger
Life at the extreme: Molecular adaptations of Halocafeteria sp. to hypersaline environments

41 *Sarah Schlosser, Vienna, Austria*
Leitsch David, Michael Duchêne
Identification of potential thioredoxin target proteins in Entamoeba histolytica

42 *Stefan Pärschke, Wuppertal, Germany*
Angelika Preisfeld
Investigation of the euglenoid ribosomal DNA operon

Posters: Parasitology
Chair: Jürgen Strassert, Berlin, Germany

43 *Heather Esson, Vienna, Austria*
Brooke Morriswood, Graham Warren
Morphological reconstruction of a cryptic cytoskeletal structure in Trypanosoma brucei

44 *Stanislaw Kazubski, Warszawa, Poland*
Further investigations on reproduction of Ophryoglena hemophaga (Ciliata, Ophryoglenidae) parasite of the mollusc Dreissena polymorpha (Pallas)

45 *Yuanjun Zhao, Chongqing, China*
Chunning Liu, Fahui Tang
Taxonomic study on three trichodinids (Ciliophora, Oligohymenophora, Mobilia) isolated from Siluriformes fishes in China

46 *Júlia Katalin Török, Budapest, Hungary*
György Csikós
Localization and density of amoeba-resisting bacteria in Arcella spp.

47 *Julia Matt, Vienna, Austria*
Michael Duchêne
DNA damage in Entamoeba histolytica after metronidazole treatment – could it be caused by parasite DNases?

48 *Mohamed Dkhil, Riyadh, Saudi Arabia*
Saleh Al-Quraishy, Mahmoud Metwaly, Abdel Azeem Abdel-Baki, Frank Wunderlich
Liver response to Eimeria coecicola infections of the rabbit, Oryctolagus cuniculus

49 *Pedro Maillo, Barcelona, Spain*
Humbert Salvadó Cabré, Adam Marques, Pilar Gracia
Myxosporeans species that infect mullets (Teleostei: Mugilidae) of the Catalan coasts (northeastern Spain)

50 *Elena Nassonova, St. Petersburg, Russia*
Gita Paskerova, Yuliya Sokolova, Yuriy Rotari, Alexey Smirnov
A new metchnikovellid microsporidium: A hyperparasite of gregarines from the polychaete Pygospio elegans

51 *Abdel-Azeem Abdel-Baki, Riyadh, Saudi Arabia*
Hussein Al-Qahtani, Mohmed Dkhil, Saleh Al-Quraishy
Ultrastructure and phylogeny of Microsporidia found in muscle tissue of Saurida undosquamis (Synodontidae) from the Arab Gulf, Saudi Arabia

52 *Ludek Korený, Prague, Czech Republic*
Julie Kovárová, Roman Sobotka, Anna Gnipová, Anton Horváth, Miroslav Oborník, Julius Lukeš
Eukaryotic life without haem: The aerobic kinetoplastid flagellate Phytomonas does not require haem for viability

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Posters: Phylogeny
Chair: Jürgen Strassert, Berlin, Germany

53 *Alexander Kolesnikov, Moscow, Russia*
Ekaterina Merzlyak, Nadezda Efimova, Gabriele Schoenian
Molecular phylogeny and species identification of Leishmania by nuclear and kinetoplast genetic markers

54 *Markus Majaneva, Hanko, Finland*
Janne-Markus Rintala, Federica Cerino, Anke Kremp, Jaanika Blomster
Family Pyrenomonadaceae (Cryptophyta): Molecular phylogeny of the genera Rhinomonas, Rhodomonas and Storeatula

55 *Katerina Procházková, Prague, Czech Republic*
Lira A. Gaysina, Martina Pichrtova, Alena Lukesova, Marek Elias
Reassessing the diversity and taxonomy of the eustigmatophyte algae of the genera Vischeria Pascher and Eustigmatos Hibberd

56 *Eliška Šrámová, Prague, Czech Republic*
Kristýna Klára Novotná, Martin Volf, Petr Šípek, Ivan Čepička, Vladimír Hampl
Diversity of the genus Monocercomonoides and its genetic codes

57 *Alexandra Jeuck, Cologne, Germany*
Frank Nitsche, Alexander P. Mylnikov, Hartmut Arndt
Long-term dynamics of planktonic choanoflagellates in the River Rhine with a description of new species

58 *Melánie Fiserová, Prague, Czech Republic*
Marek Eliáš, Katarína Nemjová, Yvonne Nemcová
The ultrastructural diversity of the algal class Eustigmatophyceae (Ochrophyta, Stramenopiles)

59 *Elena Nassonova, St. Petersburg, Russia*
Alexandra Stechmann, Alexey Smirnov, Ema Chao, Thomas Cavalier-Smith
Inferring phylogeny of Amoebozoa from analysis of hsp90 and SSU rDNA concatenated dataset

60 *Matthew Brown, Halifax, Canada*
Jeffrey Silberman, Frederick Spiegel
A contemporary evaluation of the acrasids (Acrasidae, Heterolobosea, Excavata)

61 *Vit Ceza, Prague, Czech Republic*
Tomas Panek, Ivan Čepička
Hypotrichomonas: The tip of the iceberg of trichomonad diversity

62 *Da-Peng Xu, Geoje, Republic of Korea*
Young-Ok Kim, Weibo Song
Redescription of two marine planktonic tintinnids, Eutintinnus apertus (Kofoid & Campbell, 1929) Kofoid & Campbell, 1939 and Favella campanula (Schmidt, 1901) Jörgensen, 1934

63 *Mann-Kyoon Shin, Ulsan, Republic of Korea*
Ji-Hye Kim, Jung-Min Choi
A new species candidate of Spirostomum sp. (Ciliophora: Heterotrichea, Spirostomidae) from an estuary in Jeju Island, Korea

64 *Barbara do Nascimento Borges, Belem, Brazil*
Thiago da Silva Paiva, Inacio Domingos da Silva Neto, Maria Lucia Harada
New contribution to the 18S-rDNA phylogeny of urostylid ciliates (Ciliophora, Urostylida)

65 *Geoffrey Ongondo, Innsbruck, Austria*
Andrew Wamalwa Yasindi, Bettina Sonntag, Jens Boenigk, Michael Schagerl, Steve Omondi
Intra-specific variation of Frontonia species in two shallow saline-alkaline lakes in Kenya

66 *Pablo Quintela Alonso, Cologne, Germany*
Frank Nitsche, Hartmut Arndt
Revised morphology and phylogenetic position of Microdiaphanosoma arcuatum (Ciliophora, Colpodea)

67 *Ewa Przybos-Razowska, Krakow, Poland*
Sebastian Tarcz, Alexey Potekhin,
Maria Rautian, Malgorzata Prajer
A two-locus molecular characterization of Paramecium calkinsi

68 *Chen Shao, Qingdao, China*
Alan Warren, Weibo Song
Is Prodiscocephalus a hypotrich ciliate? Phylogenetic analyses based on morphogenetic and 18S rRNA gene sequence data (Ciliophora: Spirotrichea)

69 *Mann-Kyoon Shin, Ulsan, Republic of Korea*
Choon-Bong Kwon, Seok-Won Jang
Reconsideration of endemic distritution of colpodid ciliate Corticocolpoda kaneshiroae Foissner, 1993 (Colpodea, Colpodida, Colpodidae) and new combinations of names in related species

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Posters: Special Topics
Chair: Jürgen Strassert, Berlin, Germany

71 *Gregory Antipa, San Francisco, USA*
Let your images of Protista populate the cell: An image library

72 *Gregory Antipa, San Francisco, USA*
Eddie Chang
Developmental sculpting of the thigmotactic ciliature pattern of Conchophthirus

73 *Andrzej Kaczanowski, Warsaw, Poland*
Mauryla Kiersnowska, Joanna Drozdowska,
Janina Kaczanowska
Cell cycle progression and cortical morphogenesis of Tetrahymena thermophila are affected by roscovitine

74 *Rogelio Aguilar Aguilar, México City, México*
Margarita Reyes Santos, Maricela Elena
Vicencio Aguilar, Rosaura Mayén Estrada
Patterns of biodiversity of protozoans in a megacity: Complementarity and similarity of ciliates from five localities of México City

75 *Rogelio Aguilar Aguilar, México City, México*
Rosaura Mayén Estrada
The use of biogeographical tools in the distribution of Lagenophrys (Protozoa: Ciliophora: Peritrichia): Track analysis and geographic distribution

76 *Sirma Çapar Dinçer, Ankara, Turkey*
Nazli Deniz Eyice
Contribution to the knowledge of ciliate (Ciliophora, Protozoa) fauna of Turkey

77 *Xiaofeng Lin, Guangzhou, China*
Weibo Song
The biodiversity of ciliated protozoa in mangrove wetlands of southern China

78 *Atef Omar, Salzburg, Austria*
Klaus Wolf, Wilhelm Foissner
Updating the microthoracids (Ciliophora, Microthoracida): Four new Leptopharynx species

79 *Xiaozhong Hu, London, UK*
Alan Warren
Morphology and phylogeny of a new marine ciliate, Diophrys multimacronucleata nov. spec. (Ciliophora, Euplotida)

80 *Xiaozhong Hu, London, UK*
Xinpeng Fan, Alan Warren, John C. Clamp
Morphological descriptions of three marine ciliates, with the establishment of a new genus and two new species (Ciliophora, Scuticociliatia)

81 *Thiago da Silva Paiva, Rio de Janeiro, Brazil*
Inácio Domingos da Silva Neto
A new species of the rare cliate Discomorphella (Plagiopylea, Odontostomatida) from an endangered microhabitat in Rio de Janeiro, Brazil

82 *Laure Perret-Gentil, Geneva, Switzerland*
Jan Pawlowski
Using reverse taxonomy approach to identify freshwater foraminifera

83 *Haruki Hashimoto, Tokyo, Japan*
Electron microscopic study on division of secondary plastids surrounded by four membranes in chromophyte algae

84 *Jitka Hlavacková, Prague, Czech Republic*
Anastasios D. Tsaousis, Michelle Leger, Andrew J. Roger, Jan Pyrih, Jan Tachezy, Ivan Hrdy, Vladimír Hampl
The homologue of mitochondrion in Trimastix pyriformis

85 *Sergei Fokin, Pisa, Italy*
Frequency and biodiversity of symbionts in representatives of the main classes of Ciliophora

86 *Michael Schweikert, Stuttgart, Germany*
Christioph Diebolder
Structure and function of R-bodies from endosymbiotic Caedibacter in Paramecium

87 *Erna Aescht, Linz, Austria*
Updating the "Catalogue of the generic names of ciliates (Protozoa, Ciliophora)" focused on "type" species and "type" specimens

88 *Peter Vd'ačný, Bratislava, Slovak Republic*
Wilhelm Foissner
Monograph of the dileptids (Ciliophora, Litostomatea)

89 *Helmut Berger, Salzburg, Austria*
Monograph of the Hypotricha (Ciliophora): Final spurt

90 *Maria Holzmann, Geneva, Switzerland*
Jan Pawlowski
Symbiosis in Foraminifera: An overview

91 *Karin Komsic-Buchmann, Cologne, Germany*
Lisa Marie Stephan, Burkhard Becker
The exocsyt complex is involved in contractile vacuole function in Chlamydomonas reinhardtii

92 *Catrin Williams, Cardiff, UK*
David Leitsch, Lee Dunham, Joanne Cable, Michael Coogan, David Williams, David Lloyd
Spironucleus vortens: A proteomic study of novel and traditional chemotherapeutic agents

93 *Miriam Cheaib, Kaiserslautern, Germany*
Martin Simon
Dynamic chromatin remodeling and the control of exclusive surface-antigen transcription in Paramecium tetraurelia

94 *Anush Kosakyan, Neuchâtel, Switzerland*
Thierry J. Heger, Milcho Todoroy, Edward A. D. Mitchell, Eric Verrechia, Enrique Lara
Does morphology correlate with molecular data? A case study of the Nebela tincta complex (Arcellinida)

95 *Cyril Obadia, Geneva, Switzerland*
Mikro-Dialoge

PLENARY LECTURES

► Monday, 25 July 2011

11.30 – 12.15 h Plant Physiology
Auditorium Maximum

Eukaryosis: Protist origins from bacterial communities

Margulis, Lynn
University of Massachusetts, Department of Geosciences, Amherst, MA, USA

The power of symbiogenesis to generate novelty is most pronounced, or at least most easily detected, in the Protoctista kingdom. Dinomastigote associations with red, green and chrysophyte algae and the dynamism of Hatena serve as examples in the marine environment. In fresh water Stentor polymorphus and Paramecium bursaria, large ciliates that harbor Chlorella-like green algae, proffer conspicuous evidence for symbiogenesis. Speciation, the appearance of new species either in the fossil record, in laboratory or field studies, by "gradual accumulation of random mutations in DNA" has never been adequately documented. However a multitude of high-quality scientific studies has unequivocally shown the importance of symbiogenesis in the origin of species, genera and more inclusive taxa (Margulis & Chapman, 2010; Handbook of Protoctists, in press, Jones and Bartlett, 2012). Symbiosis, an ecological phenomenon describes a physical association between "differently named organisms", i.e., members of different taxa from species to superkingdoms. Symbiogenesis is an evolutionary phenomenon. Symbiogenesis is detected when physical associations between members of different taxa generate new metabolic pathways, organelles (e.g., mitochondria, chloroplasts, kinetosomes), new tissues (e.g., the red stipulate tissue of Gunnera manicata) or new organs, etc. Lichens are new organisms generated by symbiotic associations between members of different kingdoms (e.g., fungal/phototrophic protist) or fungal/cyanobacteria). Members of at least two prokaryotic domains (a sulfidogenic archaebacterium, a sulfide-oxidizing motile eubacterium) merged in the origin of the first nucleated organisms to form the earliest protists. These were karyomastigont-bearing eukaryotes in the mid-Proterozoic Eon (c. 1,200 million years ago). Such heterotrophic, phagocytotic motile protists were ancestral to subsequent eukaryotes. The defining seme of eukaryosis, the membrane-bounded nucleus as a component of the karyomastigont, evolved from tight, permanent attachment and integration of Thermoplasma-like archaebacteria with Perfilievia-Spirochaeta-like eubacteria. They formed the motile, amitochondriate LECA (last eukaryotic common ancestor) by symbiogenesis. Phagocytotic acquisition and integration of oxygen-respiring alpha proteobacteria and subsequent integration of cyanobacterial symbionts that became plastids led to the minimal prokaryotic community that became the earliest algae cells. I will show a video of these phenomena that demonstrates there are no missing links in this scenario.

14.00 – 14.45 h Plant Physiology
Auditorium Maximum

Protists as model organisms in studies of community ecology and ecosystem functioning

Morin, Peter
Rutgers University, Department of Ecology, Evolution and Natural Resources, New Brunswick, NJ, USA

Protists have a long history as model organisms in ecology, extending back at least as far as the work of Rev. William Dallinger in the 1800s. More recent studies have used protists to explore complex dynamics, food web organization, responses to environmental change, and relations between biodiversity and ecosystem functioning. Much of what we know about biodiversity and ecosystem functioning in aquatic microbial systems comes from laboratory studies of relatively simple systems. I will summarize some of the key findings from a number of studies to provide an overview of patterns that might also be important in natural systems. Although these studies show that biodiversity has some consistent effects on various aspects functioning, including processes like decomposition and productivity, the extent to which these findings generalize to natural systems of much higher protist diversity remains unclear. An important ongoing controversy concerns the extent to which findings from low diversity laboratory systems can be extended to much more complex natural systems. Because natural patterns of microbial diversity in many systems remain incompletely known, the major obstacles to resolving this conflict involve the current state of techniques used to monitor and manipulate microbial diversity in natural settings. Finally, I will emphasize that description of the true nature and extent of protist diversity gradients in natural systems remains one of the fundamental challenges facing protistologists and ecologists.

▸ Tuesday, 26 July 2011

09.00 – 09.45 h Plant Physiology Auditorium Maximum

Comparative genomics of protists

Keeling, Patrick
University of British Columbia, Department of Botany, Vancouver, Canada

The number of completely sequenced genomes from bacteria, archaea, animals, fungi and plants has reached the point where meaningful comparative analyses are possible between both distantly and closely related species to investigate genome evolution at multiple levels. Sadly, though perhaps not surprisingly, protist lag behind. I say "not surprisingly" because, like other eukaryotes, protists have comparatively large and complex nuclear genomes (and therefore are hard to sequence), and because, like prokaryotes, they represent a comparatively large spectrum of phylogenetic diversity (and therefore are hard to sample adequately). New sequencing technologies make it relatively inexpensive to generate huge sequence databases, but how has this changed protist genomics? Small nuclear genomes can now effectively be sequenced without the support of large sequencing centres, and transcriptomics opens similar access to larger genomes. In both cases, it seems likely that we are about to see the largest burst of new data in the history of protistology, and analyzing this data is going to be the new challenge. In this talk I will review a number of comparative genomics projects ongoing in my own research group, with an emphasis on the fact that genomics is no longer the realm of mega-sequencing centres, and on the amazing potential of this approach to examine specific questions and also to explore more unpredictable topics. By comparing the complete sequences of the smallest nuclear genomes known, those of the microsporidian parasites in the genus Encephalitozoon, we are investigating how already reduced genomes can be further condensed, and what role horizontal gene transfer plays in their short-term evolution. We are also comparing the genome of the insect-parasite Helicosporidium to its green algal relatives, and contrasting this evolution with that of the photosynthetic apicomplexans (e. g. Chromera) with their more notorious parasitic relatives (e. g. Plasmodium). The effects of endosymbiosis will also be discussed, focusing on the outcome of a relatively recent partnership between a diatom endosymbiont and its dinoflagellate host in the five resulting genomes of this complex association. Lastly, single-cell genomics will be raised, because the power to sequence genomes from uncultivated protists will be necessary to truly sample protist diversity.

14.00 – 14.45 h Plant Physiology Auditorium Maximum

Viennese Waltz or Berlin Rave: How Toxoplasma injects polymorphic proteins that determine the character of its dance with the host

Boothroyd, John
Stanford University, Department of Microbiology and Immunology, School of Medicine, Stanford, CA, USA

We are interested in how infection with different strains of Toxoplasma results in dramatically different disease outcomes. In some cases, the infection is completely asymptomatic while in other instances, the disease can be fatal. In this talk, I will describe our recent results on the polymorphic rhoptry proteins responsible for these dramatic differences. One, ROP16, is a dual-specific protein kinase that works through mimicking Jak2 and altering the host immune response in very different ways, depending on the ROP16 allele the parasite expresses. Another, the ROP5 group, is a cluster of genes encoding a set of pseudokinases that collectively can impact virulence in mice by many orders of magnitude. We hypothesize that the different allelic variants of each of these effector proteins evolved as optimal solutions to the challenge of infecting a different host species. With sexual recombination, new strains have emerged, almost overnight, with a new mix of these key genes that has allowed them to rapidly take over a given ecological niche. I will also discuss recent work on the initial stages of the invasion process and implications of this on how the ROP16 and ROP5 effectors are injected into the host cell.

▸ Thursday, 28 July 2011

09.00 – 09.45 h Plant Physiology Auditorium Maximum

Membrane traffic in ciliates

Turkewitz, Aaron
University of Chicago, Department of Molecular Genetics and Cell Biology, Chicago, IL, USA

Ciliates are highly complex cells, and must have evolved specialized pathways of membrane traffic to generate and coordinate a large number and variety of membrane-bound structures. However, in most cases we understand little about the dynamic nature of these pathways and their relationship with pathways found in other eukaryotes. In recent

years, morphological and classical genetic studies on membrane traffic in ciliates have been revisited using molecular approaches. Important insights and tools to dissect both endocytic and exocytic pathways have come from the sequenced genomes of the oligohymenophoron ciliates Tetrahymena thermophila and Paramecium tetraurelia, allowing a number of research groups including our own to compare mechanisms in ciliates with those in other lineages. The approaches include both interrogation of specific pathways, using a variety of experimental tools available in these organisms, and surveys of large gene families that give a more global picture of the compartmental organization of these cells.

14.00 – 14.45 h Plant Physiology
Auditorium Maximum

Paramecium genome organization and evolution

Sperling, Linda
CNRS, Centre de Genetique Moleculair, Gif-sur-Yvette, France

Paramecium, like all ciliates, separates the germline and somatic functions of chromosomes. A diploid germline micronucleus (MIC) undergoes meiosis and transmits the genetic information to the next sexual generation. A highly polyploid somatic macronucleus (MAC) contains a version of the genome that is streamlined for gene expression. At each sexual generation, the old MAC is destroyed and a new MAC differentiates from the zygotic nucleus through reproducible DNA rearrangements that include (i) the elimination of germline repeated sequences such as transposons, (ii) the precise excision of tens of thousands of short unique copy Internal Eliminated Sequences (IES) and (iii) amplification of the DNA to a final copy number of ~800n. Sequencing of Paramecium tetraurelia MAC DNA revealed that a series of at least 3 whole genome duplications (WGD) has shaped the Paramecium genome, each of these dramatic events being resolved over evolutionary time by the loss of one duplicate for the vast majority of genes (Aury et al., 2006, Nature 444, 171-8). Analysis of the large number of paralogs identified for each WGD revealed that the major determinants of gene retention after WGD are gene balance constraints (highly expressed genes and genes whose products form complexes are preferentially retained in 2 copies) and not functional changes. I suggest that the elaborate Paramecium cell cortex may in part have arisen thanks to the WGDs through preferential retention of duplicated cytoskeletal genes followed by their diversification, driving increasing geometric complexity. Identification of a domesticated PiggyBac transposase, piggyMac, responsible for initiating developmental genome rearrangements (Baudry et al. 2009, Genes & Dev 23, 2478-83), has provided access to MIC DNA sequences since cells in which the PGM gene has been knocked down amplify zygotic DNA that has not been rearranged during sexual processes. The WGDs provide temporal markers for comparative genomic analysis of germline elements. Progress in understanding the origin and evolution of IESs will be presented. I acknowledge support from the CNRS, the ANR and Genoscope. The work presented was possible thanks to collaborations with the labs of Mireille Bétermier, Jean Cohen, Laurent Duret and Eric Meyer.

▸ Friday, 29 July 2011

09.00 – 09.45 h Plant Physiology
Auditorium Maximum

Endosymbiosis in Paramecium

Fujishima, Masahiro
Yamaguchi University, Department of Environmental Science and Engineering, Yamaguchi, Japan

Endosymbiosis is a primary force for environmental adaptation of eukaryotic cells and for evolution. The ciliated protista Paramecium species are extremely valuable cells that enable the reestablishment experiments of the endosymbiosis, which frequently bear prokaryotic, eukaryotic, or both endosymbionts in the cell. Although most endosymbiotic bacteria of Paramecium cannot grow outside the host cell as a result of their reduced genome size, these bacteria, even when isolated from the host cells, can maintain their infectivity to new host cell for a limited time. Consequently, endosymbiosis between the aposymbiotic host cells and the isolated symbiotic bacteria can be reestablished easily by mixing them through the host's digestive vacuole (DV). I show our resent studies on endonuclear symbiotic bacteria Holospora and green algae Chlorella species. Holospora penetrates the host DV membrane before lysosomal fusion to appear in the host cytoplasm, migrates to their target nucleus with a help of the host actin polymerization, distinguishes two kind of the host nuclear envelope by specific binding between their lipopolysaccharides of the outer membrane and unknown receptor substance on the nuclear envelope, invades into the nucleus 10 min after mixing and grows. Holospora induces overexpression of the host's hsp60 and hsp70 genes, and also secretes its GroEL homologs outside the bacterium. Thereby the host acquires

resistance against heat-shock and high salt concentrations and become adapted to unsuitable environments for their habitations. On the other hand, P. bursaria is the only species of Paramecium that forms symbiotic relationships with Chlorella species. The alga-free paramecia and the symbiotic algae still retain the ability to grow without a partner, and establish mutual endosymbiosis by mixing them. After lysosomal fusion to the host DVs, the algae escape from the DVs by budding of the DV membrane at 30 min after mixing. Then, the vacuole enclosing an alga differentiates to a perialgal vacuole (PV) within 15 min, which gives protection from lysosomal fusion and bind to beneath the host cell cortex. Thus, Holospora and Chlorella provide an excellent opportunity for us to elucidate not only for infection processes but also to assess the associations leading to eukaryotic cell evolution.

PROF. DR. MIKLÓS MÜLLER SYMPOSIUM

► Wednesday, 27 July 2011

14.00 – 17.00 h Theater Studies

Bacterial symbionts of termite gut flagellates: A tripartite symbiosis

Brune, Andreas
Max Planck Institute for Terrestrial Microbiology, Department of Biogeochemistry, Marburg, Germany

The most important event in termite evolution was the establishment of anaerobic flagellates in the hindgut of an ancestral cockroach. The flagellates comprise diverse lineages among the Parabasalia and Oxymonadida that occur exclusively in the evolutionarily lower termites and confer to the symbiosis the capacity to efficiently digest cellulose and hemicelluloses. They initiate a microbial feeding chain with hydrogen as a central intermediate and acetate as the major product. However, little is known about the fermentation products of different flagellate species and their metabolic response to the continuous influx of oxygen into the hindgut. Moreover, each flagellate cell is colonized by bacterial symbionts, which represent diverse bacterial lineages that occur exclusively in the hindgut of termites. The symbionts are located either on the surface or in the cytoplasm of the flagellates, often cospeciating with their particular host. None of these symbionts has yet been cultivated, but genomic studies have indicated their capacity to fix and assimilate nitrogen, to provide essential amino acids and vitamins, and possibly also to recycle nitrogenous wastes. Such contributions of flagellate symbionts to nitrogen metabolism in the hindgut underscore the emerging role of the termite gut microbiota in host nutrition, compensating for the poor quality of the lignocellulosic diet.

The hydrogenosome: Biogenesis, function and evolution

Tachezy, Jan
Charles University, Faculty of Science, Department of Parasitology, Prague, Czech Republic

Hydrogenosomes are double membrane bound organelles that produce molecular hydrogen and ATP under anaerobiosis. They were discovered in pathogenic as well as free living unicellular eukaryotes with common strategy to inhabit oxygen poor environments. Although origins of hydrogenosomes have been discussed since their discovery, today it is generally accepted that hydrogenosomes evolved from mitochondria. The transition of mitochondria to hydrogenosomes appeared several times in distant eukaryotic lineages including anaerobic ciliates, anaerobic chytrid fungi, parabasalids and heterolobosean, resulting in organelles that resemble mitochondria to a different degree. While hydrogenosomes of cockroach gut ciliate Nyctotherus ovalis retain structures resembling cristae, partial electron transport chain with capacity to generate membrane potential, and mitochondrial genome, hydrogenosomes of the human pathogen Trichomonas vaginalis loss majority of mitochondrial pathways including membrane associated respiratory chain as well as mitochondrial genome and consequently capacity for proteosynthesis. The mitochondrial pathways that are invariably retained in hydrogenosomes include (i) inner membrane carriers and outer membrane porins that mediate exchange of metabolites between organellar matrix and cytosol, (ii) machinery for assembly of iron-sulfur clusters, that is required for maturation of both hydrogenosomal and cytosolic FeS proteins, and (iii) protein import and sorting machineries that are required for correct delivery of nuclearly coded proteins into hydrogenosomal membranes and matrix. Our current proteomic survey of T. vaginalis hydrogenosomes allows detail analysis of these three pathways. The hydrogenosmal pathways revealed considerably lower complexity in comparison to known model of aerobic mitochondria of Saccharomyces cerevisiae. This trait most likely resulted from trichomonad adaptation to anaerobic conditions associated with mitochondrion-to-hydrogenosome transition. However, absence of some components might also reflect ancient eukaryotic features of Excavate group.

ORAL PRESENTATIONS, POSTERS, ARTWORKS

Only the affiliation of the first author is given.

Ultrastructure and phylogeny of Microsporidia found in muscle tissue of Saurida undosquamis (Synodontidae) from the Arab Gulf, Saudi Arabia

Abdel-Baki, Abdel-Azeem, Hussein Al-Qahtani, Mohmed Dkhil, Saleh Al-Quraishy
King Saud University, College of Science, Department of Zoology, Riyadh, Saudi Arabia

There is no knowledge about microsporidiosis in the ichthyological fauna of Arabian Gulf. In the present work, a new microsporidian infection was found in the muscle of the abdominal cavity of lizardfish, Saurida undosquamis which is one of Synodontidae member (Golani, 1993). In this study, we describe a new species based on morphology, ultrastructure, and phylogeny.

Myxosporidia infecting Nile fishes in Egypt: Light and electron microscopic study

Abdel-Ghaffar, Fathy, Heinz Mehlhorn, Abdel-Rahman Bashtar, Saleh Al-Quraishy, Khaled A.S. Al-Rasheid, Kareem Morsy
Cairo University, Faculty of Science, Department of Zoology, Giza, Egypt

Six species of economically important freshwater Nile fishes were collected from different Nile locations and investigated for myxosporean parasites. Twenty four species of Myxosporea belonging to 7 genera were detected and identified. Of these, 8 species were identified as new species. The detected parasites infected different hosts and different sites inside the hosts. The ultrastructual features of the trophozoite wall and sporogenesis of Henneguya suprabranchiae infecting the suprabranchial organ of Clarias gariepinus were studied by means of transmission electron microscopy. Investigation of the plasmodial wall and adjacent host tissue revealed that the parasite causes distortion and destruction of the whole host tissue. Sporogenesis starts by the surrounding of one generative by another resulting in the occurrence of the so-called pansporoblast stage. The enclosed cell initiates divisions until reaching 10 sporont cells which undergo differentiation, arrangement, modification and maturation resulting eventually in a mature spore. Capsulogenesis, valvogenesis and sporoplasm maturation were also described in detail.

Updating the "Catalogue of the generic names of ciliates (Protozoa, Ciliophora)" focused on "type" species and "type" specimens

Aescht, Erna
Upper Austrian State Museums, Biology Center, Linz, Austria

A precise, non-ambiguous terminology, e. g. concerning various spellings, synonymy and homonymy, together with three subsequent nomenclatural stages, viz. availability, allocation and valididy, provide an efficient tool for the computerization of databases of nomina and references to be implemented in the "Species 2000" Catalogue of Life. Apart from updating the "Catalogue of the generic names of ciliates (Protozoa, Ciliophora)" published in 2001, name-bearers were checked in more detail and a first evaluation of "type" material is provided. A detailed logonymic list allows a connection between the different historical stages of the ciliate taxonomy and highlights persisting problems, viz. concerning unresolved homonyms, problematic designations of type species and "forgotten" senjor synonyms. Following Vorticella in 1767, 2797 generic names have been proposed based on 2201 "type" species by 459 authors (only senjor ones embraced) in 1168 (94 since 2000) publications. 334 (about 12 %) are unavailable for various reasons, of the 2463 available genera, 1767 are considered as valid, whereas 28.2 %, viz. 696, names are treated as invalid, i. e. junior homonyms or synonyms. The great majority of the 245 genera newly created since 2000 resulted from new combinations of species (viz. 149), while 96 were based on new species discoveries. Only about 10 % of "type" species proposed are substantiated by holo-, syn- or neotypes. Consequently, a shift from concepts (diagnoses, though increasingly improved) to the (re-)investigation and deposition of specimens, which are the objective and permanent link between the world of orgnaisms and the world of language, is urgently needed. Two monographs embracing "all" ciliate taxa were published in Russian by Jankowski in 2007 and in English by Lynn 2008, however, only 1324 genera (apart from 46 established later) are listed in both, while about 400 genera are inconsistently mentioned.

Diversity and biogeography of oligotrichid and aloricate choreotrichid ciliates (Protista, Ciliophora, Spirotricha, Oligotrichea) in marine and brackish sea water

Agatha, Sabine
University of Salzburg, Faculty of Natural Sciences, Department for Organismic Biology, Salzburg, Austria

Oligotrichids and choreotrichids are ciliate taxa episodically dominating the marine microzooplankton and contributing to the multi-step microbial food web. The global diversity and distribution of aloricate Oligotrichea are unknown. Here, the geographic ranges of the 140 accepted species and their synonyms in marine and brackish sea water are analyzed, using hundreds of taxonomical and ecological studies; the quality of the records is simultaneously evaluated. The aloricate Oligotrichea match the moderate endemicity model, i. e., the majority (95) of morphospecies has a wide, occasionally cosmopolitan distribution, while 45 morphospecies show biogeographic patterns: they are restricted to single geographic regions and probably embrace 13 endemic morphospecies. These endemics are found in the Antarctic and Black Sea, whereas the "flagship" species Strombidinopsis cercionis is confined to the Caribbean Sea. Concerning genera, again several geographic patterns are recognizable. The recorded species richness is distinctly lower in the southern hemisphere than in the northern probably due to the scarcity of studies. Since the aloricate Oligotrichea represent a functionally heterogeneous group of heterotrophic and mixotrophic organisms with species-specific preferences, the extinction of a few species might threaten the functional integrity of the whole community. Accordingly, the aloricate Oligotrichea should not any longer be ignored in conservation issues. The ecophysiological diversity is considerably larger than the morphological, and even tops the richness of SSrRNA and ITS haplotypes, indicating that 83 – 89 % of the diversity in aloricate Oligotrichea are unknown. The huge challenge to discover all these species can only be managed by combining the expertises of morphological taxonomists, molecular biologists, ecologists, and physiologists. Supported by the Austrian Science Foundation (FWF; Project P20461-B17).

Patterns of biodiversity of protozoans in a megacity: Complementarity and similarity of ciliates from five localities of Mexico City

Aguilar Aguilar, Rogelio, Margarita Reyes Santos, Maricela Elena Vicencio Aguilar, Rosaura Mayén Estrada
Universidad Nacional Autónoma de México, Facultad de Ciencias, Departamento de Biolología Comparada, México City, México

As result of a recent survey work on protozoans of Distrito Federal (Mexico City), 279 ciliate species were recorded. This survey shows the vast diversity of this taxon inhabiting the scarce and highly disturbed environments characteristic of this megacity. In order to quantitatively analyse this biodiversity, the complementarity index (CI) of Colwell and Coddington, and the similarity index of Jaccard (Ji) were applied to distributional data of 193 species of free-living and associated ciliates inhabiting five localities into Mexico City: Chapultepec (species richness = 94), Ciudad Universitaria (103), Rio Magdalena (35), Tezozómoc (26) and Xochimilco (47). Only the ciliate species Aspidisca cicada, Litonotus fasciola, Podophrya fixa, Paramecium aurelia and P. caudatum (3.8 % of the taxa) were recorded at all sites, while 68.4 % of the taxa were recorded in one of the sampled localities. Values of complementarity were high (CI > 0.75) for all combinations, suggesting that ciliate assemblage is different for each locality. Being a supplementary analysis, the low value (Ji < 0.25) of the Jaccard index for each combinations confirm this results, grouping all of the localities close to the root of the resulting tree diagram (Ji = 0.14). According with the analysis of similarity two main clusters were obtained, one of them grouping Tezozómoc artificial lake and Río Magdalena, and another clustering Xochimilco (very disturbed and reduced natural lake), Chapultepec and Ciudad Universitaria (artificial lakes). Patterns of diversity in the studied areas can partially be explained by the heterogeneous sampling effort, which has been more intensive in those localities conforming the second cluster. In this sense, further sample work probably increase the values of similarity among localities, notwithstanding, we consider that each area will keep a core of characteristic species of ciliates due their particular environmental features.

The use of biogeographical tools in the distribution of Lagenophrys (Protozoa: Ciliophora: Peritrichia): Track analysis and geographic distribution

Aguilar Aguilar, Rogelio, Rosaura Mayén Estrada
Universidad Nacional Autónoma de México, Facultad de Ciencias, Departamento de Biolología Comparada, México City, México

Panbiogeography is a methodological approach in historical biogeography, which consists in plotting distributions of species on maps, connecting their disjunction distribution areas with lines to produce individual tracks, and looking for coincidence among them to detect biogeographical patterns. In order to determine the suitability of distributional data derived from protozoan taxa, and to detect global and regional distributional patterns, we analysed the worldwide geographic distribution of species of Lagenophrys under a panbiogeographic track analysis, which is a method that has not been used previously for any ciliate group, Based on track analysis of 28 species of this genus, five generalized tracks were found: (1) Euroamerican; (2) American; (3) Caribbean; (4) Tasmanian; and (5) South American. Two panbiogeographic nodes were found at the intersection of generalized tracks 1 and 2, and 2 and 5. The distribution of Lagenophrys species showed complex patterns that mainly could be related to trophic opportunities, habitat and distribution of hosts. The distribution patterns suggest a degree of allopatric events and regionalization for the taxa, and allows us to consider them, seldom used in historical biogeography, as useful for biogeographical analysis.

Dictyostelium discoideum: A bioethical model for the study of the effects of Extremely Low-Frequency Electromagnetic Fields (ELF-EMF)

Amaroli, Andrea, Bruno Bianco, Maria Giovanna Chessa
University of Genova, Department for the Study of the Territory and its Resources, Genova, Italy

Dictyostelium discoideum: a bioethical model for the study of the effects of Extremely Low-Frequency Electromagnetic Fields Andrea Amaroli1, Bruno Bianco2, MariaGiovanna Chessa1 1 DIPTERIS, 2 ICEmB at DIBE, University of Genova, Italy Numerous organisms have been proposed as biotests to evaluate contamination caused by anthropogenic environmental stressors. In this context, the peculiarities of protozoa could be even more interesting when we consider how the test on protozoa can assuage public opinion, more and more sensible to bioethical matters, and meet the requests of both the Interagency Coordinating Commitee on the Validation of Alternative Methods and the European Centre for the Validation of Alternative Methods for compliance with the 3Rs strategies. In previous works, we showed that the presence of cholinesterase (ChE) activity in single-cell amoebae of Dictyostelium discoideum is involved in cell-cell and cell-environment interactions, as its inhibition affects cell aggregation and differentiation. In this work, we have exposed single-cell amoebae to an extremely low-frequency (50Hz) electromagnetic field (ELF-EMF) with a magnetic induction about of 300mTesla, from 1 h to 48 h at 21 °C and we have observed the possible effects on the life cycle of D. discoideum and on the presence and activity of its enzyme ChE. A delay in the early phase of the differentiation was observed in 3 h-exposed cells, and a significant decrease in the fission rate appeared in 24h-exposed cells. Furthermore, the exposition from 1 h to 48 h affected, in different way, the activity of ChE. The trends of the ChE activity values shown at increasing exposure times to ELF-EMF could be a result of the adaptative process to these experimental conditions. In the attempt to restore homeostasis, the cells could be involved in an overcompensation mechanism, a phenomenon similar to the hormesis described in higher organisms. Finally, the result of the image analysis of immunoblot analysis indicate that the higher activity observed in the exposed cells than the control, it is due to a major presence of the cholinesterase enzyme in the exposed samples. However, such effects appeared to be transient and all the parameters observed returned to the values of the control after 24 h under standard conditions.

Biodiversity as indicator of environmental risk factors in molecular epidemiology studies: The case of Toxoplasma gondii

Angelici, Maria Cristina, Pietro di Pinto, Cristina Giuliani, Antonella Vimercati, Michelina Pugliese, Valentino Terio, Elisabetta Monteduro, Angela di Pinto, Giuseppa Marilia Tantillo
Istituto Superiore di Sanità, Department of Environment and Primary Prevention, Rome, Italy

Toxoplasma gondii is one of the most common parasitic protozoa in mammals, human included. Oocysts deriving from sexual stage of T. gondii corresponding to the infectious stage of the parasite and are spreaded by cat faeces in environment. Human may be infected by ingestion of oocysts potentially in all kinds of environments were prey-predator mechanism is maintained. Marine and fresh water environments with neighbouring humid areas

can be contaminated by Toxoplasma oocysts. Shellfish living in aquatic environments are able to retain microorganisms by concentration after filtering a large volume of water, in fact these filter feeding organisms have been found to be contaminated by protozoan parasites such as Giardia and Cryptosporidium. When these animals are bred in restricted marine environments polluted by contaminated rivers, they may become a food borne disease resource. This risk is further amplified by the eating habits in different Italian regions, chiefly in the South, where there is large consumption of raw bivalve molluscs mainly for pregnant women and immune-compromised people. In the present research we have investigated the presence of T. gondii in bivalve shellfish produced in Apulia Region. We performed techniques of PCR-RFLP by the use of specific primers for B1 and SAG3 Toxoplasma genes to detect the parasites in shellfish hepatopancreas. The Toxoplasma DNA was founded in several samples bred in restricted basins. The SAG3 gene RFLP showed the presence only of T. gondii genotype II, the most common agent of congenital toxoplasmosis allowing to identify a probable risk factor for pregnant women in the Apulia Region. Techniques to study organisms biodiversity, as DNA-RFLP on Toxoplasma protozoa, allow the characterization of an environmental risk for an infection with growing frequency in relation to increasing environmental pollution.

Let your images of Protista populate the cell: An image library

Antipa, Gregory
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We need your images of Protista so they can get their long overdue and well deserved respect. Let me help you populate the library with your high resolution, high quality images as I am directly involved with the early development of this cell biology resource. The ASCB Cell Image Library provides research quality images to and for Researchers, Educators, Students, and the Lay Public by allowing the viewing and download of data. As a worldwide site, it is expected to allow for collaboration among researchers who might not know each other. In this way the synergy of these images accomplishes objectives already achieved by GenBank. Since the site is in it's infancy, your research and research program cannot help but to benefit from it's exposure. My involvement in this project is, in part, due to my desire to more broadly share and recognize the contributions of protistan research to our understanding of basic cell biology. Please consider sharing your images through me, and I will try to give your work the best possible exposure. To take a look at the library go to www.cellimagelibrary.org.

Developmental sculpting of the thigmotactic ciliature pattern of Conchophthirus

Antipa, Gregory, Eddie Chang
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The ciliature of the holotrichously ciliated Conchophthirus curtus is distinctive and regionally differentiated. Here we report verification of earlier observations of morphogenesis and provide the morphological details that lead to final pattern formation within the distinctive thigmotactic field region of the opisthe. Individual dividing cells were fixed, embedded, oriented, and sectioned for electron microscopy. Cortical morphogenesis in Conchophthirus represents a 'point-of-no-return' process. Within the nine stages of morphogenesis described earlier, we were able to distinguish two major phases for development of the opisthe thigmotactic field. The first phase spans from stage 1 to stage 5 and principally involves formation of a replication band by the duplication of basal bodies within all kinetal rows around the midsection of the mother cell. This band eventually separates the presumptive proter from the opisthe. Initially, individual basal bodies (monokinetids) replicate to produce groups of doublets, triplets, then groups of four. Eventually, a dense band of basal bodies encircles the cell. All new basal bodies become ciliated during these six stages. Up to this point, basal body development and ciliogenesis resembles that described for other holotrichous ciliates. The second phase begins at stage 6 and involves only remodeling within the replication band only on the left side of the organism and only within the presumptive opisthe thigmotactic field. Here, one additional round of basal body proliferation produces one additional basal body which results in the characteristic zig-zag arrangement of basal bodies for thigmotactic field of this organism. Each new basal body becomes ciliated by stage seven. Thus this region becomes the thigmotactic field of the opisthe. The proter inherits the parental thigmotactic field.

Phylogeny and ecology of bicosoecids

Arndt, Hartmut, Áron Kiss, Cecile Reed, Melanie Müller, Nicole Nopper, Claudia Wylezich, Frank Nitsche
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Colourless heterokont flagellates are probably the most abundant heterotrophic eukaryotes in the biosphere. Recent phylogenetic information revealed from ribosomal RNA genes directly or indirectly amplified from marine, freshwater and soil habitats have shown that the diversity of heterotrophic flagellates has been underestimated by orders of magnitude. Bicosoecids are small (generally smaller than 8 µm) heterokonts and are among the most common heterotrophic flagellates in aquatic habitats. Most species have a high growth potential and function as important bacterivores. We will review the current systematics of bicosoecids based on a comparative study of diverse aquatic habitats in limnetic surface and groundwater, marine surface and deep-sea waters as well as soil habitats. We will add a considerable number of new or up to now not yet sequenced and morphologically described taxa allowing a higher resolution of systematic positions even of very common taxa.

Free-living heterotrophic flagellates from intertidal sediments of Saros Bay, Aegean Sea (Turkey)

Aydin Dede, Esra Elif, Won Je Lee
Hacettepe University, Department of Biology, Institute of Zoology, Ankara, Turkey

This is the first study of free-living heterotrophic flagellates in intertidal sediments of Saros Bay, Aegean Sea (Turkey). In order to contribute to an understanding of the geographic distribution of free-living marine heterotrophic flagellates, we investigated the diversity of heterotrophic flagellates occurring in the Bay from 25th June 2010 to 10th Oct. 2010 (n = 5). Thirty six species from 25 genera of heterotrophic flagellates are reported with uninterpreted records based on light-microscopy. The records include accounts of two unidentified taxa, and consist of, 1 apusomonads, 1 cercomonads, 1 choanoflagellates, 2 cryptomonads, 12 euglenids, 1 Heterolobosea, 1 kathablepharids, 3 kinetoplastids, 8 stramenopiles, and 6 uncertain affinities. Most flagellates described here appear to be cosmopolitan. We are unable to assess if these two unidentified species are endemic because of the lack of intensive studies elsewhere.

Conceptual progress in protistology: Conclusions and ways forward

Bass, David
Natural History Museum, Systematic Biology, London, UK

The final talk in the Concepts in Protistology symposium will have three main elements. Firstly, informed by the presentations earlier in the session I shall discuss the implications of ideas and opinions relating to species delineation for derived concepts such as biogeography, biodiversity measurement, and comparative ecological analyses. Secondly, I will consider the roles that next generation sequencing can play in refining concepts of what protist species are and how lineage differences can most meaningfully and usefully be measured. Finally I will present some recent case studies and possible experimental approaches that could be used to help define biologically informative OTU or species boundaries in large numbers of groups simultaneously, and/or in poorly known groups.

Species Taxonomy of Protists: Morphological and molecular perspectives from flagellates

Bass, David
Natural History Museum, Systematic Biology, London, UK

Modern protist taxonomy is closely allied with lineage discovery, whether that is via morphological or molecular techniques. In this talk I will discuss the criteria for deciding species boundaries and for describing new taxa in four groups of abundant gliding flagellates – cercomonad and glissomonad Cercozoa, the amoebo-flagellate Granofilosea, and 'Apusozoa'. Historically, all of these have been represented in the literature by a small number of species, which in any case were often misidentified. Therefore, the information provided by historical literature for these groups is of limited value for understanding their true diversity, ecology, and biology, except at the most basic level. The integration of molecular data into the study of such cryptic groups offers a paradigm shift in describing and quantifying protist diversity, but also brings challenges and risks. The interplay of these factors in our recent taxonomic revisions of these groups will be discussed, including insights into the ecology of 'newly diverse' groups, defining species boundaries in asexual taxa or those for which sexuality is unknown, and practical considerations for using contemporary taxonomic treatments as a baseline for future discoveries (i.e. how morphological and

molecular data can be combined to produce robust and enduring taxon diagnoses).

Monograph of the Hypotricha (Ciliophora): Final spurt

Berger, Helmut
Consulting Engineering Office, Salzburg, Austria

Recently, the preparation of the last volume of the Monograph of the Hypotricha has started. The hypotrichs are one of the three major groups (euplotids + (oligotrichs + hypotrichs)) of the spirotrichous ciliates which have a prominent adoral zone of membranelles and a replication band as synapomorphies. The final (= sixth) volume deals with all taxa not yet treated in volumes 1 – 5, that is, the "spiralled" genera (e.g., Hypotrichidium, Strongylidium, Stichotricha) and a relatively high number of little known genera each comprising only one, two, or three species. In addition, volume 6 will contain a key to the taxa revised in all volumes. It is estimated that about 700 valid species of hypotrichs are described, which corresponds 5 – 10 % of the known ciliate diversity (Berger 2012/2013, Nomenclator Ciliophorum, in prep.). When the monograph is complete, the hypotrichs will be the sole very large group of ciliates which is revised in detail. The review is an up-to-date overview about this highly interesting taxon of ciliates which is very common in marine, limnetic, and terrestrial habitats. The series is mainly addressed to taxonomists, cell biologists, ecologists, molecular biologists, and practitioners. The Monograph of the Hypotricha is published by Springer in the book series Monographiae Biologicae (MB): Berger (1999, Oxytrichidae, MB 78, 1092 pp; 2006, Urostyloidea, MB 85, 1319 pp; 2008, Amphisiellidae and Trachelostylidae, MB 88, 753 pp; 2011, Gonostomatidae and Kahliellidae, MB 90, 755 pp; 2012, Uroleptidae and Paraholostichidae, in prep.; 2014/2015, Strongylidiidae and remaining genera, in prep.). The preparation of volumes 2 – 6 was and is supported by four grants of two Austrian research funds. The financial support for volume 6 by the Austrian Science Fund (FWF; Project P23415-B17) is greatly acknowledged.

Diversity and phylogeny of Endomyxa, the possible ancestors of Foraminifera and Radiolaria

Berney, Cedric, David Bass
Natural History Museum, Systematic Biology, London, UK

Endomyxa is a possibly paraphyletic assemblage of various lineages at the base of phylum Cercozoa (part of the eukaryotic supergroup Rhizaria). The morphological, ecological and genetic diversity of Endomyxa is huge: they include at least three major and very distinct lineages of amoeboid organisms (the vampyrellids, Filoreta, and Gromia), two major lineages of parasitic organisms (the phytomyxean plant pathogens and the ascetosporean shellfish parasites), plus a collection of lineages known so far only from environmental surveys. Given the current uncertainty about the exact position of Foraminifera and Radiolaria within Rhizaria, it is possible that one or both of these major groups of amoeboid eukaryotes originated from an endomyxan ancestor. As part of an ongoing project about the diversity, ecology and evolution of large, naked, ramose or reticulose amoebae, we isolated several new organisms belonging to Endomyxa, mostly among vampyrellids. Here we present an updated and comprehensive phylogeny of Endomyxa including all our new isolates, and the results of an intensive screening of sequences in NCBI and BioMarKs databases to identify additional endomyxan clones from environmental libraries and metagenomic surveys. In light of our results, we discuss possible higher-level relationships and major evolutionary trends within Rhizaria.

A pluralistic approach to interpreting protistan diversity using Next Generation Sequencing data: The case of Haptophyta

Bittner, Lucie, Elianne S. Egge, Sarah Romac, Ian Probert, Stéphane Audic, Bente Edvardsen, Colomban de Vargas, The Biomarks Consortium
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During the last 200 hundred years of protistology, taxonomists have described, counted and classified groups designated as 'species', firstly based on morphological data, then on single genetic sequences (mostly rDNA), and finally using multiple genetic sequences. To measure diversity, 'species' are delineated and then counted. However if a conflict happens between species taxa (ST) delineated by different species concepts (SC) (e.g. genetic SC, phylogenetic SC, biological SC) or by data from different nature using the same SC (e.g. genetic ST

inferred from one molecular marker versus one other), the most common, monistic behavior, is to favor one dominant criterion to determine the 'good' species statement. Considering the amount of genetic data emerging from NGS (Next Generation Sequencing), conflicts between genetic SC will likely be a common problem. In the framework of the EU project BioMarKs (Biodiversity of Marine euKaryotes), environmental SSU and LSU sequences of Haptophyta were generated through NGS. A critical and pragmatical approach is then proposed to interpret diversity of Haptophyta, based on the following guidelines. (1) A priori accepted ST of protists must be evaluated to determine when genetic SC truly agree on their delineated units or when such an agreement cannot be tested due to a lack of comparison data. (Comparison of diversity inferred from LSU and SSU data for Haptophyta will be used as a case study.) (2) Monistic interpretation of diversity using single unified species concept (USC) can often lead to biased conclusions, ignoring part of the evolutionary processes that generate diversity. (3) Use of alternative, pluralistic approaches to interpret diversity does not allow to count 'unified species' (that most of the time correspond uniquely to phylotypes for one kind of markers) but can offer finer description and more accurate account on the evolutionary processes. Considering the current dramatic lack of knowledge on protistean diversity, a pluralistic interpretation appears to be a more reasonable and pragmatical strategy to increase our understanding on the mechanisms of diversification at play in evolutionary protistology.

A tale of twins: One addicted to sugared candy and the other not

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Toxoplasma and Plasmodium are obligate intracellular parasites, which utilize host glucose for their energy and biosynthetic needs. To this end, both pathogens express a pan-hexose transporter in their plasma membrane that is capable of importing glucose, mannose, galactose and fructose. Unexpectedly, this permease is not essential for Toxoplasma survival and virulence. In contrast, it is absolutely indispensable in Plasmodium throughout the parasite life cycle. Restricted access to glucose prompts T. gondii to glutaminolysis and gluconeogenesis for sustaining its central carbon metabolism, which partly explains its metabolic robustness and versatile intracellular parasitism. This metabolic capacity appears rather different from Plasmodium. Though the annotations for enzymes of glutaminolysis exist in Plasmodium database, our in silico analysis did not identify a complete gluconeogenesis in P. falciparum and P. berghei. The lack of gluconeogenesis probably renders glycolysis essential in Plasmodium, and offers hexose transporter (PfHT1) as an excellent drug target against human malaria. To permit a high-throughput screening of inhibitors targeting PfHT1 and their subsequent in-vivo assessment, we have generated a Saccharomyces cerevisiae mutant expressing PfHT1, and a PfHT1-dependent P. berghei strain, respectively. Our work now aims to construct a comprehensive metabolic map of both parasites for a better understanding and comparison of their central carbon metabolism. Such a model will also permit high-throughput in silico analyses for a rational drug design following its experimental validation.

Concepts in Protistology: Protist classification and diversity in the crossfire of evolutionary differentiation and historic misconceptions

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Despite two centuries of efforts in elucidating biodiversity surprisingly few general patterns across all groups of organisms, including animals, plants, fungi and microorganisms, have been identified. This lack of generalizability may well be related to the different conceptual and methodical approaches to diversity in different biological disciplines. There is currently a fundamentally important debate within protistology about the practical and theoretical basis of protist diversity, in particular with respect to the concept of species and the megadiversity of eukaryotes. Recent methodological progress has highlighted severe inconsistencies between the conceptual and the practical historic approaches to species and biodiversity. The dispute is currently stirred up by inconsistencies between molecular phylogenies on the one hand and morphological species denominations and traditional classification concepts on the other. Further, the conceptual conflict embraces the differences between zoological, botanical, and microbiological concepts of species. Various problems result from partially inconsistent species concepts, insufficient

taxonomic coverage of the recent diversity, and frequently lacking knowledge on exact species boundaries. This basic problem has many consequences, and represents a serious obstacle to understanding key aspects of protist biology and ecology, for example the 'everything is everywhere' dispute and the perception of protist biodiversity. As different methodological approaches are – in part – linked to the different concepts of species and of diversity the dispute on protist species, protist diversity, and protist systematics often failed to differentiate differences in the conceptual basis from methodological limitations and real variation. For instance, the existence of uncovered cryptic species is obviously resulting in increasing biodiversity estimates. By contrast, OTU-based diversity studies as often applied for microorganisms tend to fail to resolve species and thus tend to underestimate biodiversity. We feel that the time is ripe to revive the discussion on the conceptual basis of and the methodological approaches to species, and on their impact on derived concepts such as that of diversity and biogeography.

Compare and contrast: Pseudopodia of Foraminifera, Radiolaria and Gromia

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Understanding evolutionary relationships among protists remains an elusive goal. Currently, the supergroup Rhizaria is defined exclusively by molecular sequence criteria, although some authors have attempted to correlate this molecular data with structural features – especially pseudopodial characters – of representative species. Pseudopodia of the Foraminifera are the best characterized rhizarians in terms of ultrastructure, protein composition, and membrane surface dynamics, and therefore serve as a useful basis for comparison. Although the structural data set remains very incomplete, profound differences between gromiid and foraminiferan pseudopodia provide little support for grouping them together. We are aware of only a single ultrastructural study of radiolarian pseudopodia which, by contrast, did reveal some similarities with the Foraminifera. We are confident that more sophisticated structural studies will identify morphological correlates of the inferred genetic relationships within the Rhizaria.

Foraminiferan protists as pontiffs of evolution

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In the 19th century, certain philosophers used the natural beauty and architectural elegance of foraminiferan (foram) shells, particularly those of multi-chambered agglutinated and calcareous taxa, as evidence of "divine design." Since that time, micropaleontologists have charted the structural changes of fossilized foram shells in well-characterized stratigraphic sequences, providing a clear picture of the evolution of the group. Contemporary interdisciplinary research has combined this fossil record with molecular phylogenetic analyses of extant species to yield an even more compelling, highly convergent view of not just foram evolution, but also the mode(s) and tempo(s) of evolution in general. In short, foraminiferal shells are a superb and unusually well-documented example of elegant design as an emergent property of evolutionary processes. Today, foram aesthetics should be used to herald evolution as the rational basis of the life sciences.

The InterfaCE of Art and Science

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In this Art/Science collaboration, Claire Beynon's artwork, inspired by her two month stay in Sam Bowser's Antarctic field camp, was used as a template to produce topographically-rich growth substrates for rhizarian protists. The motile behavior of benthic foraminifera and Gromia on these microfabricated substrates tested hypotheses and prompted new artistic renderings. One aim of cycling of information through artistic, scientific, and protistan processes is to effectively communicate scientific principles, particularly the concept of scale (nano-to-macro and vice versa), to general public audiences. Supported in part by National Science Foundation grant ANT0944646.

A Protistan Explosion coincident with the Cambrian Explosion of animal life: In search of Precambrian rhizarians and their relatives

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Modern protists are deeply adapted to living in a world influenced by metazoans with a through-gut. Such a system did not evolve after until about 550 million years ago. Before that time, the quality of the fossil record was very much better (not worse, as Darwin once thought) but we still seek in vain for our modern protists groups. Why are they so hard to find in the excellent Precambrian fossil record? Some molecular phylogenies place Rhizarians such as Foraminifera and Radiolaria close to the Stramenopiles (e.g., diatoms, brown algae) and Alveolates (e.g. dinoflagellates). The fossil record suggests that this SAR clade, and the green algae, is likely to have evolved during the 'Boring Billion' between 1.8 and 1.2 billion years ago during several episodes of symbiogenesis. Rhizarian ancestors could even have been the hosts of these symbioses. The search is now on for the early fossil record of Rhizarians in the Precambrian. Hitherto, they have been firmly recognised near the base of the Cambrian some 540 million years ago. Before this, however, their record becomes more controversial. Some have argued that giant rhizarian-like protists dominated the seafloor during the Ediacaran Period, some 630 to 580 Ma BP. Our review will critically evaluate these ideas, and reveal new fossil materials from an interval stretching back through the new Ediacaran Period to the Gunflint Chert some 1900 million years back. Current evidence suggests that the characteristics of most crown group protists did not develop until surprisingly near the start of the Cambrian itself. In other words, it suggests that both ecosystems, and cytological architecture, were radically re-organized in an avalanche of novelty that was precipitated by the evolution of the metazoan mouth and anus, after about 550 Ma BP. Before that time, we should not expect to find protists organized in their modern manner.

18S + 28S rDNA phylogeny divides Radiolaria into Polycystina and Spasmaria and supports the Retaria hypothesis

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Radiolarians are marine planktonic protists that belong to the eukaryote supergroup Rhizaria together with Foraminifera and Cercozoa. Radiolaria has traditionally been divided into four main groups based on morphological characters; Acantharia, Phaeodaria, Nassellaria and Spumellaria. 18S rDNA phylogenies have however shown that Phaeodaria belongs to Cerocozoa, and that the previously heliozoan group Taxopodida should be included in Radiolaria. 18S rDNA phylogenies have not been able to resolve the sister relationship between the main Radiolaria groups, but nevertheless suggests that Spumellaria is a polyphyletic group. Very few sequences other than 18S rDNA have so far been generated from radiolarian cells, mostly due to the fact that Radiolaria has been impossible to cultivate and single cell PCR has been hampered by low success rate. To overcome these problems we used a novel molecular approach by combining single cell whole genome amplification with gene-targeted PCR. We successfully obtained both 18S and 28S rDNA genes from several species of Radiolaria and from all the main groups. In my talk I will present results from combined phylogenetic analyses of 18S and 28S rDNA. The molecular phylogeny divides Radiolaria into two main groups: Polycystina (Spumellaria+Nassellaria) and Spasmaria (Acantharia+Taxopodida). Foraminifera robustly groups with Radiolaria, but not within any of these groups. The clustering of Radiolaria and Foraminifera is in accordance with the Retaria hypothesis.

Radiolaria revealed as a reservoir for marine alveolates

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Radiolarians are marine planktonic protists known to harbor many symbiotic species. In order to understand the radiolarian symbiont diversity we have sampled single specimens of Radiolaria and generated 18S rDNA sequences by whole genome amplification and gene targeted PCR. In my talk I will present results from the molecular work and

phylogenetic analyses of 18S rDNA sequences obtained from single radiolarian cells. We recover a surprisingly large diversity of intracellular protist symbionts related to the enigmatic marine alveolate groups (MAG). Hence, a significantly larger MAG diversity only known from environmental sequencing surveys can now be linked to intracellular symbionts. The phylogeny groups all the radiolarian MAG symbionts into 5 distinct clades (named RAS 1-5). Similarly, other MAG sequences with a known host origin cluster according to their host type, e. g. phaeodarians, fish, copepods, ciliates or dinoflagellates. This host-specific clustering pattern of the symbiont sequences implies several independent colonizations of Radiolaria species and of other host lineages. The large diversity of symbionts identified here reveals radiolarians as an important reservoir for MAG species and therefore a key group for understanding the impact of MAG symbionts on the marine ecosystem.

Ciliate community composition in phytotelms of the bromeliad Aechmea distichantha Lem. from riparian vegetation of the Upper Paraná River, Brazil

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Phytotelmata, such as tree holes, pitcher plants, and tank bromeliads, provide physically distinct habitats for several aquatic organisms, by collecting and accumulating rain water and litterfall, which provides both nutrient reserves and refugia for protists and small metazoans. Although obviously being common and abundant, only recently the ciliates of tank bromeliads have received some attention. In this way, many new species and genera have been described, and some of them occurring exclusively in these tanks. In this study, we aimed to describe the ciliate community composition in the tanks of Aechmea disticantha, in Upper Paraná River, Brazil, and verify the effect of pluviometric seasonality (rainy and dry seasons) on species composition. A total of 72 plants were collected in the two different seasons. The samples were analyzed in vivo, within 6 h after sampling, using optical microscopes. The identification was performed using the differential interference contrast system and silver impregnation techniques. In order to summarize the differences in species composition between the two periods, we conducted a Detrended Correspondence Analysis (DCA). Considering both periods and all sampling dates, we recorded 92 ciliate species of 14 different orders. The most representative order was Hymenostomatida, with 18 species, followed by Hypotrichida with 12 species. From the total number of species, a greater number was recorded in the rainy season. In this way, 40 species occurred in both periods, whereas 34 occurred exclusively in the rainy season, and 18 occurred exclusively in the dry season. In both periods, the most frequent species were Colpoda steinii Maupas, 1883 (85 % of samples), and Cyrtolophosis mucicola Stokes, 1885 (80 % of samples). The tank bromeliad-specific ciliates Bromeliophrya brasiliensis Foissner, 2003 and Bromeliothrix metopoides Foissner, 2010 also presented high frequencies, the first in dry season whereas the second in both periods. The results of DCA showed a substantial difference between the two periods of study, evidencing the relevance of pluviometric level in structuring ciliate community in tank bromeliads.

A contemporary evaluation of the acrasids (Acrasidae, Heterolobosea, Excavata)

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Sorocarpic amoebae (or cellular slime molds) are amoeboid organisms that individually aggregate and work together to form a fungus-like fruiting body, a sorocarp. Historically, they have been referred to as acrasids. Use of this term implies a phylogenetic relationship; however, the sorocarpic fruiting organisms have polyphyletic origins. To alleviate confusion of terms, we suggest limiting acrasid to refer only to the Acrasidae in Heterolobosea. Acrasis is a sorocarpic amoeba with limited cellular differentiation in the sorocarps. Here we study the diversity of Acrasis, using a combination of morphological characteristics and small subunit rRNA gene sequences. A total of eight isolates of Acrasis and an example of Pocheina were examined from worldwide locales. Acrasis/Pocheina form a well-supported monophyletic group that is the highly supported sister to a clade containing Allovahlkampfia and several uncharacterized amoebae. Four distinct morphologic and molecular lineages of Acrasis were resolved; each represents a species, two of which are novel. Unexpectedly, an isolate identified as Pocheina rosea, is nested within a clade containing isolates of the taxon A. rosea, in which P. rosea should tentatively be subsumed.

One member of the tightly knit Allovahlkampfia clade was induced to fruit. Therefore we expand Acrasidae to include this entire clade.

Evolutionary history of aggregative multicellularity: Insights from phylogenomics of Guttulinopsis

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Over the course of eukaryotic evolution, multicellular eukaryotes have arisen multiple times independently from a variety of protistan lineages. Well-recognized eukaryotes like animals, plants, and fungi represent only a miniscule fraction of multicellular organismal diversity. In fact nearly every major protistan lineage has given rise to multicellular forms. One form that is particularly widespread is the aggregative fruiting life cycle (also known as the cellular slime mold habit), whereby individual cells in the environment aggregate to form a "fungus-like" sorocarp. Sorocarpic protists are currently known in four of the six eukaryotic supergroups. One such organism, Guttulinopsis vulgaris, discovered in 1901, has eluded proper classification, making it truly an organism of unknown affinities. Here using phylogenomic analysis of a 171-protein dataset from a transcriptomic project we show that, surprisingly, G. vulgaris robustly groups within the supergroup Rhizaria. These results refute all previous classification schemes based on gross cell morphology and ultrastructure that placed G. vulgaris with the heteroloboseid lineage of the supergroup Excavata. Furthermore, this is the first rhizarian described to be capable of aggregative multicellularity, a life cycle that requires the complex interaction of thousands of cells to work in concert for a common goal. Perhaps even more interestingly, the fruiting of G. vulgaris appears to involve cell differentiation into stalk and spore cell types, and the former are often sacrificed through degeneration. We anticipate that further investigations of the molecular processes underpinning aggregation and fruiting will clarify how convergent evolution has so frequently generated this widely distributed mode of multicellularity.

First record of Epistylis procumbens Zacharias, 1897 (Ciliophora, Peritrichia) as epibiont on planktonic copepods in a neotropical floodplain lake

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Epibiosis is a facultative association between two organisms: the epibiont, which colonizes the surface of live substrates, and the basibiont, which hosts the epibionts. While numerous accounts exist for ciliates as epibionts of zooplankton, little information is available on the prevalence of epibionts in these populations, and data on seasonality and geographic distribution of epibionts is almost lacking. During a survey of ciliated protists attached to zooplanktonic copepods in the Upper Paraná River floodplain, we report the occurrence of Epistylis procumbens Zacharias, 1897 on Thermocyclops minutus (Lowndes, 1934) in one floodplain lake among 36 environments that were been studied. The zooplankton community was sampled in this lake, using a 100 µm plankton net, to obtain an integrated sample (2,000 L) from the water column at day time, and preserved with Bouin's fixative. Both zooplankton and ciliates abundances were determined by counting 10 % (77 colonies) of the sample in a Sedgewick-Rafter counting chamber using an optical microscope. The ciliates as well the planktonic copepods were identified using specialized taxonomic literature. T. minutus, the most abundant copepod species, reached an abundance of 4,925 ind/m^3, with 8 % carrying epibionts. E. procumbens colonized the entire body of the hosts, and the overall infestation density and load were higher on adults than on copepodites (2 %). This result could be explained by the fact that adult copepods constitute a more stable substrate for epibionts, since they do not molt and can accumulate a higher density of epibionts. On the other hand, the colonies in copepodites are shed with the cast skin every molt, forcing epibionts to find another substrate. Despite several studies have focused on the ecology of Upper Paraná River floodplain zooplankton, no data are available about the relationship between ciliate epibionts attached to planktonic organisms in this system, being this the first record of this kind of epibiotic relationship. Financial support: CNPq/PeId and CAPES.

Dissecting the microsporidian secretome: An interface between host and parasite

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Pathogenic fungi of the phylum microsporidia thrive as obligate intracellular parasites of humans and many species of economic importance. This group of over 1300 parasitic species are known to successfully infect vertebrates, invertebrates and protists despite highly reduced genomes and corresponding proteomes of around 2,000 proteins. The molecular basis of the host-parasite interaction is at present poorly characterised, however infection, survival and transmission from the host cell are undoubtedly dependent on both membrane bound and extracellular proteins to modify and manipulate the host cell environment. Emerging sequence data has allowed us to bioinformatically predict and compare the secretome of three microsporidian species; human infective Encephalitozoon cuniculi, honey bee parasite Nosema ceranae and Spraguea lophii a parasite of fish of the genus Lophius. This has allowed identification of a set of 25 'conserved' secreted proteins between the three distantly related microsporidian species. We have also used in vitro spore germination in S. lophii and quantitative mass spectrometry to identify the range of proteins secreted into the extracellular medium during transition from dormant spore to infective parasite. A large percentage of conserved secreted proteins between the 3 microsporidian species have at present only 'hypothetical' functional prediction or no functional prediction at all. However one conserved protein of particular interest has 7 predicted transmembrane domains, and also contains a HlyIII functional domain. We have demonstrated that the E. cuniculi HlyIII displays hemolytic activity in recombinant expression systems in both Escherichia coli and Saccaharomyces cerevisiae. We have also conducted phylogenetic analyses of homologs of HlyIII gene from both prokaryotes and eukaryotes. This shows a surprising motif shift present in fungi excluding the microsporidia. Due to its presence in distantly related and reduced microsporidian genomes we believe the microsporidian HlyIII gene may have crucial and core function in microsporidian development and may be one of the first 'virulence factor' proteins characterised in the phylum.

Protozoa as bioindicators in a Shortcut Biological Nitrogen Removal (SBNR) process treating high ammonium loaded wastewater

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The bioindicator capability of protozoan species inhabiting a pilot treatment plant with Shortcut Biological Nitrogen Removal (SBNR) Moving Bed Biological Reactor (MBBR) process treating high ammonium loaded wastewater has been studied. The SBNR-MBBR process consists in two consecutive reactors, anoxic and aerobic, with suspended carriers where the biofilm develops. Samples from mixed liquors and biofilms were collected every 2 weeks during 9 consecutively months. Operational parameters, biomass structure and quantification of protozoa species were carried out. Despite the high concentrations of $N\text{-}NH_4^+$ (average of 162 ± 80 ppm and 74 ± 71 ppm in anoxic and aerobic tank respectively), ciliates, flagellates and amoeba were observed. Results showed a system with a low diversity, with clear differences between protozoan communities of both biolfims. The 94 % of protozoa taxa were observed in aerobic biofilm, while in anoxic biofilm were only observed the 38 %. Aerobic biofilm seems to play an important role of reservoir in the system, from which mixed liquor is colonized. Epistylis sp has been the only specie that presented constant and abundant population, almost exclusively in aerobic biofilm. Due to lack of competition by other ciliates, Epistylis sp population dynamics has presented an exceptional relation with $N\text{-}NH_4^+$ variations, allowing us to consider Epistylis sp as a great tool as bioindicator of nitrification process in this system. The ciliates Cyclidium glaucoma and Colpoda, observed only in the mixed liquor, have also presented tolerance to $N\text{-}NH_4^+$. Both ciliates, in processes without biomass recirculation, can be indicators of high hydraulic retention times (HRT ranged from 5'6 to 8'3 days). Small flagellates were more abundant in the mixed liquor than in biofilms, specially in anoxic mixed liquor, demonstrating a considerable capacity to survive long times in anoxic conditions. The study of protozoan communities in new wastewater treatment processes allows us to offer new biologic management parameters, and also sheds light of species ecology in conditions hardly observed in widely used wastewater treatment processes.

Contribution to the knowledge of ciliate (Ciliophora, Protozoa) fauna of Turkey

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Considering the limited data about Anatolian Diagonal, the purpose of this study is to contribute to the knowledge of ciliates recorded from two new habitats. Samples were collected from different sites of a mesotrophic shallow and an oligotrophic deep lake which were from the northwest of Turkey. All the samples for this study were collected from lakes by using 10 µm plankton net and artificial subtrats. Plankton net samples were identified live and after fixation with 2 % lugol. As an artificail substrat glass slides were clipped to a PVC frame, and were immersed in the water at depth of about 0.5 m below the surface for the shallow and with 10 m intervals for deep the lake. All were stayed for about 1 month. Morphological characters for all the species were identified by live observation and observation with impregnation methods. The species were defined by the evaluation of morphometric measurements and counts which were performed digitally by IM50 image manager system and Q-win measurement program, few were with optical scale measure. Illustration of the specimens were by free-hand sketchs and micrographs. At the end of 2008 internationally recognised ciliate taxa number of Turkey were 175. Date from 2009, totally 115 additional taxa were recorded only from 2 habitats. Micrographs and drawings of someremarkable species according to the different sampling sites are shown and general habitat knowledge is given.

From malaria pathogens to sexually transmitted bugs: Comparative genomics of parasites

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Eukaryotic microbes are essential to many aspects of human health and the environment. The work in my lab focuses on comparative genomics of two protist lineages: apicomplexans, obligate parasites that cause severe human disease such as malaria (Plasmodium spp.) and cryptosporidiosis (Cryptosporidium spp.); and trichomonads, symbiotic, parasitic or free-living flagellates, one of which (Trichomonas vaginalis) causes 'trich', the most common non-viral sexually transmitted infection. The completion of the P. vivax genome (Carlton et al., Nature 2008) has led to more functional genomics studies, and we are currently using arrayCGH technology to identify loci involved in drug resistance in India, while sequencing more world-wide samples to construct a haplotype map for association mapping. Sequencing the T. vaginalis genome (Carlton et al., Science 2007) has enabled us to identify genetic markers for assessing the diversity and population structure of the parasite in women attending STD clinics in New York City. These studies reveal the prevalence of two phylotypes, and we plan to undertake metagenomic studies of vaginal samples to ascertain the association between these types and the normal vaginal microbiota. These projects will be discussed in the context of the importance of these group of organisms to human health.

Three protist meditations

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High resolution electron images of termite gut protist structure – Musical accompaniment by Alice Coltrane, Pharoah Sanders, et al.

Unraveling protist-bacterial symbioses in the termite gut: Inferences from structural and experimental studies with scanning and transmission electron microscopy and NanoSIMS

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Symbioses between large cellulolytic parabasalid protists and bacteria in the hindguts of lower termites are hypothesized to be important, if not essential for the survival and functioning of both partners, as well as the termite itself. Such associations are often very extensive, with the bacterial symbionts commonly forming dense communities on protist cell surfaces and within cytoplasmic vacuoles. Evidence for the symbiotic nature of these associations includes ultrastructure and more recently, completed genomes of bacterial partners that suggest a role in providing the protist with nitrogenous nutrients, while receiving glucose from cellulose breakdown by the protist in return. However, the great majority of protists likely to have bacterial associations (possibly symbioses) remain unexamined by SEM and TEM, and predictions based on genomic data await testing with experimental methods. To augment our knowledge of the diver-

sity of these protist-bacterial associations and their fine-scale structure (along with possible functional implications), we are examining protists from numerous species of lower termites with SEM and TEM. This reveals several new classes of protist-bacterial associations in different taxa of parabasalids and oxymonads. These include associations between protist flagella and spirochetes; associations of rod-shaped bacteria with protist posterior feeding zones; the presence of extruded towers of protist cytoplasm surrounded by rod-shaped bacteria, and several new associations of spirochetes with surfaces of various protists. In many of these associations data suggest an important role for the glycocalyx in mediating metabolite exchange, and possibly bacterial adhesion. Density and patterning of bacteria in protist resistant stages suggest mechanisms by which protist surfaces are periodically cleared and recolonized by bacterial symbionts. To test functional hypotheses of carbon and nitrogen exchange between protists and bacterial symbionts we have initiated in-situ stable isotope probing of live termites with 13C-cellulose and 15N-dinitrogen. We are using high-resolution imaging mass spectrometry by NanoSIMS to directly image the incorporation of 13C and 15N by the protists and bacteria to characterize these relationships. New results from these NanoSIMS studies will be presented.

Extensive molecular and morphological diversity of free-living trichomonads

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Trichomonads (Parabasalia) are members of the eukaryotic supergroup Excavata. Although most of approximately 400 known trichomonad species are intestinal symbionts of termites and other animals, few free-living representatives have been described as well. It has been assumed until recently that the free-living trichomonads constitute a single lineage, the Honigbergiellidae. However, three additional lineages only distantly related to Honigbergiellidae were identified in 2010 (Tetratrichomonas undula, Lacusteria cypriaca and Psedotrichomonas keilini). Interestingly, all known free-living trichomonads belong to the class Trichomonadea and are likely to be descendants of endobiotic ancestors. Such a switch between the endobiotic and free life style is quite rare among eukaryotes. To investigate the diversity of free-living trichomonads more deeply, we have recently isolated and cultivated six strains obtained from fresh-water and brackish sediments. Phylogenetic analyses of the SSU rRNA gene showed that some isolates were more or less closely related to the already-described P. keilini or L. cyprica. However, two isolates formed deep isolated lineages in the SSU rRNA gene tree. Light- miscroscopic and TEM study of the two strains revealed that they display a number of features unusual for the other trichomonads. Most notably, the strain LAGOS2D bears flagellar vanes which may represent the first known excavate feature of trichomonads. Therefore, the strain LAGOS2D might be a primarily free-living trichomonad and may represent a key organism for understanding the origin of parabasalids.

Hypotrichomonas: The tip of the iceberg of trichomonad diversity

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The genus Hypotrichomonas Lee, 1960 belongs to the smallest parabasalian class Hypotrichomonadea. Typical morphological features of the genus Hypotrichomonas include three anterior flagella, rudimentary costa and fully developed undulating membrane. Although five Hypotrichomonas species have been described from intestine of lizards and birds, most of the descriptions were too brief and incomplete. Only H. acosta, the type species, has been observed repeatedly. During our previous studies, we have created a collection of approximately 250 trichomonad strains obtained from various different hosts. Preliminary SSU rDNA screening showed that 15 strains contain members of the genus Hypotrichomonas. We have sequenced their SSU rDNA and stained them with protargol. Phylogenetic and morphological analyses showed that these isolates represent eight distinct species, six of which being novel. Two of the species showed unusual morphology, such as a reduced undulating membrane or a fiber reminiscent of costa. In addition, seven unclassified SSU rDNA sequences from GenBank obtained from beetles clustered with Hypotrichomonas as well, possibly representing two more species. Our strains were isolated from taxonomically wide range of hosts including cockroaches, frogs, tortoises, lizards, snakes, marsupials, pigs, rodents, and primates. The genus Hypotrichomonas thus contains at least ten species which differ in morphology, phylogenetic position and host range. From our results we conclude that the diversity of the genus Hypotrichomonas as well as of the whole Parabasalia is still only poorly understood. The lineages described so far likely represent only

a small fraction of the true diversity of parabasalids.

Dynamic chromatin remodeling and the control of exclusive surface-antigen transcription in Paramecium tetraurelia

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Chromatin-Immunoprecipitation (ChIP) is a powerful technique to investigate DNA-associated proteins, such as histones, chromatin-modifying enzymes, or other modulating factors which influence the chromatin condensation level. We established a ChIP protocol to characterise Paramecium macronuclear DNA. Analysing the surface antigen-multigene family, we found that these genes are under control of mutual exclusion mechanism controlling exclusive transcription by two classes of short interfering RNAs. Chromatin-Immunoprecipitiation demonstrated that transcriptional activation of surface antigen genes is correlated with accumulation of H3K4me3 and H3K9ac in the 5'-region of the genes. Consequently, transcriptional silent genes reveal these post-translational histone modifications predominantly in their 3'-regions. We therefore conclude that transcriptional control of surface antigen genes is controlled by the initiation of transcriptional activity. We then analysed RDR3 silencing lines, showing a surface antigen co-expressive phenotype with a loss of a single siRNA species. The direct comparison of nucleosome modifications in a single part of a gene with the appearance and loss of short interfering RNAs allowed then to characterise the effectiveness of these molecules.

Diversity of Pelomyxa species (Archamoebae, Pelobiontida)

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The fauna of pelomyxas from freshwater basins of the North-West Russia was investigated. 11 species of the genus Pelomyxa were found and studied using the light and transmission electron microscope. Besides P. palustris, four species: P. prima (Gruber, 1884), P. binucleata (Gruber, 1884), P. tertia (Gruber, 1884), P. belevski Penard, 1904) were reisolated firstly after their description. Moreover, four species P. gruberi Frolov et al., 2006, P. stagnalis Chistyakova et al., 2010, P. flava Frolov et al., 2010, P. corona Frolov et al., 2004 were described for the first time. And beside that three species were studied but don't identified yet. All investigated Pelomyxa species are well distinguished even with light microscope. Next features can be concerned as the most important differential characters of pelomyxas: shape of the cell during locomotion, cytoplasm structure and colour depending on food preferences of pelomyxas and structure of the nuclei. The number of species-specific characters of Pelomyxa species considerably increases when using TEM data. The most substantial differences are found in the organization of basal part of flagella, cell surface structure, organization of the nuclear envelopes and glycogen bodies as well as in the composition of bacterial endobionts. According to the molecular phylogenetic analysis based on 18S rRNA sequences, three Pelomyxa species (P. stagnalis, P. palustris and P. belevski) have quite significant differences in the sequences of 18S rRNA gene (similarity doesn't exceed 53 %). Meanwhile according to the molecular phylogenetic analysis data they constitute a group of organisms most closely related to each other among all eukaryots studied so far. Interestingly, the sequence we obtained for P. stagnalis and that of P. palustris deposited in Genbank by Milyutina et al (2001) have only 1 % difference. Taking into account the extremely high variability of 18S rRNA gene in pelomyxas this difference may be considered insignificant. So, results of comparative morphology analysis of pelomyxas as well as molecular phylogenetic analysis supports the hypothesis of species diversity in the genus Pelomyxa.

Photosensitized inactivation of protozoa in the medical and environmental control of water-borne diseases

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Photodynamic therapy (PDT) takes advantage of the properties associated with a number of tetrapyrrolic or phenothiazine derivatives compounds to localize and be retained by a variety of cells. These compounds, termed photosensitizers, are intrinsically nontoxic for the biological systems; however, upon excitation by selected visible light wavelengths, they generate reactive oxygen species, which cause irreversible oxidative modification of cell constituents. The effectiveness of PDT as a novel mode in treating some diseases caused by bacterial and eukaryotic micro-organisms has been

demonstrated, offering alternative approaches in the medical and environmental control of pathogenic agents. PDT with aminolevulinic acid (ALA), Al(III)-phthalocyanine or phenothiazinium analogues was proposed to treat cutaneous leishmaniasis as well as retinochoroiditis of Toxoplasma gondii origin. The combined action of visible light and photosensitizers was also demonstrated to disinfect blood products through the photoinactivation of Plasmodium falciparum and Babesia divergens. Among the pathogenic protozoa, the free-living soil and freshwater amoebae Naegleria fowleri, Acanthamoeba spp., can become highly pathogenic and responsible for opportunistic and non-opportunistic infections, such as granulomatous encephalitis. A tetracationic Zn(II)phthalocyanine efficiently inactivated Acanthamoeba palestinensis cultures in both the cystic and vegetative stages upon illumination with 600 – 700 nm light: the two forms exhibited a comparable dependency on the photosensitizer concentration, however cysts required a significantly longer irradiation time to give a similar degree of inactivation. A phthalocyanine concentration as low as 0.5 μM, followed by 20-min irradiation, induced 50 % inhibition of excystment in trophozoites. The photosensitization via cationic phthalocyanines appeared to represent an efficient and safe approach for a close control of the cystic and trophozoitic cultures of a pathogenic protozoan such as Acanthamoeba, opening new perspectives for the disinfection of microbiologically contaminated waters as a tool for the prevention of water-borne infectious diseases. Findings dealing with the photoinactivation of cultures of Colpoda inflata and Tetrahymena thermophila indicated that also these free-living ciliates are sensitive to the photocidal action of several meso-substituted porphyrins, the most recently used being meso-tri(N-methyl-pyridyl), mono(N-dodecyl-pyridyl) porphine (AquaFrin). Thus an attentive modulation in the concentration of photosensitizers used in disinfection of waters by pathogenic protozoa must be performed.

Initial insights from the Naegleria fowleri genome initiative

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The "Brain-Eating Amoeba" Naegleria fowleri is the causative agent of primary amoebic meningoencephalitis, a water-borne disease of the central nervous system. Disease progression is extremely fast and fatal if untreated. Incidence of infection is low, but increasing. N. fowleri is found globally, but grows preferentially at high temperatures (up to 45 °C), and thus is found more frequently in warm climates, such as the southern US. However, also exists in temperate regions in association with thermal springs. Climate change may additionally increase the prevalence of this pathogen in more northern regions. N. fowleri is currently best treated with amphotericin B, a severely toxic antibiotic. A better understanding of pathogenicity in this species forms a first step in the discovery of more effective and safer therapies. N. fowleri has a non-pathogenic, non-thermotolerant relative, Naegleria gruberi, for which the genome sequence has recently been determined. The N. gruberi genome sequence forms the perfect background to investigate the genetic basis of pathogenicity in N. fowleri through comparative analysis. We have produced a100X coverage of the N. fowleri nuclear genome using a combination of 454 pyro-sequencing and Illumina Hi-Seq technologies. We here report an initial meta-comparison between the ~50 KB mitochondrial genomes and a contig of nuclear genomic DNA of approximately equivalent size of the two Naegleria species. These show widely different patterns of conservation and degrees of synteny. Our initial meta-comparison lays the groundwork for a full-scale genomic comparison allowing eventual identification of molecular markers to be used for improved diagnosis, and putative genes involved in N. fowleri pathogenesis. Together these will greatly improve our understanding of this globally distributed amoeba and how it kills.

A new species of the rare cliate Discomorphella (Plagiopylea, Odontostomatida) from an endangered microhabitat in Rio de Janeiro, Brazil

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Discomorphella Corliss 1960 is a curious genus of odontostomatid ciliates which have the ciliature reduced and possess conspicuous spines on the body surface. Currently, there are three known morphotypes included in this genus, namely the type species D. pectinata and its varieties bidenticulata and lauterborni, of which taxonomic status differs among authors. A new species of this genus is characterized by measuring in vivo ~ 55μm x 40μm (lateral view); body dorsoventrally compressed with left side flat and right side irregularly bulged;

frontal and short ciliary fringes bearing ~ 15 and 5 (rarely 6) rows of cilia, respectively short fringe located conspicuously below the level of the frontal fringe; invariably one frontal spine, two long spines in the right side and one slight shorter spine close to the rear end of the left side of the body, attached in between the two leftmost ciliary rows of the short fringe. The oral ciliature, ventral cilia, the three posterior cirri, macronucleus and contractile vacuole are as known from D. pectinata. The type locality of the new species is an abandoned artificial pond located within the campus of Universidade Federal do Rio de Janeiro, RJ, Brazil. The pond is a hot-spot for microscopic biodiversity, thriving with dozens of species of autothropic and heterotrophic protists and small metazoans. Sadly, given the lack of conservation programs directed to microorganisms and that the referred pond is generally seen as a reservoir of filth water by laymen eyes, it may not last long. Financial support: CNPq - PROTAX 562366/2010-5.

Molecular phylogenetics and evolutionary history of planktonic Acantharia (Radiolaria)

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Acantharia are marine protists taxonomically affiliated to the super-group Rhizaria. In the Ocean, they are ubiquitous and commonly outnumber their rhizarian counterparts (e.g. Foraminifera, Polycystinea). They are active grazers and substantially participate to primary production through endosymbiotic relationships with microalgae. Despite their key position in marine ecosystems, our knowledge on the biology of Acantharia is still in its infancy. The evolutionary history of these marine protists is unknown since there is no fossil record. The first classifications based on the skeleton's morphology have been done by Johannes Muller in 1858 and his student Ernst Haeckel in 1888. In 1926, Schewiakoff included several cytoplasmic features to produce the main and currently used taxonomic framework for Acantharia. Since this time, this has not been revised and no molecular tools have been applied. Based on a morpho-molecular approach on single cells, our study aims at re-assessing the taxonomy of Acantharia. To do so, we isolated about 250 cells from different oceans, took pictures of each specimen for morphological identification, and sequenced the small (18S) and large (28S) subunit ribosomal DNA sequences for phylogenetic analysis. Here we propose an amended taxonomical framework for Acantharia combining morphology and molecular markers. We also assess the divergence time of Acantharia via a molecular clock analysis comprising sequences of Acantharia and some eukaryotic lineages. This study improves our understanding on this poorly known yet important protistan group by exploring their molecular and morphological diversity, as well as, their evolutionary history.

Rhizaria: The simultaneous rise of protistology and Art Nouveau

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Rhizaria, with their marvelous skeletons, are most likely the first protists that appeared to human eyes. Found in Egyptian pyramids, they retained the attention of Pliny the elder and Herodotus during the Antiquity. Much later in 1796, the famous german writer and scientist Johann von Goethe, pioneer of the Romantic Era, coined the word "morphology", inventing a novel science. Goethe was intrigued by the astonishing variety of organic forms. His study of natural morphologies aimed at unveiling implicit bonds between the arts and science, paving the way to aesthetics. The poet had great influence on many scientists, in particular Johannes Muller and Ernst Haeckel, who both became fascinated by the morphologies of marine creatures. Haeckel, one of the fathers of embryology, became obsessed with the beauty of Radiolaria. He restlessly drew and described hundreds of radiolarian forms, naming them and trying to resolve their phylogenetic relationships. His inspiring work gave simultaneous birth to two major fields: protistology and Art Nouveau. Haeckel invented the word "protist", and his compelling artistic talent was decisive for the rise of bionic architecture and "Art Nouveau". The rhizarian architectures, incredibly complex for single-celled creatures, undoubtedly awoke our sense of aesthetics. Despite the fundamental role of Rhizaria in the second half of the 19th century, these organisms became largely overlooked as for their ecological and evolutionary significance.

Evolutionary dynamics of the protist communities living in the hindgut of lower termites

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Several protist groups are necessary for the survival of lower termites because these gut symbionts are required for the degradation of lignocellulose. On the other hand, these protists are usually strictly anaerobic and cannot survive outside their gut habitat. Therefore, one would expect that such interdependent associations would be evolutionary stable, exhibiting a general pattern of co-cladogenesis. Previous studies revealed, however, that species composition of protist communities can vary between termite families and genera, suggesting that strict cospeciation between termites and their symbionts have not occurred. In my talk, I will first present an overview of the co-evolutionary patterns of the associations between protists and lower termites. In a second part, I will present recent results focusing on the protist communities of the subterranean termites of the genus Reticulitermes (Rhinotermitidae). The obtained data set reveals that, even during the diversification of a single genus of termite, protist – termite associations seem to be evolutionary unstable and in perpetual dynamics. The possible reasons for such evolutionary dynamics of these symbiotic systems will be discussed.

Fermentation products of termite gut flagellates and the effect of oxygen

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The gut of lower termites contains a complex microbial ecosystem that is densely populated by symbiotic flagellate protists belonging to the phyla Preaxostyla and Parabasalia. Although these flagellates are considered to be oxygen-sensitive organisms that ferment lignocellulose to acetate, hydrogen and carbon dioxide, they have been implicated also in the production of lactate, a major intermediate in hindgut fermentations. However, since there are no cultures of termite gut flagellates, information on their metabolism is limited. Here we demonstrate that small and large flagellate species produce different fermentation products. The major metabolites in the hindgut of Reticulitermes santonensis and Zootermopsis nevadensis are acetate, lactate, succinate and butyrate. When the termites were treated with antibacterial drugs, which resulted in a substantial reduction of the large flagellate species, only acetate und lactate were detected, suggesting that the small flagellates are a source of lactate. Physical enrichment of the larger flagellates (mainly Trichonympha spp.) of Zootermopsis nevadensis yielded a suspension that produced acetate as the only soluble product under anoxic conditions. Under microoxic conditions, however, also formate, succinate and glycerol were formed, indicating a strong effect of oxygen on the fermentation products of Trichonympha spp. We are currently elucidating hydrogen production and the capacity to reduce oxygen of particular flagellate species.

Liver response to Eimeria coecicola infections of the rabbit Oryctolagus cuniculus

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Coccidiosis causes important economic losses in livestock and poultry production in domestic animals including rabbits. The study aimed to investigate the pathological effects of E. coecicola on liver of rabbit. Haematological, biochemical, histological and genetic alterations due to inoculation of rabbits with 5 × 104 sporulated oocysts, were investigated. Maximal shedding of 1.1 million oocyst per gram faeces of rabbit occurred on approximately day 7 p.i.. Infection was associated with upregulation of 363 genes and downregulation of 381 genes of the liver, as evidenced by cDNA microarray analysis. Infection also induced inflammation and injury of the liver. This was evidenced (i) as increases in inflammatory cellular infiltrations, dilated sinusoids, and vacuolated hepatocytes, (ii) as increased mRNA levels of inducible nitric oxide synthase (iNOS) and of the cytokines interferon gamma (IFN-γ), and interleukin-6 (IL-6), (iii) as increased plasma levels of alanine and aspartate aminotransferases, alkaline phosphatase, γ-glutamyl transferase and total bilirubin, (iv) as increased production of nitric oxide derived products (nitrite/nitrate) and malondialdehyde, and (v) as lowered glutathione levels and decreased activities of catalase and superoxide dismutase, respectively. Identification of rabbit genes induced or repressed following Eimeria infection offers a powerful tool to enhance our understanding of host-parasite interactions leading to protective immunity.

The role of acidocalcisomes in the stress response of Trypanosoma cruzi and T. brucei

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Acidocalcisomes are acidic phosphorus- and calcium-containing organelles widely distributed from bacteria to humans (Patel and Docampo, Trends Cell Biol. 20, 277-86, 2010). Phosphorus is stored mainly as pyrophosphate (PPi) and polyphosphate (poly P) and acidity is maintained through the activity of two proton pumps, a vacuolar H^+-pyrophosphatase and a vacuolar H^+-ATPase. A Ca^{2+}-ATPase is involved in calcium uptake and an aquaporin is important for water transport in T. cruzi. Acidocalcisomes function as storage sites for cations and phosphorus, participate in PPi and poly P metabolism, and volume regulation, and are essential for virulence. Enzymes involved in the synthesis and degradation of PPi and poly P are present within the organelle. Poly P synthesis was unclear until recent work demonstrated that vacuolar transport chaperone 4 (VTC4) catalyzes its synthesis in yeast. T. brucei possesses a VTC4 homologue (TbVTC4, Tb11.01.4040), which we detected in a proteomic analysis of acidocalcisomes of T. brucei. T. cruzi also possesses a VTC4 homologue (TcVTC4, Tc00.1047053511127.100). Localization studies using antibodies against TbVTC4 revealed its co-localization with the vacuolar proton pyrophosphatase, a marker for acidocalcisomes, in both parasites. Both proteins were expressed in bacteria and shown to have poly P synthase activity. Ablation of TbVTC4 expression by RNA interference (RNAi) in procyclic forms (PCF) led to decreased growth and poly P content, and increased PPi content, while overexpression of this gene led to the opposite results. We obtained conditional double knockout mutants in both T. brucei PCF and bloodstream forms (BSF). Phenotypic characterization of BSF mutants indicated that TbVTC4 gene ablation produced a decrease in parasite growth rate while gene overexpression did not have any effect on growth. Our data suggests that TbVTC4 and TcVTC4 are essential enzymes involved in poly P synthesis. Since VTC4 is absent in vertebrates, this enzyme could be a potential drug target. In T. cruzi a signaling pathway involving cyclic AMP (cAMP) generation is important for fusion of acidocalcisomes to the contractile vacuole, transference of aquaporin, and volume regulation. This pathway is also an excellent target for chemotherapy as shown by the effects of phosphodiesterase C (PDEC) inhibitors.

Similarity in widely separated communities of planktonic protists (tintinnid ciliates)

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Tintinnids are a monophyletic group of planktonic ciliates (mainly marine). They are, compared to many groups of protists, relatively homogenous in terms of ecology and morphology. However, tintinnids are a very species-rich group; several hundred species have been described and in a single locale, at one time, dozens of species can be found. The co-existence of so many apparently ecologically similar species remains difficult to explain. Given a rough ecological equivalency of species, community composition should vary with distance between the communities, in the absence of distributional barriers or environmental gradients. Here 3 tintinnid communities, widely separated geographically, are compared. Assemblages were sampled in two oligotrophic gyres of the Western and Eastern basins of the Mediterranean, separated by 2500 Km. A third set of samples was obtained from the California Current System, a productive upwelling area, of the Eastern Pacific Ocean. The two geographically distant Mediterranean assemblages showed very similar species richness (36-42), overall species catalogues, as well as identities of core (found in all stations) and 'occasional species'. Furthermore, the patterns of relative abundance distribution, whether in terms of species or size-classes (based on oral diameters), were nearly identical. The California Current assemblage was less species-rich (12 - 29 species per station). Remarkably though, the most abundant California Current species were nearly the same as those found in the Mediterranean. In all 3 assemblages, the size-class of 'mouth-size' (lorica oral diameter) which contained species whose mouths range between about 20 and 30 microns accounted for most of the species and the individuals. While different tintinnid species occupy distinct niches, at least that of a particular size-class, widely separated areas can harbor very similar assemblages of morphologically distinguished species. The genetic similarity of widely separated populations of a single tintinnid morphotype remains to be investigated. Nonetheless, some morphotypes appear to be globally common while others are globally rare. Research supported by the ANR Biodiversité program Aquaparadox. www.obs-vlfr.fr/LOV/aquaparadox/

Comparative study of cercomonad community structure at different soil sites by high-throughput parallel tag sequencing

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New molecular methods facilitate intensive studies of community structures. Morphological studies constitute the basis of protozoan diversity studies, but determination of protozoans is time consuming and requires long-term experience to identify the organisms morphologically reliably. Over the last few years molecular studies supplemented morphological studies thus making a certain identification of polymorph organisms possible even without ultra structural studies. Morphological determinations from monoclonal cultures in combination with molecular studies resulted in SSU rDNA sequence barcodes as an accurate tool for species identification. We applied 454-sequencing, also called high-throughput parallel tag sequencing to determine the diversity of cercomonads in forest soils, including 40 different forest sites in Germany with different degree of anthropogenic influence. Cercomonads are important components of soil microbial food webs and inhabit morphologically very variable organisms. We analyzed the cercomonad community structure based on the highly variable V4-region of SSU rDNA.

New contribution to the 18S-rDNA phylogeny of urostylid ciliates (Ciliophora, Urostylida)

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Urostylids are flexible-body ciliates with a "hypotrichous" body architecture which present a mid-ventral complex of paired ventral cirri organized in a conspicuous zigzag pattern. Even though having generally weak phylogenetic signal to resolve deep divergences within the core Stichotrichia, the 18S-rDNA marker was shown to resolve the internal relationships of the so-called "core urostylids" with relative stability and high statistical support (Paiva et al., 2009 Gen. Mol. Res., 8: 233-246). We herein hypothesize the phylogeny of six urostylid 18S sequences (Caudiholosticha sylvatica, Hemycicliostyla sphagni, Nothoholosticha sp., Pseudokeronopsis sp., Pseudourostyla levis, and a novel [undescribed] urostylid genus) sampled from Brazilian locations. The DNA was isolated using phenol-chloroform method and the 18S gene was isolated via PCR using specific primers. The obtained sequences were assembled along with others from major stichotrichian taxons (Sporadotrichida, Stichotrichida and Urostylida) plus euplotids as outgroup, and aligned with the software ClustalX. Maximum likelihood and Bayesian phylogenetic analyses were performed using PhyML and MrBayes respectively. Node support was accessed via 1,000 bootstrap pseudoreplicates and Bayesian posterior probabilities. The six new sequences all group within the core urostylids, which generally exhibited high support, thus corroborating the literature. C. sylvatica grouped as sister taxon of P. levis + P. cristata, which were distantly placed from P. franzi. This last formed a monophylum with H. sphagni, thus suggesting the polyphyly of genus Pseudourostyla. Notoholosticha spp. formed a consistent monophylum, which was placed within the cluster of Pseudokeronopsis. As this whole cluster has strongly supported nodes, it is perhaps safe to suppose Notoholosticha is a pseudokeronopsid with a reduced bicorona. The new genus clustered with Urostyla grandis with high support, which is plausible because they share great morphological similarity. Other traditional urostylid lineages, such as those of uroleptids, Holosticha and Parabirojimia, departed from other stichotrich nodes, corroborating previous 18S analyses in which a core and some peripheral urostylid lineages were recovered. Interestingly, the branch lengths in the core urostylids are often slight longer than the observed in unstable stichotrich nodes. This is consistent in the literature, and may suggest some heterogeneity in the 18S evolution rate.

In vitro activity of methylgerambullin from Glycosmis mauritiana against Entamoeba histolytica and Giardia intestinalis

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The microaerophilic protist parasites Entamoeba histolytica and Giardia intestinalis are the cause of important health problems in many countries of the world. The drug of choice for treatment of both parasites is metronidazole and although it has been used successfully for more than 40 years, it also possesses adverse properties such as potential teratogenicity. We therefore examined the anti-amoe-

bic and anti-giardial activity of 14 newly isolated substances from tropical plants, from Rutaceae, Meliaceae and Stemonaceae. E. histolytica strain HM-1:IMSS and G. intestinalis strain WB C6 were grown anaerobically in 96-well microtiter plates. Tests were performed in triplicate and repeated three times, at compound concentrations between 1 µg/ml and 20 µg/ml. After 24 h and 48 h, life and dead cells were counted. Methylgerambullin from Glycosmis mauritiana (Rutaceae) showed activity against both parasites. The mean EC50 values (24 h treatment) were 6.09 µg/ml for anti-amoebic and 6.14 µg/ml for anti-giardial activity. These values varied significantly between the repeats, the activity being lower in freshly prepared media. This effect depended on the level of reduced cysteine in the medium, which is supported by testing methylgerambullin in four freshly prepared media with defined cysteine concentrations. This led to the hypothesis that chemical reaction of the compound with thiol groups in the parasite may be important for its mode of action. The effect of methylgerambullin on the E. histolytica proteome was analysed by two-dimensional gel electrophoresis. Four extra protein signals were reproducibly found in treated cells, which could be identified as isoforms of alcohol dehydrogenase and pyruvate:ferredoxin oxidoreductase shifted to an acidic isoelectric point. These findings are the very first steps to unravel the mode of action of this unusual new compound. Supported by the "Österreichische Forschungsförderungsgesellschaft" FFG.

Re-inventing the wheel: Overcoming low Reynolds numbers convergently in the termite hindgut

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The communities of protistan symbionts of the hindguts of some termites may be considered as manifold replicates in Galapagos island-like isolation within each of about a thousand termite species. The evolution of those replica communities has unfolded in diverse ways over the course of 300 million years, filling niches within the constraining parameters of crowded, viscous, and nearly anoxic conditions. The relatively stable nutrient and oxygen levels within each hindgut, seem repeatedly to have been auspicious conditions for the diversification of cell morphologies, including the baroque and bizarre. One particular parameter of termite hindguts (or of any microbial habitat) is a perception (by the microbes) of overwhelming viscosity, sometimes described by low Reynolds numbers. Nonetheless, termite symbionts move with exhuberance, whether churning in place or shoving past and through the swarming masses of other cells, themselves churning and shoving. Clearly low Reynolds numbers are being overcome, presumably due to several types of adaptations, including increases in cell size, stream-lining of shape and acquisitions of symbionts as well as modifications of shapes, sizes, and functions of motility structures that enhance efficiency. The growing body of DNA sequence analyses of termite symbionts, allows some of those modifications to be interpreted as examples of convergent evolution. The many island-like (experiment-like) replicates in which termite symbiont communities are found, enhance and reinforce those interpretations and even allow heuristics to be derived as to the ranges, limits and possibilities of motilities at low Reynolds numbers. This paper focuses primarily on interpreting convergences in the larger, charismatic protists of termites, especially those recently reclassified according to DNA sequence analyses by Moriya Ohkuma, Eric Viscogliosi, Vladimír Hampl, Patrick J. Keeling and colleagues: Trichonymphaea, Trichomonadea, Spirotrichonymphaea, and Cristomonadea (lophomonads, calonymphids, and devescovinids.) Unaware of the admonishment to not "reinvent the wheel", the termite "megamicrobes" have done just that. In the island-like isolation of diverse hindguts, many convergent inventions and variations have evolved by which microbes have increased their size and enhanced their mechanisms of motility.

Spatial distribution of kinetoplastid flagellates in hypersaline anoxic deep-sea basins in the Eastern Mediterranean

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The combination of nearly saturated salt concentration and corresponding high density, high hydrostatic pressure, absence of light, anoxia, and a sharp chemocline make the deep hypersaline anoxic basins in the Eastern Mediterranean Sea some of the most polyextreme habitats on Earth. Using kinetoplastid-specific primers, we detected kinetoplastid flagellates in some of the harshest deep-sea environments known to date, including some whose small subunit ribosomal RNA gene sequences are not closely related to cultured representatives. Kinetoplastids, including presumably novel

representatives appear to be specialists of halocline environments in the Eastern Mediterranean, and comprise a significant fraction of the protist communities in the brines and haloclines of several basins. Fluorescent in situ hybridization data indicate a novel 'unidentified' sequence clade of kinetoplastids related to bodonids represent as much as 10 % of the total protist community in the Discovery Basin halocline. Since different kinetoplastid groups are unevenly represented in the different basins and habitats we sampled, their spatial distribution appears to be in line with the moderate endemism hypothesis for protists.

The conceptual basis of species

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A general goal in biology is to find the correct definition of 'species'. However, there may be no such definition. Biologists, including microbiologists, offer numerous and conflicting species concepts. Those concepts pick out different types of taxa: some species are groups of sexually reproducing organisms, others are phylogenetic lineages, and still others are groups of organisms occupying distinct niches. Given the variety of taxa called 'species', we have good reason to believe that there is no species category in nature. Consequently, we should stop looking for the correct definition of 'species'. This suggestion may sound radical, but it offers a practical and theoretically sound answer to the species problem. Furthermore, this suggestion may be Darwin's approach to the species problem.

Archamoebae (Amoebozoa) as case examples to illustrate diverse protistan evolutionary history

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The supergroup Amoebozoa includes naked and testate lobose amoebae, pelobionts, entamoebids, and mycetozoans. Increasing phylogenomic and phylogenetic data support the classification of these clades (Wegener-Parfrey et al. 2010). The anaerobic Archamoebae (Entamoebae and pelobionts), were wrongly considered 'primitive eukaryotes' that lacked mitochondria and placed at the base of the eukaryotic tree. Single gene analyses of metabolic genes (e. g. alcohol dehydrogenase adhe) have contributed to conflictive phylogenetic depictions, due to horizontal acquisition (horizontal gene transfer -HGT) of genes from prokaryotes and unicellular eukaryotes. Classic SSU rDNA phylogenies have been unreliable to resolve the relatedness between amoeboid protists (Pawlowski & Burki 2009). Phylogenomic analyses of 100 genes support the grouping of three highly divergent amoebae Dictyostelium, Entamoeba, and Mastigamoeba within the class Conosea (Bapteste et al. 2002). The protein sequences of ADHE from Entamoeba terrapinae, Entamoeba invadens, Entamoeba moshkovskii, and Entamoeba histolytica branch together next to a cohesive cluster of low G+C Gram positive and alpha-proteobacteria (i. e. Streptococcus spp., Mannhelmia sp., Pasteurella sp., and Actinobacillus sp.; Andersson et al. 2006), suggesting that ancient amoeba most likely ingested, via phagotrophism, prokaryotes capable of glucose fermentation, and later integrated early bacterial metabolic genes into the ancestral Entamoeba genome (Espinosa et al. 2001, Paz-y-Miño C. & Espinosa 2010). Close to 70 Entamoeba genes, seven of which are involved in energy metabolism, have sequence features consistent with HGT origin (Alsmark et al. 2009). Combining molecular, biochemical, ecological and behavioral analyses can help discover the natural groups within Archamoebae. Specific examples are used to discuss how evolutionary processes have given rise to extant Amoebozoa and alternative views that claim a 'Designer' as responsible for protistan diversity are unfounded.

Morphological reconstruction of a cryptic cytoskeletal structure in Trypanosoma brucei

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Trypanosoma brucei is an important euglenozoan parasite whose cytoskeletal morphology has been extensively studied using electron microscopy. A novel cytoskeletal structure (provisionally referred to as the "bilobe") that contains a homologue of centrin, a calcium-binding protein ubiquitously found in microtubule organizing centres, was recently discovered. Further work identified and localized, using immunofluorescence, other protein components (namely TbMORN1 and TbLRRP1) but 40 years of ultrastructural studies have failed to reveal any structure that corresponds to the immunofluorescence data. Taking advantage of the bilobe's strong association with the flagellum, we extracted cells stably expressing YFP-tagged TbMORN1 with detergent to produce cell ghosts (comprising the outer corset microtubules, the flagellum, and asso-

ciated structures). Further treatment with high salt resulted in the purification of isolated flagella and associated structures. We then labeled these two preparations with antibodies against YFP:TbMORN1, TbCentrin4, TbLRRP1, and TbBILBO1, prior to negative staining for whole mount transmission electron microscopy (TEM). Data obtained from these preparations revealed a complex, asymmetrical structure that is associated with important cytoskeletal elements including the flagellar pocket collar, the microtubule quartet, and the flagellum attachment zone. Data obtained through whole mount TEM were combined with data from previous work using immunofluorescence, thin section TEM, and tomography in order to produce a morphological model of the bilobe. While the bilobe's function remains unknown at this point, its position and complexity suggest an important role in the regulation of cellular processes such as motility or nutrient uptake and/or organelle biogenesis and cell division. Searching for similar structures in related taxa (such as free living kinetoplastids, including the genus Bodo) may give additional insight into the bilobe's evolution and ancestral, if not current, function.

Exploring the species richness of ciliated protists in Lake Zurich (Switzerland)

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Ciliates (Ciliophora) represent an integral component of the freshwater microbial food web and affect different trophic levels due to their broad food range. Whereas there is a lot of data about bacteria, algae and metazooplankton in Lake Zurich, knowledge about ciliate diversity and their quantitative importance in the lake is scarce and the only data originate from the 1950's and 60's. Ever since, the status of the lake changed drastically, from highly overloaded with nutrients to a more natural condition nowadays. Our study aimed to get first insights into the pelagic and benthic (sediment) ciliate assemblages and to investigate their role during the productive algal spring bloom period in the year 2009. Pelagic ciliates responded immediately to the first rise of phytoplankton and were the most important consumers of primary production in that period. Our intensive sampling scheme (2 – 3 day intervals) allowed for the observation of several short-living blooms of single quantitatively dominating species. Thus, a clear succession of broader taxonomic groups and of functional groups could be described. Ciliate diversity and abundance was strongly coupled to the composition of algal assemblage and we observed parallel changes in both, prey and predators diversity indices. After the decline of the algal bloom ciliates decreased mainly due to impact of grazing zooplankton. In total, 34 pelagic and 120 benthic ciliate species were identified during a relatively short sampling period. This highlights the importance of ciliates for the total species richness of Lake Zurich.

Functional and comparative analysis of the plastid proteome of the glaucophyte Cyanophora paradoxa and the red alga Cyanidioschyzon merolae

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Oxygenic photosynthesis evolved more than 3 billion years ago in the ancestors of modern cyanobacteria. Acquisition of the ability to perform photosynthesis by eukaryotes occurred by engulfing and stably integrating a photoautotrophic prokaryote. This event, referred to as primary endosymbiosis, occurred only once in the ancestor of the protoalga, giving rise to the Archaeplastida. This endosymbiotic integration produced three major photoautotrophic lineages: the glaucophytes, the red algae and the green algae, including their descendants, the plants. The establishment of the plastid was accompanied by a massive gene transfer from the cyanobacterium to the nuclear genome. This all implicated the evolution of a protein import apparatus in order to relocate the gene products back to the original compartment. Furthermore, a key feature of early success for the endosymbiosis must have been the integration of the metabolism of the host and the endosymbiont. The key to this process would have been solute transporters that coordinate the exchanges of metabolites across the envelope membrane. Contributions to elucidate the transport mechanisms have been made based on computational tools such as TargetP to identify plastid-targeted proteins and by proteomic surveys in order to generate a collection of plastid-localized proteins. However, most of the data available to date are restricted to the Viridiplantae, whereas the knowledge on red algae and Glaucophytes is rather limited. Previous work performed in our lab demonstrated that prediction programs are not applicable on these two lineages. A proteomic approach is therefore necessary to generate a comprehensive list of the plastid proteome. Plastids are isolated from the glaucophyte

Cyanophora paradoxa and the red alga Cyanidioschyzon merolae, and stroma and envelopes are fractionated in order to obtain an enriched fraction. MS identification of the isolated proteins will provide information on the protein import and the metabolite exchange mechanisms in these two lineages.

Ubiquitin and turnover of the trypanosome surface

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The African trypanosome evades the mammalian immune response through a mechanism of antigenic variation. A succession of GPI-anchored immunologically distinct variant surface glycoproteins (VSG) are expressed at the cell surface at a superabundant level, but many other proteins are also present. We wished to understand how the surface proteome of trypanosomes, which is clearly essential for infection and hence survival, is maintained. We found that the major classes of transmembrane domain surface proteins, termed ISGs on account of being invariant, are turned over by a ubiquitin-dependent mechanism that involves trafficking via the multivesicular body. This system accounts for the efficient segregation of ISGs from VSG, the very differing half lives and also how TMD proteins can be endocytosed in a cell that lacks the AP-2 complex.

Environmental metatranscriptome analysis of a hypersaline anoxic deep-sea basin (Urania, Mediterranean)

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Polyextreme habitats like Deep Hypersaline Anoxic Basins (DHABs) are amongst the most hostile environments on Earth and therefore pose a challenge to resident organisms, also featuring high pressure, total darkness and high chemical concentrations. Until now, little is known about specific adaptations that allow the survival and thriving of eukaryotic life under these conditions. Here, we describe a pilot metatranscriptome analysis performed on eukaryotic planktonic organisms sampled from the oxic/anoxic-layer of the mediterranean Urania DHAB in a depth of 3.540 m. The established strategy comprised the construction of a normalized cDNA-database and adjacent pyrosequencing, leading to 166.084 reads of which up to 859 of 6.228 assembled contigs could be assigned to known gene products by annotation with the NCBI protein database. 573 sequences were categorized in different functional groups using the KOG database. Both indicate that the identified sequences are exclusively assigned to housekeeping genes. However, the majority of the sequences remained unidentified due to insufficient database contents, which leaves room for further research approaches. Using different genetic codes for translation, most of the genes could be identified with the ciliate-specific code. Taxonomic analysis were conducted by amplification of the SSU rRNA V4-region and fluorescence in situ hybridisation using clade-specific probes. Both techniques account for ciliates being in fact the dominant planktonic organisms in the oxic/anoxic layer of the Urania basin, which is likely compared to the community structure of other DHABs. Although only less than 50 % of the sequences could be identified, the developed strategy is suitable for further investigations, offering insights into the structure and function of planktonic communities.

Species concept in Myxogastria (Amoebozoa): Morphology, mating and molecules

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The plasmodial slime-moulds (Myxogastria) are a group of common and widespread unicellular organisms that have a sexual as well as an entirely asexual life cycle, both culminating with the formation of macroscopic fruiting bodies that disseminate spores. Recently it has been found that Myxogastria are one of the major components of protistan soil biodiversity. While in most amoebozoans sexuality has never been reported, the recently recognized clade called "macromycetozoa" composed of Dictyostelia, Ceratiomyxa and Myxogastria is characterized by alternating asexual and sexual reproduction. Most members of this clade form macroscopic fruiting bodies that efficiently disperse spores. The distinctive fruiting bodies of Myxogastria have been intensely collected worldwide and ca. 900 morphospecies are recognized. Myxogastria have a complex life cycle with alternating sexual and asexual, haploid and diploid stages. Laboratory studies based on mating compatibility have shown that each morphospecies may be composed of an intricate pattern of core

sexual strains, surrounded by a swarm of asexually reproducing clones that may differ genetically and morphologically. This complex genetic structure challenges classical taxonomy. Current models on evolution of sexuality are based largely on multicellular organisms such as plants, fungi and metazoans, which represent only a very small fraction of the genetic diversity of eukaryotes. Myxogastria present unique advantages for studying factors shaping the proportion of sexual/asexual protistan strains in nature. We investigated the genetic variability of several species in natural populations. We will present our first results, discuss their implications for both morphological and biological species concept and suggest more ways to assess the occurrence of sexual/asexual strains in natural conditions.

The ultrastructural diversity of the algal class Eustigmatophyceae (Ochrophyta, Stramenopiles)

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The algal class Eustigmatophyceae was separated forty years ago from the class Xanthophyceae on the basis of prominent ultrastructural differences in both vegetative cells and zoospores. The class currently comprises 11 genera and some 25 species and our recent phylogenetic analyses indicate its division into two deeply diverged clades (possible separate orders), with the larger clade (comprising most traditional eustigmatophytes) further divided into three strongly supported monophyletic lineages (possible redefined families) provisionally denoted Vischeria-clade, Monodopsis-clade, and Pseudellipsoidion-clade. To improve our understanding of the ultrastructural diversity of eustigmatophytes, we investigated the ultrastructure of eight strains representing these three lineages. The typical eustigmatophyte features such as a plastid without a girdle lamella, a reddish globule, or lamellate vesicles, were confirmed in all strains studied. The appearance of the lamellate vesicles was found to change during the life cycle. Younger, intensively-growing cells possessed vacuoles filled with lamellae, but older, less active cells had vacuoles full of storage products. The most conspicuous ultrastructural characters differentiating the three investigated clades concern the pyrenoid. A large stipitate pyrenoid protruding out of the plastid seems restricted to the Vischeria-clade. The recently described Pseudotetraëdriella kamillae exhibits a bulk pyrenoid located inside the plastid similar to previously studied members of the Monodopsis-clade. Finally, members of the Pseudellipsoidion-clade appear to be devoid of any pyrenoid.

Identification of protein transport channel in the mitosomes of Giardia intestinalis

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Giardia intestinalis is not only an important human pathogen but also an excellent model organism for studying mitochondrial evolution. Due to its anaerobic lifestyle it has reduced mitochondrion (mitosome). Mitosomes contain very few proteins and lost all its genome. There has been only single mitosomal function proposed so far – FeS cluster biogenesis. However, import pathway to the mitosomes remains elusive. While Tom40 homologue is present in outer mitosomal membrane, co-chaperones Pam18 and Pam16, are present in inner membrane. In classical mitochondria, these chaperones (together with Hsp70) constitute a motor complex for the inner membrane translocase Tim23. However, no homologue of Tim23 has been found in G. intes-tinalis genome, neither in proteome of isolated mitosomes. Hence, G. intestinalis might employ unique translocase adapted to import of extremely small set of proteins (14 known so far) and lack of measurable membrane potential. To characterise this translocase and other components of the import apparatus in G. intestinalis we developed two systems for isolation of such channel. One system is based on DHFR properties, which we used to introduce a "molecular plug" into the channel. Second one is based on antibodies against Pam16 and Pam18, which should be in physical contact with the translocase.

Intraclass evolution and classification of the Colpodea (Ciliophora)

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Using nine new taxa and statistical inferences based on morphological and molecular data, we analyze the evolution within the class Colpodea. The molecular and cladistic analyses show four well supported clades: platyophryids, bursariomorphids, cyrtolophosidids, and colpodids. There is a widespread occurrence of homoplasies, affecting

even conspicuous morphological characteristics, e. g. the inclusion of the micronucleus in the perinuclear space of the macronucleus and the silverline pattern. The most distinct changes in the morphological classification are the lack of a basal divergence into two subclasses and the split of the cyrtolophosidids into two main clades, differing mainly by the presence vs. absence of a vestibulum. The most complex clade is that of the colpodids. We partially reconcile the morphological and molecular data by using evolutionary systematics, providing a scenario in which the colpodids evolved from a Bardeliella-like ancestor and the genus Colpoda performed an intense adaptive radiation, giving raise to three main clades: Colpodina (a new suborder), Grossglockneriina, and Bryophryina. Within these taxa, several curious clades occur, consisting of Colpoda stem species and new genera budding from them, for instance, Colpoda henneguyi, Bresslaua and Bresslauides as well as Colpoda steinii and Bromeliothrix metopoides. The classification of such taxa is one of many unsolved problems. The data available indicate that the colpodean evolution and classification are far from being understood because sequences are lacking from most species and half of their diversity is possibly undescribed. (Supported by the Austrian (FWF, projects 19699-B17, 20360-B17, 22846-B17) and the German (DFG, project 414/3-1) Science Foundations as well as the Alexander von Humboldt Foundation.)

How resting cysts, spatial constraints, time and endemics structure protist communities

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There is a widespread believe that air currents, water, and animal vectors disperse micro-organisms to a cosmopolitan mass, in the sense that they are present if the species-specific environmental conditions are met. However, this has been disproved by flagship species, microscopic pests, mushrooms, and mosses, all having restricted distribution in spite of the high number and minuteness of their spores. I shall show that restricted distribution and the structure of protist communities are heavily influenced by the morphology and physiology of the dormant stages (resting cysts), spatial constraints, and time. However, we must not expect that protist communities are as distinct as those of animals and flowering plants because most have, indeed, cosmopolitan distribution. To find the endemic species is easy in flagships but difficult in ordinarily-looking endemics, which are very likely more numerous than flagships. How protist communities really look-like is poorly known due to the lack of appropriate investigations, but rough patterns are known from flagship groups, such as desmids, diatoms, and dinoflagellates. What is needed to get more and better data? First, we need what is common in animals and flowering plants: detailed and reliable distribution data on all scales and for many species. Then, all the fine statistical methods which were developed in the past years can be successfully applied. This sounds simple, but isn't because of the decreasing number of people interested in morphological identification work. I do not believe that sequences alone can be a substitute because, in case of the protists, half or more of their diversity is undescribed and sequences tell us nothing on the appearance of the organisms. (Supported by the Austrian Science Foundation, FWF projects 19699-B17, 20360-B17, 22846-B17.)

Species taxonomy of protists: Morphological and molecular perspectives

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Since the beginning of species recognition, there have been debates on their reality and usefulness. More than 20 species concepts can be found in the contemporary literature, but only six have been discussed and used by more than a few specialists: the "Biological Species Concept" of Ernst Mayr, the "Hennigian Species Concept" of Willi Hennig, the "Phylogenetic Species Concept" sensu Mishler and Theriot, the "Phylogenetic Species Concept" sensu Wheeler and Platnik, and the "Evolutionary Species Concept" of Wiley and Mayden. Today, most anticipate Mayr's concept: "biological species are groups of interbreeding natural populations that are reproductively isolated from other such groups". As far as I know, a molecular species concept is not available. The daily work of taxonomists usually follows a basically phenetic concept: "A species is the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of characters in comparable individuals". Why this big gap between theorists practicians? The answer is quite simple: the requirements of the concepts mentioned above hardly can be met in practice. Is species taxonomy then a science at all? I believe "yes" for two reasons: (i) the success of the phenetic approach is overwhelming, i. e., more

than one million species have been described and (ii) about 80 % of these are out of doubt. The last fact is frequently forgotten in species discussions, and the remaining 20 % are inflated to more or less complex theories and are, sometimes, the reason to deny species at all. Species recognition in protists is basically not different from that of plants and animals, but is plagued by more poorly described taxa and less distinctive characters. So, one question remains: why is it important to know the morphology of species? Wouldn't it be sufficient to have a sequence? (Supported by the Austrian Science Foundation, FWF projects 19699-B17, 20360-B17, and 22846-B17.)

Frequency and biodiversity of symbionts in representatives of the main classes of Ciliophora

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Analysis of own sampling ciliate's material connected mainly with Paramecium spp. was performed. The sampling was made during 20 years in different parts of Europe, Asia, Australia, Africa, North and South America. Some samples provided by colleagues and friends. Altogether, it was about 5000 units. Some ciliates from classes Heterotrichea (I), Armophorea (II), Sprirotrichea (III), Litostomatea (IV), Phyllopharyngea (V), Prostomatea (VI), Nassophorea (VII), Colpodea (VIII), Plagiopylea (IX) and Oligohymenophorea (X) (including Paramecium spp. kept as clone cultures) were studied primarily for detection and investigation endo- (EnS) and ectosymbionts (EcS), both prokaryotic and eukaryotic (not algae) nature, some with TEM and fluorescent in situ hybridization. Literature data also were taken in consideration. The main Paramecium spp. were studied first of all. Apparently, ability to keep symbionts is different in various Paramecium spp. as well as generally in different classes of ciliates. For example, till now no EnS were found in P. woodruffi, but close related species P. nephridiatum has a lot. Very few bacterial EnS were recorded for P. putrinum, P. polycarium and P. jenningsi but, in opposite, number microorganisms could populate P. aurelia and P. caudatum cells. The main part among prokaryotic EnS detected belongs to Alphproterobacteria. Holospora or Holospora-like infectious bacteria were found in representatives of I-II, V-VI and mainly of X classes. Bacteria associated with bacteriophage capsids were found in ciliates of I and X classes. The same combination was found for bacteria with R-bodies. Quite rare type of EnS – motile bacteria were found in ciliates of I and X classes as well either in the cytoplasm (I) or in the macronucleus and its perinuclear space (X). The EcS are more common in classes I, II and IX, but were never found in V, VII and VIII). Among eukaryotic Enb very few representatives of Microsporidia (I, III, VIII, IX and X) and Trypanosomatida (III and X) were recorded. Apparently, heterotrichs and oligohymenophoreans are most promise groups of ciliates for symbiosis investigation.

New Holospora-like bacterium from the Paramecium genus

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The prokaryotic endocytobionts (Eb) of Paramecium genus representatives are much more abundant (more than 60 types of bacteria) and better investigated in comparison with other ciliate-prokaryote systems. This is true in particular for bacteria belonging to the genus Holospora, highly infectious endocytobionts of Paramecium (Fokin and Görtz, 2009). Although all representatives of the genus Holospora share life cycle and many morphological features, some strains described until now should probably be excluded from the genus according to some biological features and fluorescent in situ hybridization (FISH) indications. Some Holospora-like microorganisms were also recorded in other group of ciliates but, since now, none of these bacteria have been studied with molecular approaches. In this investigation we performed the morphological, ultrastructural, life-cycle and molecular characterization of a new Holospora-like bacterium (Hlb) found in the macronucleus (Ma) of Paramecium jenningsi isolated from a sample taken in Thailand (Samui Island). The microorganism has typical holospora's life cycle with infectious (4 – 7 μm) and reproductive (1 – 3 μm) forms. Experimentally, new host cells could be infected in 1 – 2 h. In these experiments, the entrance of the bacteria into the target nucleus was also recorded for P. schewiakoffi, P. caudatum and P. aurelia, but only in the last species the Hlb could complete its life cycle. During division of infected Ma, the Hlb never produced the special "connecting piece" (an equatorial part of the dividing nucleus where the majority of infectious forms collects), a feature manifested by "classical" holosporas. The results of FISH reaction, using probes specific for Alphaproteobacteria and "classical" holosporas confirmed that the Hlb belongs to Alphaproteobacteria but suggested it could be not a "classical" Holospora. Indeed, the characterization of 16S rRNA gene sequence of this

novel HIb and its comparison with that of H. obtusa, a "classical" Holospora, showed only a 90 % identity and confirmed that the probe for "classical" holosporas does not work on it. A probe specific for these novel HIb have been designed and validated. Obtained results suggest that the newly characterized HIb should be considered the first representative of a novel genus belonging to the family Holosporaceae (Alphaproteobacteria).

X-cell parasites of Atlantic cod are basal dinoflagellates

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Unusual tumour-like pathologies containing mysterious cells, termed 'X-cells', have been reported from numerous cold-water associated marine fish groups worldwide. After nearly a century of research, and numerous differing explanations for the cause of the conditions, X-cells are now known to be protozoan parasites. However, histological and ultrastructural investigations are unable to reveal typical features seen in other protozoan groups, and phylogenetic analyses, using small subunit ribosomal DNA sequences, fail to robustly place the group within the protozoa. In the present study, the large subunit ribosomal DNA was sequenced for X-cells from epidermal pseudotumours in flatfish from northern Japan, from X-cells causing gill filament lesion in European dab in Scotland and from X-cells in pseudobranchial pseudotumours from Atlantic cod from Iceland. In addition the Hsp90 gene was sequenced for X-cells from the Atlantic cod. Phylogenetic analyses confirm that X-cell parasites are protozoan parasites and firmly place them as members of the alveolate group. Concatenated ribosomal DNA and Hsp90 gene data for the X-cell parasite from Atlantic cod reveal that it forms a sister branch to the perkinsids and is located at the base of the dinoflagellates clade.

Neospora caninum is associated to abortion in Algerian cattle

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Neospora caninum is a major cause of abortion in cattle worldwide. The situation in Algerian cattle was unknown. 799 cattle belonging to 87 farms of the north and north east of Algeria were investigated. First a seroprevalence study was performed. The global seroprevalence in IFAT was 19.64 %. The cattle were divided into modern cattle corresponding to imported cattle, local cattle corresponding to local breeds and improved cattle corresponding to breeding between modern and local cattle. The seroprevalence was 16.04 %, 18.64 % and 34.28 % in modern cattle, improved cattle and local cattle, respectively. A case control study has been performed to investigate the link between the seropositivity to N. caninum and the abortion in cattle farms. There is a clear significant ($p < 0.01$) association between the seroprevalence against N. caninum and the presence of abortion in farms (OR = 12.03). These association was also significant at the individuals levels (OR = 2.79). For the several populations, the association is more contrasted, there is a clear association between seroprevalence and abortion in modern and improved cattle but the association was not significant for local cattle even if it was the population where the seroprevalence was the highest. Finally, in order to show an objective direct link between N. caninum and abortion in cattle, the brain of 5 aborted fetuses was analyzed by histology and by PCR to detect the presence of protozoal-associated lesions and/other DNA N. caninum in target organ. One sample was positive both by histology and by PCR, 2 samples were positive by PCR but not by histology and 1 sample was negative for both tests.

Transfer of the human infectivity trait by sexual reproduction in Trypanosoma brucei

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Human trypanosomiasis in East Africa is caused by Trypanosoma brucei rhodesiense, a parasitic protist transmitted by tsetse flies. The trait for human infectivity is encoded by a single gene that confers resistance to the trypanolytic factor in human serum (SRA). Lacking SRA, T. b. brucei infects a wide range of wild and domestic mammals, but is not human infective. We have successfully crossed T. b. rhodesiense with different strains of T. b. brucei in the laboratory and tested whether the SRA gene can be transferred to different genetic backgrounds. A proportion of the hybrid progeny from each cross inherited the SRA gene, demonstrating that the gene is present as a single allele in the T. b. rhodesiense parent. Hybrids carrying the SRA gene were resistant to lysis by human serum in vitro,

showing that they have the potential to infect humans. These results demonstrate that new genotypes of T. b. rhodesiense can be formed after genetic exchange between T. b. rhodesiense and T. b. brucei in the lab. Extrapolating to the epidemiological context, we speculate that the observed heterogeneity of T. b. rhodesiense in East Africa is a result of sexual reproduction and that the number of different T. b. rhodesiense genotypes is potentially unlimited.

Phylogenetic position of Lophomonas and implications for character evolution in Parabasalia

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Lophomonads, e.g. Lophomonas, Joenia, Deltotrichonympha, and Kofoidia, are multiflagellate parabasalids with a single apical flagellar area that degenerates during mitosis. However, molecular phylogenetic analyses have shown that lophomonads are not monophyletic. We have determined the SSU rRNA sequence of L. striata, from the gut of the omnivorous cockroach Periplaneta americana, and we find that it branches sister to the Trichonymphida with strong support. This is surprising because all other lophomonads sampled to date branch within the Cristamonadida, and the order Trichonymphida (e.g. Trichonympha, Eucomonympha, Hoplonympha) is coherent both morphologically and in molecular phylogenetic analyses. Trichonymphida, unlike the lophomonads, share a bilateral symmetry, in which their multiple flagella occur in two (or sometimes four) regions, and rather than degenerating upon mitosis, half of the flagella are passed to each daughter cell. We also determined the SSU rRNA sequence of Kofoidia loriculata, which was considered upon its discovery to be the closest relative of Lophomonas. Kofoidia loriculata branches within the Cristamonadida, but not as a deep branch like other lophomonads sampled to date, rather it is the sister lineage to the genus Devescovina. The single apical flagellar region characteristic of lophomonads is therefore either plesiomorphic or it has arisen multiple times in parabasalids. Our results also suggest that parabasalid gut symbionts may have been vertically transmitted in cockroaches since before the common ancestor of Cryptocercus and termites.

Flagellar energy metabolism in protists and a possible loss of flagellar glycolysis during trypanosomatid evolution

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The '9+2' microtubule axoneme is the principal structure of most motile cilia/flagella and is an iconic structure in eukaryotic cell biology. The motility function imparted by flagella underpins cell swimming, cell feeding and/or reproduction in many eukaryotes. As a consequence of proteomic investigations, there have been unexpected descriptions of flagellum-associated metabolism in several protists. For instance, in the green alga Chlamydomonas reinhardtii there are flagella-specific isoforms of enzymes required for the ATP-generating phase of the glycolytic pathway. Although we and others have speculated on the purpose of a flagellum-associated metabolism in Chlamydomonas or other protists (e.g. Nature Reviews Microbiology 6:838-50), the physiological role(s) conferred by this novel metabolism remain uncertain. However, there is a key difference between the protists and various animal sperm, where the existence of axoneme-associated metabolism has been known for many years – in protists flagella metabolism is a consequence of protein isotype targeting since flagella (and cilia) are organelles that are distinct from those contained within the cell body, whereas sperm are flagellate cell-types essentially devoid of cytosol. Here, I will discuss our recent characterisation of two trypanosomatid-specific flagellar proteins resembling the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase (PGK). Provocatively, these proteins could provide a molecular scar of when glyocolysis was an energy-generating pathway in the free-living ancestors of trypanosome and Leishmania parasites: an adaptation to parasitic niches limited in carbohydrate availability could have underpinned the degeneracy of flagellar glycolysis, although the unique re-compartmentalisation of glycolytic enzymes to peroxisomes that occurred during kinetoplastid evolution, coupled to consequent changes in glycolytic regulation, provide another strong selective pressure for loss of a flagellar glycolytic pathway. Alternatively, the trypanosomatid GAPDH- and PGK-like proteins may owe their origins to the duplication and divergence of genes that ancestrally combined moonlighting functions with better-known metabolic functions.

Biogeography of amoebae: A case study of seven naked lobose amoebae species isolated from North-American habitats

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Although naked lobose amoeba are rather abundant in a majority of marine, freshwater and soil habitats, their global-scale distribution pattern remains very poorly known. Lack of reliable biogeographic data is related with the difficulty of species identification and evident undersampling of this group of protists. While species problem in amoebae remains unsolved, the primary way to establish the amoebae distribution pattern is a direct comparison of amoebae fauna from geographically distant sites. To contribute to this problem, we studied seven amoebae species isolated from the Niagara River and Ontario Lake (North America). Light microscopic, transmission electron microscopic and molecular studies showed that all of them could be assigned to known amoebae genera – Hartmannella, Ripella, Cochliopodium, Acanthamoeba, Vexillifera, Mayorella and Flamella. However even the comparative analysis of such a distinguishable morphological features as general shape of locomotive form, cyst wall structure, cytoplasmic inclusions, number and appearance of pseudopodia, uroidal structures, trophozoite and cyst sizes indicated that all these species didn't match any named ones, being new for science. The 7:0 "new species"/"known species" ratio for the firstly investigated habitat shows that the morphospecies diversity of naked amoebae is still heavily underinvestigated and may be an argument against the world-wide distribution of the most of known naked amoebae species.

Diversity and ecology of Apusozoa (Protozoa): A mysterious phylum of free-living zooflagellates

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Apusozoa is a recently established protozoan phylum primarily comprising biciliate gliding zooflagellates classified within three orders: Apusomonadida, Planomonadida, and Mantamonadida. Using sequences of known strains, we designed PCR primers specific to each order and obtained clone libraries from a range of natural environments. We found evidence of many new apusomonad and planomonad lineages, as well as novel mantamonad ITS1 ribotypes, from marine, freshwater, and soil environments. We show that Apusozoa is more diverse and cosmopolitan than revealed by culturing alone and that some lineages may be more ecologically sensitive than others. Our results suggest that Mantamonas plastica, the only described mantamonad species, thought to be exclusively marine, also exists in freshwater and soil. We searched online sequence databases (CAMERA Portal, EMBL) and found environmental sequences belonging to new apusomonad and planomonad lineages. However, a similar search for mantamonads revealed no new related sequences, reinforcing our hypothesis that this group is distinctly difficult to detect using conventional molecular techniques. We present these findings in the context of ongoing large-scale molecular marine surveys, where preliminary results suggest an unprecedented scale of apusozoan diversity.

Detection of two spliceosomal proteins in the divergent eukaryote Giardia intestinalis

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The protist Giardia intestinalis (G. intestinalis) is a human parasite which causes diarrheal disease throughout the world. It is frequently described as an ancient protist and an excellent model for studying complex cellular process due to its early divergence and genomic reduction. Few years ago, it was thought that G. intestinalis did not have introns, but in 2002 one was identified; now there are four introns known in the whole genome. Moreover, many proteins of the spliceosome have been identified through bioinformatic tools. Despite it is assumed that introns are removed through spliceosome, this big molecular machinery has not been characterized. In the present research we wanted to study central proteins of the ribonucleoparticles which are part of the spliceosome: the proteins Sm. Firstly we expressed proteins SmB and SmD3 of G. intestinalis in E. coli using pGEX vectors, then we purified GST-tagged recombinant proteins with Glutathione Agarose under native conditions. Purified SmB and SmD3 were used to raise polyclonal antibodies in mice. The antisera were used to detect proteins with western blot on cytoplasmic and nuclear extracts of G. intestinalis trophozoites. Immunofluorescense assays were also done on trophozoites. Serum of a different mouse was used as negative control in both cases. Immunofluorescense assays showed detection of proteins SmB

and SmD3 in trophozoites of G. intestinalis, both of them have discrete localization near the nuclei. In western blot we detected the proteins SmB and SmD3 only in the nuclear extract. Antibodies against SmB recognized two close size bands of approximately 31KDa, whereas antiserum SmD3 recognized one band near 66 KDa. It has been reported in most eukaryotic organisms except yeast, that SmB, SmD1 and SmD3 contain methylated arginine residues in its C terminal, we hypothesize the higher band with antiserum SmB corresponds to the protein SmB with its arginine residues modified by methylation. This is the first time that two proteins of the spliceosome are detected in this protist, suggesting that the complex machinery for removing introns is not only present in the genome but also in the transcriptome and the proteome of this ancient eukaryote.

Internal targeting motifs are common in hydrogenosomal proteins of Trichomonas vaginalis and found in multiple domains

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The human pathogen Trichomonas vaginalis harbors mitochondria-related organelles known as hydrogenosomes, which produce ATP by substrate-level phosphorylation. The organelles contain neither a genome nor a translation machinery and all proteins are hence imported from the cytosol. In contrast to the import machinery of Saccharomyces mitochondria, very little is known about how proteins are targeted to and transported across the two hydrogenosomal membranes. Recently evidence emerged that next to more canonical N-terminal targeting signals, internal targeting signals seem present in some hydrogenosomal proteins. We have extended the search for proteins imported in the absence of their N-terminus and were surprised to find that all proteins tested – such as PFO:A, IscA and Ferredoxin – were imported in vivo without their N-terminal targeting motif. Hence we commenced to analyze the import behavior of individual domains of the hydrogenosomal proteins (ferredoxin, thioredoxin reductase and SCSalpha) and provide the first evidence that internal targeting motifs are not only far more common than previously thought, but furthermore not restricted to a single domain. Although import recognition of hydrogenosomal proteins seems somehow flexible it must still be stringent, as six glycolytic enzymes tested were not imported and exclusively localized to the cytosol. Given their common ancestry with mitochondria, it appears that the import information for hydrogenosomal proteins in T. vaginalis is shifting from the more canonical N-terminal motif to internal regions and by reductive evolution leading first to short and then entirely obsolete N-terminal motifs.

Charged repeat motifs: Common characteristic of eukaryotic cytoskeleton proteins

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Single-celled eukaryotes such as the ciliate Tetrahymena thermophila often have a more complex cytoskeleton than an individual metazoan cell. Intriguingly, intermediate filament proteins such as desmin, vimentin or lamin – next to actin-based microfilaments and microtubules one of the three pillars of the eukaryotic cytoskeleton – have not been clearly identified from the many known genomes of single-celled eukaryotes. In some cases this might be due to the poor sequence conservation of intermediate filament proteins, but it fails to account for the overall little knowledge we have about them in protozoa. A mass spectrometric and subsequent bioinformatic analysis of the cyto- and membrane skeleton of T. thermophila led to the discovery of a conserved feature. A multitude of proteins contain charged repeat motifs (charged repeat motif proteins = CRMPs). Prominent members of CRMPs that were identified in the proteome data and at the same time are known to be components of the pellicle, are for example members of the dynein family, kinesins, basal body proteins, the tetrins of the oral apparatus, alveolins and EPC1, a major component of the ciliate cytoskeleton. CRMPs are found in all eukaryotes and preliminary analysis of ten such proteins has shown that some are associated with microtubules, generate filaments and built up invasion-related structures in apicomplexen parasites. They form entirely new classes of cytoskeletal proteins, are difficult to identify by standard sequence analysis such as BLAST – also due to their rapid evolution – and represent previously unrecognized, but important and sometimes highly expressed structural proteins of protozoa, if not of every eukaryotic cell.

The surface antigen multigene family of Paramecium tetraurelia

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Members of the surface antigen multigene family were described to share several characteristics. Next to a high degree of sequence homology in the marginal regions of the open reading frame, especially the highly conserved cysteine-periodicity of the proteins is not only a feature of Paramecium's antigens but was also shown for several parasitic species. In P. tetraurelia, eleven serotypes were described by immunological analysis, however, sequence data existed only for few of them. By database mining, we identified 75 genes exhibiting the characteristics of surface antigens and we were able to annotate the sequences of the antigens 51I, 51J and a candidate for 51Q. Within the large group of candidate genes, we can separate a core group by size, domain characteristic, signal peptide and GPI-anchoring signal, and we conclude that these genes represent the classical antigens. This core group is not evenly distributed in the genome but closely related to the macronuclear telomeres and the open reading frame is directed towards the telomere. This special localization may be involved in the regulation mechanism of these genes by the formation of subtelomeric heterochromatin.

Environmental diversity of an intracellular parasite group, the Microsporidia

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Microsporidians are intracellular, spore-forming parasites able to infect any eukaryotic organsim, ranging from protists to vertebrates. They are severe pathogens of immunocompromised humans, cause economic losses in aquaculture and lifestocks, and are implicated in the loss of bee colonies. Furthermore, microsporidia may also be able to modulate ecosystem functioning, as they can distort sex-ratio and alter the behaviour of key species in food webs. To date, approx. 1200 microsporidian taxa have been described, however, their wide host range and their long-term survival in water systems indicate that microsporidian abundance and diversity has been underestimated. We aim to address the lack in our knowledge of environmental microsporidian abundance and diversity by using Roche's FLX 454 Next Generation Sequencing technology on samples from marine, freshwater and terrestrial ecosystems. DNA was extracted from size-fractionated filtrates from sea- and freshwater collected from different European sites, and from invertebrates and protists collected from forest soils and bogs. PCR amplification of the small subunit (SSU) of the ribosomal RNA gene was carried out with different primer pairs to ensure that the widest possible diversity of microsporidian SSU sequences is amplified. Environmental DNA samples that tested positive for microsporidia were amplified and sequenced using 454 FLX Titanium systems with primer sets that include habitat-specific tags. With these tags microsporidian SSU sequences can be assigned to their place of origins, which aids a location-dependent phylogenetic analysis. Thus, this project provides the first environmental survey of the microsporidia, and examines the hypothesis that microsporidian genetic diversity is related to location or environment.

Phylogeny and classification of parabasalids: Situation in 2011

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Parabasalids represent distinct group of protists containing approximately 450 species that can be defined by the presence of hydrogenosomes, a parabasal apparatus (a Golgi body attached to striated fibers), and nuclear division by a closed pleuromitosis with an extranuclear spindle. Besides a few free living and a few parasitic species, parabasalids are gut commensals or symbionts. The main part of their diversity can be found in the guts of wood eating termites and cockroaches. Phylogenetically parabasalids belong into the subgroup of anaerobic protists (Metamonada) of the kingdom Excavata. The closest relatives to parabasalids are Preaxostyla (oxymonads and Trimastix) and Fornicata (e. g. diplomonads, retortamonads). The relationships among parabasalids have been studied repeatedly but particularly the backbone of the tree and the position of the root are still not well resoled. The major challenge in resolving the parabasalian phylogeny is the existence of several long branching clades – trichonymphids, teranymphids, spirotrichonymphids and hexamastigids. The talk will present the complete up-to-date phylogeny of parabasalids based on the most complete data set analyzed by the most sophisticated methods available today. It will focus primarily on resolving the position of long branching clades and the root of parabasalids. Phylogenies based on the gene for

small subunit rRNA and well sampled proteins will be discussed in the context of the new parabasalian classification (Čepička et. al 2010) and in the context of morphological evolution of parabasalian groups.

Life at the extreme: Molecular adaptations of Halocafeteria sp. to hypersaline environments

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Microbial life in environments near salt saturation has evolved to withstand the osmotic stress that would otherwise kill the cells. Typically, Halobacteriaceae (the famous haloarchaea) and Halanaerobiales (anaerobic eubacteria), preferentially import some ions into their intracellular environment to equilibrate the osmotic balance. This adaptation has led to a molecular signature that includes a highly acidic and hydrophilic proteome. Most other microbes including protists like Dunaliella salina cope with the osmotic stress by exporting the salt, coupling it with import or synthesis of compatible solutes like glycerol. However, virtually nothing is known about halophilic heterotrophic eukaryotes that thrive in hypersaline habitats all over the world. Among them, the stramenopile Halocafeteria seosinensis has been recently isolated and characterized. Here we conduct transcriptomic investigations under optimal and maximal salt concentrations for growth to unravel the molecular adaptations of this bacterivorous nanoflagellate. The genomic and proteomic content will be characterized in terms of preferential codon usage, amino acid composition and isoelectric point of predicted proteins. Description of differential gene expression will also allow us to determine the strategy that H. seosinensis uses to sustain its biological functions in these extreme environments.

Electron microscopic study on division of secondary plastids surrounded by four membranes in chromophyte algae

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Secondary plastids in chromophyte algae are surrounded by four membranes: the inner double envelope membranes, periplastid membrane (PPM) and the outermost epiplastid rough ER (EPrER). In this study, division of secondary plastids of Nannochloropsis oculata (Eustigmatophyceae) and of Heterosigma akashiwo (Raphodophyceae) was examined by electron microscopy focusing on the division of PPM and EPrER. Cleavage of PPM and EPrER are far behind the constriction of the inner double envelope membranes. As far as examined, electron-opaque structures like plastid dividing-rings that are observed on the double envelope membranes of dividing primary plastids could not be detected on either EPrER or PPM at the cleavage sites of the secondary plastids. These observations imply that PPM and EPrER constrict under distinct mechanisms from that of constriction of the double envelope. Three-dimensional observations of the cleavage site of dividing plastids of N. oculata and H. akashiwo have revealed that a number of vesicles (periplastid vesicles, PPVs) are present in the periplastid compartment (PPC) between the outer envelope membrane and PPM, and that the vesicles are fused to form tubular vesicles encircling the extremely constricting neck of the double envelope membranes. It is also suggested that the vesicles might be generated by invagination of the PPM. PPM separates during the plastid division into two entities that containing the daughter plastid respectively. One hypothetical model is that the separation of PPM might take place through growth of the PPV from tubular to disc-like form and following fusion of the disc-like PPV and PPM. This model somehow resembles cell plate formation in the cytokinesis of higher plant cells. Another possibility is that nuclear-encoded proteins including proteins involved in the plastid division might be imported from the cytosol into the compartment surrounded by the double envelope membranes by vescular trafficking mediated by the PPVs.

Protistan parasites of freshwater algae

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Several eukaryotic micro-organisms have a parasitic or parasitoid lifestyle and infect microalgae. In freshwater systems Chytridiomycota are well known to infest certain microalgae, but also unicellular protists are able to attack freshwater microalgae and are – similar to parasites – dependent on specific host algae. Since the 19th century scientists have described these protists and discussed their position in the system of life. Nowadays we don't know much about such parasites, because they are difficult to cultivate, and many species have never been found again since their original description. Furthermore, some descriptions are

doubtful, because the criteria for species definition used in the past differed from those we use today. Using modern techniques of isolation and culturing some strains of protistan parasites of freshwater algae (mainly Zygnematophyceae) have been established, especially vampyrellid amoebae (Vampyrella, Hyalosdiscus and Leptophrys) and amoeboflagellates. Three isolates with cercozoan affiliation (termed PC-strains), an unknown flagellate organism (UFO) with its very peculiar swimming behavior and an isolate of the old and doubtful genus Pseudospora have been studied regarding morphology, infection cycles and their phylogenetic position using the nuclear-encoded SSU rDNA. Furthermore, some preliminary results regarding host specificity have been obtained and microscopical time lapse techniques have been used to unravel the pattern of the very slow amoeboid locomotion of these parasites.

The homologue of mitochondrion in Trimastix pyriformis

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Trimastix pyriformis (Preaxostyla, Excavata) is a poorly studied free-living protist from anaerobic environment. Instead of typical mitochondria, the cytoplasm of Trimastix contains double membrane bounded vesicles without cristae. Canonical mitochondria are absent in many groups of eukaryotes, but it has been shown that all these lineages harbour organelles – hydrogenosomes or mitosomes – that are homologous with mitochondria. 19 genes for typical mitochondrial or hydrogenosomal proteins have been identified among Trimastix ESTs and localisation of their products into the Trimastix organelle would confirm that it is a homologue of mitochondrion. We tried to localise proteins IscU, IscS, Hsp70, Cpn60, Tom40, glutaredoxin, IscS, Hsp70, Isd11, frataxin, PFO and hydrogenase using heterologous antibodies and [FeFe] hydrogenase, maturase HydG and Cpn60 using homologous antibodies. We tested the set of antibodies on immunoblot of a Trimastix lysate. Only antibody against IscU of Giardia intestinalis produced a specific signal – single band of the expected size. The signal increased, when we used a lysate of Trimastix cell fraction enriched with the double membrane organelles. Unfortunately, the IscU antibody did not show convincingly organellar localisation on immunofluorescent slides with fixed Trimastix. The antibodies against PFO and HydG showed apparently a vesicular localisation on the immunofluorescent slides. The antibody against [FeFe] hydrogenase seems to localise into the cytosole, but we cannot exclude organellar localisation. We work on co-localization of these antibodies. We tried to detect the organelles by different kinds of mitochondrial specific dyes (Mito Tracker RED FM; Mito Tracker GREEN FM; Rhodamine B, hexyl ester, perchlorate), some of them staining organelles with electric or pH potential on the membrane. None of these dyes showed specific signal in Trimastix. Our results indicate that the organelles of Trimastix do not have a membrane potencial and may contain PFO, [FeFe] hydrogenase, HydG and IscU. [FeFe] hydrogenase is also localised in the cytosole. To further characterise the organelle, we are preparing antibodies against TOM40, P-protein of glycine cleavage system and pyridine nucleotide transhydrogenase.

Species concepts

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Among the biological research disciplines, first of all biodiversity research and ecology rely on safe identifications of species and on reproducible species counts, but often this requirement is not met. This is especially true for microscopic life. Due to ambiguous, contradictive and/or inconsistent species descriptions, species numbers obtained from biodiversity surveys using the traditional morphospecies concept are not comparable to those obtained from environmental DNA. Also the biological species concept cannot be applied to all groups of organisms. A combination of traditional morphology-based methods with molecular phylogenetic analyses helps to solve problems in systematics at species-level. For comparative analyses of morphological traits and their congruence with phylogenetic trees, a larger number of clonal strains is required. If inappropriate (e. g. plesiomorphic or variable) characters have been chosen for species identification, they can be identified. If species-specific characters are present, but have been ignored before, they also can be identified. However, it will not always be possible to apply the morphospecies concept in congruence with phylogenetic trees. A lack of resolution in morphological characters may impede an identification of species resulting in cryptic species complexes. Under such circumstances, molecular markers can be used as diagnostic characters to obtain unambiguous spe-

cies descriptions. The internal transcribed spacer 2 of the eukaryotic ribosomal operon is an option to establish a reproducible species concept. Compensatory base pair changes in conserved parts of the seconday structure of ITS2 proved to be a safe predictor of sexual incompatibility in many groups of organisms. A species concept based on this molecular marker, thus, provides an approach toward biological species limits to some extend and will result in a consistent systematics. In asexual groups with a lack of distinctive morphological characters, molecular signatures may be the only possibility to define species.

Symbiosis in Foraminifera: An overview

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Foraminifera are a major group of protozoa that successfully colonizes aquatic habitats since Cambrian times. Several groups of benthic and planktonic foraminifera evolved symbiotic relationships with different groups of algae. Recent symbiont bearing foraminifera belong to eight different families and are host to a surprising diversity of algal types that comprise chrysophytes, dinoflagellates, diatoms, rhodophytes and chlorophytes. Symbiotic foraminifera are also common fossils in Mesozoic and Cenozoic sediments and are used in micropaleontology for stratigraphical and environmental analyses, to establish biozonations Their evolutionary history, characterized by rapid radiations and adaptations is relatively well known and the acquisition of a symbiotic lifestyle is regarded as a major driving force of evolution in this heterogenous group. The amazing diversity of symbiont types has prompted a concept of flexible symbiotic relationships stating that single host specimens are able to house several species of closely related algal symbionts. However, molecular studies on benthic foraminifera show quite specific relationships between endosymbionts and hosts. Comparison with sequences available from the algal DNA database suggests that foraminiferal endosymbionts are rarely found outside their hosts. The establishment of symbiosis appears to be an infrequent event in the evolution of foraminifera but has proofed to be very successful whenever it took place.

Biodiversity patterns in dinoflagellates

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Dinoflagellates are common to abundant in both marine and freshwater environments. The spatial distribution patterns of dinoflagellates are similar to other aquatic protists of similar size. 90 % of the species are marine and 10 % occur in freshwater. They are particularly diverse in the marine plankton. About 2500 extant species have been described, including auto-, mixo-, and heterotrophs. Northern and southern hemispheres contain virtually identical communities within similar latitudes, separated by a belt of circumtropical species. A few endemics seem to be present in tropical and polar waters. Some benthic dinoflagellates are exclusively tropical, including a distinct epiphytic community. It is obvious that differing species concepts have a strong impact on the consideration of biodiversity patterns and biogeography. Examples for species community, genus, and species level patterns in dinoflagellates will be presented for discussion. Current (methodological) problems will be highlighted. How to deal with the situation that not only one dinoflagellate morpho-species can contain several genotypes (cryptic species?), but also one genotype can contain two morpho-species/genera?

Iron-inducible transcription in Tritrichomonas foetus

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Iron has been shown to regulate transcription of genes coding for both hydrogenosomal and cytosolic proteins in trichomonads. Shortage of iron causes decreased expression of key enzymes of pyruvate metabolism in hydrogenosomes (pyruvate: ferredoxin oxidoreductase, hydrogenase and ferredoxin – Fdx), which is compensated by increased conversion of pyruvate to ethanol in cytosol with upregulation of cytosolic pyruvate decarboxylase (PDC). In trichomonads, regulation of protein-coding gene expression is controlled by a conserved core promoter region that assure accurate transcription initiation and by distal regulatory elements that regulate the level of transcription. The main core promoter element is a highly conserved DNA initiator (Inr) that surrounds the start site of transcription in ~75 % of trichomonads genes. Inr is bound by 39-kDa transcription factor (IBP 39) that mediates transcription initiation. Several Myb

recognition elements were found in the distal promoter regions of trichomonad genes, some of them playing role in the iron-dependent regulation of transcription. To identify distant control regions that regulate the level of transcription of Tritrichomonas foetus genes coding for Fdx and PDC according to iron availability, 5'flanking sequences of both genes were analyzed. Series of constructs with progressive deletions in upstream regions were fused with luciferaze gene as a reporter were generated. Six constructs were prepared with Fdx upstream regions of 584bp to 50bp upstream of the transcription start site and four constructs of PDC with upstream sequences from -301 to -70bp. These constructs were used for stabile transfection of T. foetus cells and luciferase activities were compared in cells maintained under iron-rich (150 uM iron nitrilotriacetate) or iron-restricted (100 uM 2,2-dipyridyl) conditions in vitro. An iron-responsive regions were located between -235 bp and -100 bp in the Fdx upstream region and between -230 bp and -140 bp in the PDC upstream region.

Algal symbionts of Paramecium bursaria: Origins and diversification

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Paramecium bursaria is a single-celled protozoan that maintains several hundred green algal cells within its cytoplasm, lending it a green color. The symbiotic algae are usually cloned, single species within each Paramecium, and the species depends, in part, on where the P. bursaria was collected. Piecing together collection reports, P. bursaria collected from countries along the Pacific Rim contain Chlorella variabilis, whereas many of the P. bursaria collected in western to northern Europe contain Micractinium reisseri. When isolated from Paramecium experimentally, each algal species demands organic nitrogen and is rather sensitive to host-specific lytic viruses (i.e., Chlorella variabilis virus and Micractinium reisseri virus). Both algae have already lost the ability to live in natural water resources, and seem to be "old" natural symbionts. Chlorella vulgaris and Scenedesmus sp. have also been found as other symbionts of P. bursaria. The genetic discrepancies among these symbionts indicate multiple origins of the symbioses. So, how were the various symbionts obtained? Since P. bursaria has lost none of its ability to take in algae to be new symbionts, the following two modes of algal switching are conceivable: loss of the natural symbiont and subsequent ingestion of another suitable alga, or, more than one symbiont lives in a P. bursaria cell sympatrically, and one is "chosen." Some studies suggest the latter scenario.

Morphological descriptions of three marine ciliates, with the establishment of a new genus and two new species (Ciliophora, Scuticociliatia)

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Three marine scuticociliates, namely Falcicyclidium fangi nov. gen., nov. spec., Falcicyclidium atractodes nov. spec., and Cristigera media Kahl, 1928, isolated from a sandy beach near Qingdao, China, were investigated using live observation and silver impregnation methods. The genus Falcicyclidium is distinguished by the combination of (i) dorsoventrally flattened body, (ii) hook-like (falciform) paroral membrane, and (iii) multiple caudal cilia. Falcicyclidium fangi, the type species of the new genus, can be distinguished by the combination of its large size, extremely flattened (3:1) body, ten somatic kineties, and broad buccal area occupying 60 % of the body length. Falcicyclidium atractodes is mainly characterized by a unique spine at the anterior end of the body and a distinct protrusion at the posterior end. Cristigera media is redescribed based on the Qingdao population and more details, especially with respect to its living morphology, are documented. This work was supported by the Natural Science Foundation of China (Project number 40976075), and a Marie Curie Incoming International Fellowship within the 7th European Community Framework Programme.

Morphology and phylogeny of a new marine ciliate, Diophrys multimacronucleata nov. spec. (Ciliophora, Euplotida)

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The morphology in vivo, infraciliature, and SSU rRNA gene sequence of Diophrys multimacronucleata nov. spec., isolated from a salt mash at Blakeney, UK, were investigated. The new species is recognized by the following characters: body elliptical in outline and slightly greyish to yellowish in colour; size in vivo about 60 – 180 × 50 – 150 µm; pellicle flexible, with underlying granules densely arranged in lines; ciliature comprising about 40 – 49 adoral membranelles, 6 – 9 frontoventral,

5 transverse cirri, 2 left marginal cirri, 3 caudal cirri, and 5 dorsal kineties; nuclear apparatus comprising 7 – 19 spherical to ellipsoidal macronuclear nodules scattered throughout the body and 1 – 4 spherical micronuclei; marine habitat. Comparisons with similar congeners support the validity of the new species. Phylogenetic trees inferred from SSU rRNA gene sequence data show that the new species is most closely related to Diophrys oligothrix and that the genus Diophrys s. l. is paraphyletic. We gratefully acknowledge financial support from a Marie Curie Incoming International Fellowship within the 7th European Community Framework.

Long-term dynamics of planktonic choanoflagellates in the River Rhine with a description of new species

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We quantified and examined planktonic choanoflagellates in the River Rhine at Cologne for more than ten years. The seasonal cycles of the choanoflagellates were studied using the life-counting-technique and video recordings. In addition, we carried out molecular studies to identify species. New species were characterized using electron microscopy studies and video enhanced microscopy of living choanoflagellates.

Investigation on cryo-conservation of free-living heterotrophic flagellates

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Protists are ubiquitously distributed all over the world, probably dispersed by air, water or carried by animals. Survival of extremely low temperatures is essential for air transport and an important feature for cryo-conservation and longtime storage of cultures for research. We examined the freezing tolerance of 25 different flagellate species of 8 different phyla by cryoconserving them at -180°C in a nitrogen tank with an anti-freezing mixture (DMSO), and observing the growth rate after thawing at two different temperatures (room temperature (RT) and 10°C). It appeared that most species survived freezing. In general recovery was highest at room temperature. Only some chrysomonads and one cercomonad did not survive freezing.

Karyoklepty and the reduced endosymbiont of Mesodinium rubrum: A tertiary plastid in the making?

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Mesodinium rubrum is a widely distributed ciliate in coastal marine ecosystems that possesses cryptophyte organelles. However, differences have been reported in the organization of these organelles and the stability of the cryptophyte nucleus, resulting in a debate as to whether the association should be regarded as a permanent endosymbiosis or organelle retention. An Antarctic strain of the ciliate has been described as practicing a novel trophic phenomenon, termed karyoklepty, which involves the sequestration and retention of a functional nucleus from its cryptophyte prey, Geminigera cryophila. The acquired prey nucleus (= kleptokaryon) is capable of gene expression but becomes lost from M. rubrum populations though cytokinesis and is rarely observed to undergo karyokinesis. When the kleptokaryon is present, the ciliate grows phototrophically, synthesizing chlorophyll and dividing plastids. In the present study we investigated the ability of M. rubrum to regulate its plastids, by measuring expression of photosynthetic proteins and its photoacclimation potential. Plastids in the Antarctic M. rubrum strain can express photosystem proteins, even in the absence of the cryptophyte nucleus. Recently fed M. rubrum cells, can photoacclimate to a broad array of irradiance levels, but are best suited for sustained growth in low light. The plastids of this M. rubrum strain are highly stable but still require the periodic sequestration of the G. cryophila nucleus in order to acquire their full biochemical and genetic potential. Thus while the Antarctic strain still practices organelle retention, it maintains its plastids in a quasi-endosymbiotic state. Together, these observations suggest that functional phototrophy may evolve through organelle retention. With certain strains of M. rubrum possessing a stable endosymbiont and others requiring the periodic replacement of the cryptophyte nucleus, the ciliate is a useful model for understanding early events in the establishment of a tertiary plastid.

Cell cycle progression and cortical morphogenesis of Tetrahymena thermophila are affected by roscovitine

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In mammalian cells the centrosome duplication is indispensable for the formation of the bipolar mitotic spindle. At the start of cell cycle, the duplication of centrioles requires activation of the cyclin-dependent kinases (Cdks) and roscovitine at high doses blocked this duplication. However, the low doses of roscovitine applied to various mammalian cells (Whittaker SR et al. 2007, Cell Cycle 6:3114-3131) cumulated these cells at the G2/M transition with overduplication of centrosomes. It has been shown that the basal body of Tetrahymena substitutes functions of centrosome in mammalian cells (Heidemann SR and Kirschner MW 1975, J Cell Biol 67:105-117). In this study we asked how the low doses of roscovitine affect divisional cortical morphogenesis, basal body proliferation, and dynamics of cell cycle in Tetrahymena thermophila. In Tetrahymena the basal bodies are distributed in ciliary rows along the longitudinal bundles of microtubules and in the subapically located oral apparatus (OA1). The low doses of roscovitine applied to T. thermophila temporary arrested cells before cytokinesis, delayed cell division and induced increase of basal bodies along the ciliary rows, but roscovitine did not affect position of the new oral apparatus (OA2) for posterior daughter cell. In the majority of cells both: the ring of double basal bodies (couplets) and the centrin apical ring of filaments (ARF) that form the fission line, were shifted posteriorly and the cell division was highly asymmetric. Therefore, delayed in formation fission lines run across, or even posterior to the fully developed OA2. This resulted in an appearance of Tetrahymena dividing cells demonstrating variable asymmetric positioning of cytokinesis. In conclusion: the presumed inhibition of Cdks with roscovitine had three different effects on cortical divional morphogenesis in T. thermophila (i) the overduplication of basal bodies in ciliary rows, (ii) the posterior shift of differentiating couplets and ARF forming the fission line, (iii) highly unequal cell division. However positioning of OA2 was similar to that in control cells. This work was supported by the research grant of the Polish Ministry of Science and Higher Education NN303 091134 to Janina Kaczanowska.

Ciliate assemblages and their vertical distribution in two alpine lakes of contrasting transparency

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Knowledge on ciliate assemblages in alpine lakes and factors regulating the species distribution in the water column are scarce. We assessed the composition of the ciliate community in two remote alpine lakes of contrasting transparency (z 1 % 320nm = 5.3 vs. 24.4 m, turbidity: 0.2 vs. 3.2 nephelometric turbidity units) caused by the inflow of glacier melting water in one of them. Species composition was analyzed by using morphologic and molecular data (SSUrRNA). Further, to understand the species vertical distribution in the lakes, optical characteristics, abiotic parameters, and biotic factors such as chlorophyll (chl a), bacteria, phytoplankton and zooplankton abundance were also measured. In both types of lakes, the dominant ciliates were the prostomatids Balanion planctonicum and Urotricha spp. (90 % of the total abundance in the turbid and up to 99 % in the transparent lake). Other species found in the turbid lake were the haptorids Enchelys sp., Mesodinium cf. acarus and Askenasia sp. whereas in the transparent one, only Askenasia sp. was observed. In the transparent lake, B. planctonicum was restricted to deep water layers and its distribution was strongly correlated with chl a ($r^2 = 0.8$) indicating food dependence. In contrast, in the turbid lake B. planctonicum was found in the whole water column suggesting that this species is UV sensitive. Our results suppose that the drastic changes in lake characteristics caused by glacier retreat affect the composition and vertical distribution of the planktonic ciliate assemblage. Supported by the Austrian Academy of Sciences (DOC-fFORTE fellowship) and the Austrian Science Fund FWF (P21013-B03).

Evolutionary relationships of green euglenoids inferred from taxon-rich analyses of 5 genes

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Since the genus Euglena was first described by Ehrenberg (1830) green euglenoids classification by various authors has largely been based on morphological features such as: chloroplast type and distribution, flagellar length, shape and distribu-

tion of the storage product, and cell surface features. Early molecular evidences noted that some genera (Euglena, Phacus and Lepocinclis) were polyphyletic and led to several taxonomic revisions made in last decade. Our study uses 68 taxa and an expanded dataset of five genes (nuclear SSU and LSU rDNA, hsp90 and psbO and plastid 16S rDNA) to resolve relationships between genera. We used for the first time sequences of protein coding genes in combined analyses of green euglenoids. Combined analyses showed strong support by both Bayesian and ML analysis for 10 major in-group clades with 10 corresponding genera. The majority of the species sampled in the genus Euglena grouped into a large, well-supported clade. However, support for Euglena proxima as sister clade to Euglena is weak. More surprisingly, a position of Euglena archaeoplastidiata was established as sister to Euglenaria clade. Relationships between main clades remained similar to the previous results. The only exception is clade Colacium, though; in present analysis, it is placed as sister clade to Trachelomonas, Strombomonas, Monomoprhina, Cryptoglena, Euglenaria clades. Based on this well-resolved tree, we trace morphological characters and found that most of the characters evolved many times independently (i. e. rigid cells, big paramylon grains or mucocysts). Nonetheless, the evolution of chloroplast morphology and presence of pirenoids still remain unclear.

Further investigations on reproduction of Ophryoglena hemophaga (Ciliata, Ophryoglenidae) parasite of the mollusc Dreissena polymorpha (Pallas)

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Further investigations on reproduction of Ophryoglena hemophaga (Ciliata, Ophryoglenidae) parasite of the mollusc Dreissena polymorpha (Pallas) Stanisław L. Kazubski, Museum and Institute of Zoology of Polish Academy of Sciences, 00-679 Warsaw, 64 Wilcza Str., Poland The announcement concerns further investigations on reproduction of ciliate Ophryoglena hemophaga Malloy et al., 2005, presented together with dr V.I. Yurishinets on The V European Congress of Protistology in St. Petersburg 2007 (Protistology 5, 1, p.40). Presented evaluations concentrated mainly on morphological analysis of morphology of ciliates from Dreissena polymorpha from Licheńskie and Gosławskie lakes (near Konin), Szczecin Lagoon and Niegocin lake near Giżycko (Masurian Lake Region) and experimental cultures of single specimens of O. hemophaga grown in a drop of water on microscopic slides, kept in moist chamber (box). During the years 2008 – 10 more then 200 of such experiments were realized. Among the specimens of O. hemophaga, from mentioned locality, there were many very close to those described in 2007; they were small, rather wide, frequently with irregular posterior end. These specimens, named by me infants, were found on silver stained preparations as well as living in fresh smears from many molluscs. In material from Konin lakes the infants were found in 21 % infected dreissens. In experimental cultures there was obtained a transformation of specimens O. hemophaga into feebly moving oval and then into spherical form. Many of them formed envelops where three divisions of cell took place originating eight tomites. They remained in the cyst for about 6 – 10 hours, wriggled constantly and then went out as the elongated therons, measuring 110 – 120 x 40 – 45 µm. At the same time in many experiments spherical forms did not form envelopes and the movement of the cilia was well visible. Majority of them disintegrated attacked by bacteria. But one such specimen (in Exp. 154) divided three times and one of the offsprings from the third division; broad and ciliated, begun to move. By form and dimension it was very close to the above mentioned infants. In my opinion, above presented data confirmed a hypothesis that Ophryoglena hemophaga can reproduce in its host where the life conditions are much more suitable.

Protist themed jewelry

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Wooden pieces, mostly jewelry; technique is to build an image in cross-section and slice it up, like millefiore glass; some are based on abstract images of protist morphology; some are based on 'Sleigh Diagrams' of cytoskeletons.

Anaerobic protists with mitochondrion-related organelles as evidence of evolution

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The supergroup Chromalveolata includes Apicomplexa, Stramenopiles, Haptophytes, and Cryptophytes (Simpson and Roger 2004), whereas the

supergroup Excavata includes Diplomonads and Parabasalids. Increasing phylogenomic and phylogenetic data support the classification of these clades (Wegener-Parfrey et al. 2010). The anaerobic Diplomonads and Parabasalids include Giardia intestinalis and Trichomonas vaginalis which were erroneously placed at the base of the eukaryotic tree were considered 'primitive eukaryotes' lacking mitochondria. Amongst the Chromalveolata, Cryptosporidium parvum and Blastocystis hominis were also thought to be "amitochondriates". It has been established, however, that many of these protists do possess mitochondrion-related organelles (MRO), mitosomes, or hydrogenosomes which are derived from an ancestor with mitochondria. It became apparent in the late 1990s that hydrogenosomes, discovered in 1973 (Lindmark and Müller), were in fact anaerobic forms of mitochondria that produced hydrogen and ATP. This placed the origin of mitochondria much earlier in the Eukaryotic Tree of Life. Furthermore, a variety of protist lineages possess reduced mitochondria eg. mitosomes and MRO. Perhaps the most reduced of the MRO are those of the apicomplexan C. parvum. This organelle is structurally distinguished from hydrogenosomes and mitosomes of other anaerobic protists both by its association with the crystalloid body, an unique apicomplexan organelle of unknown function, and the absence of cristae junctions with the mitochondrial inner membrane. The only function ascribed to this organelle is typical of all eukaryotic mitochondria – the assembly and maturation of iron sulfur clusters. Core metabolism in C. parvum is also distinctive. This protist has an unique pyruvate-ferredoxin oxidoreductase (PFO) resembling that in the anaerobic protists Entamoeba histolytica, G. intestinalis, T. vaginalis, except that it is fused to a C-terminal NADPH-cytochrome P450 reductase (CPR). Unlike them, however, Cryptosporidium has both a cytosolic and organellar form of this fusion protein, and it is not localized to the MRO, but to the mysterious crystalloid body (Keithly 2007). Thus, reductive evolution in organelles amongst the anaerobic protists seems to have endless variety. This is another example of how evolutionary processes have given rise to multiple types of MRO, dispelling the view that an overall design is responsible for protist diversity.

Tintinnid species as biological sensors for monitoring the Kuroshio Extension in Korean coastal waters

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Tintinnids are the best planktonic group to use as indicators of water mass movements. Three approaches, the cruises from the warm pool in North West Pacific to Korea/Tsushima Strait, the biweekly or monthly monitoring in Korea/Tsushima Strait, and the daily monitoring in a near shore of southern Korea have been simultaneously done to identify the seasonal and annual fluctuation of Kuroshio effect on the southern coastal water in Korea. Annual pulses of Kuroshio indicative species were detected regularly in Korea/Tsushima Strait. Maximal number of the indicative species was recorded in September 2008. Climacocylis scalaroides, a representative Kuroshio indicator species, occurred simultaneously at a near shore of southern Korea as well as Korea/Tsushima Strait. It is assumed that Kuroshio in Korean coastal areas exhibits the most effective extension in Sept. 2008 during the study years (2006 – 2009). Sharp decreases of species diversities were noticeable in the transition zone between Japan Kuroshio zone to East China Sea. Epiplocyloides reticulata reported previously as a Kuroshio indicator, is considered as an indicative species for the water mass of East China Sea (ECS) because this species was not detected in the Northwest Pacific central zone but found abundantly in ECS. Tintinnid species shown temporal and spatial sensitivity of their occurrence can be used as biological sensors to identify mixtures of different water masses and as a valuable tool for supporting the result obtained from physical oceanography.

Phylogenetical, morphological and ultrastructural diversity of novel soil cercomonad species and genera: Few correspondences are found between ultrastructure and 18S rDNA phylogeny

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With the recent dispersal of molecular ecological methods, the correspondence between the morphological, structural and ecological characteristics of species and their molecular phylogeny has become an important question in protistology. Cercomonads are important grazers in soil and freshwater

habitats, with huge morphological and phylogenetical diversity and large ecological significance. In the present study we investigate the morphology, ultrastructure and phylogeny of cercomonads in a German grassland soil habitat, and search for correspondences between the ultrastructure and phylogenetic position of species. From 23 strains we describe 10 new species, including two new genera. Three Cercomonas species from different clades, two Eocercomonas species, and three Paracercomonas species are described. Based on large phylogenetical distance and distinct morphology, we erect two new genera, more basal from genus Paracercomonas, near the root of the cercomonad tree: Nucleocercomonas and Metabolomonas. Nucleocercomonas bears a number of characters unusual for cercomonads: Its anterior flagellum is extremely long, it is not able to glide, in its most frequent life stage its cell body does not attach to the substratum but produces pseudopodia into the water body, and it has a unique nucleus structure with a peripheral nucleolus attached to the nuclear envelope opposite to the basal body connection. Metabolomonas is extremely metabolic with a very high beating frequency of flagella, fast gliding, very fast changing of shape, and strong cytoplasmic streams. We searched for correspondences between morphological and ultrastructural characters and the phylogenetic position of species among our strains, comprising several genera, as well as among previously described cercomonad species. We found only few characters which are exclusively present among the species of a genus, while absent in other genera. Most morphological and ultrastructural characters differ within species of a genus, or are present in various genera. The lack of close correspondence between morphological, behavioural and ultrastructural characters and the 18S rDNA phylogenetic position of species implies that little information can be extrapolated reliably from already described species to molecular ecologically detected undescribed sequences.

Transmitted light super-resolution nanoscopy: The real-time ultrastructure of living protists

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The development of super-resolution microscopic techniques to exceed the resolution limit of conventional light microscopes and reach nanoscale resolutions has resulted in some very promising methods in the past few years. Most super-resolution systems are based on fluorescent microscopy, are relatively slow and extremely expensive. We developed and present a new super-resolution microscopic system, which is based on transmitted light, thus image formation is not time-limited and specimens can be investigated in real-time. The method gives a very nice relief contrast by imaging phase objects in a pseudo three-dimensional way. It particularly enhances and prodigiously images the small object details. The method has the best modulation transfer function among optical contrast techniques. The contrast enhancement intensity of phase objects is lower than that of high quality DIC systems; however, it is perfectly combinable with DIC to sum the contrast enhancement potential of both techniques. The practically achieved highest resolution of the system after image analysis is ~90nm with white light illumination. Conventional light microscopes can be upgraded with this technique relatively simply and for low costs. The high resolution of the system, the excellent imaging of small details and the strong contrast enhancement of phase objects, in combination with videomicroscopy, allows the investigation of the ultrastructure of living protist cells in real-time. For ultrastructural investigations the resolution of the system is lower than transmission electron microscopic studies using ultra-thin sectioned preparations, but there is an enormous advantage: living cells can be investigated without fixation artefacts. The function of several structural components become investigable, and a brand new field bridging fine structural, functional, behavioural and ecological studies opens for researchers. With the method we could successfully observe in-vivo ultrastructural components, like basal bodies, several different microtubular roots, striated roots, fine intranuclear details, chromosome segregation during division, microbodies, paranuclear bodies, Golgi cysternae and single vesicles, mitochondria, extrusomes, flexible external sheaths, etc.

Control mechanisms of establishment of the endosymbiosis between Paramecium bursaria and symbiotic Chlorella sp.

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Endosymbiosis is a primary force for eukaryotic cell evolution in function and their environmental adaptation. Recent studies of algal evolution have shown that endosymbiosis has occurred several times and has brought a biodiversity of protists. Despite the importance of this phenomenon, molecular mechanisms of establishment of the endo-

symbiosis between different protists (secondary symbiosis) are still not well known. We aimed to clarify control mechanisms of establishment of the secondary symbiosis between alga-free Paramecium bursaria and its symbiotic algae. Irrespective of the mutual relationships between them, both retain an ability to grow independently and can be experimentally reinfected synchronously by mixing them. This phenomenon provides an excellent opportunity to elucidate cell-to-cell interactions between the protozoa and algae during establishment of the secondary symbiosis. Using pulse-label of the alga-free paramecia with the isolated symbiotic algae and chase method, algal reinfection process to the paramecia was well defined. Furthermore, we found 4 important cytological events needed to establish endosymbiosis. (1) Three minutes after mixing, some algae show resistance to the host lysosomal enzymes in the digestive vacuoles (DVs) even if the digested ones are coexisted. (2) Thirty minutes after mixing, the alga starts to escape from the DVs as a result of the budding of the DV membrane into the cytoplasm. (3) Within 15 minutes after the algal escape, the vacuole enclosing a single green alga differentiates into the perialgal vacuole (PV) membrane from the DV membrane. The PV gives protection from the host lysosomal fusion. (4) After that, the alga wrapped by the PV membrane localizes in the primary lysosome-less region beneath the host cell cortex by an affinity of the PV membrane to unknown structures of the host. At about 24 hours after mixing, the alga increases by cell division and establishes endosymbiosis. Although molecular analyses of these four phenomena have just begun, the results of such analyses will dramatically promote the study of endosymbiosis control mechanisms in the near future.

Molecular phylogeny and species identification of Leishmania by nuclear and kinetoplast genetic markers

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The molecular phylogeny of 12 Leishmania species from three subgenera based on nuclear and kinetoplast genetic markers was reconstructed. The primary structures of RNAP(RPOIILS), DNAP(POLA), 12S RNA, 9S RNA, ND8, cyb, A6, ND3, rps12 genes and fragments of divergent region proximate to 12 S RNA(DR fragment) were used as genetic markers. The alignment of investigated genes sequence provides strong support for the discrimination between Leishmania subgenera. The phylogeny reconstructed from nuclear and kinetoplast genes is very similar. The significance and possible application of these results for the using in molecular systematic of trypanosomatids, including the rank estimation of individual markers will be discussed. We suggest that sequence of the DR fragment may be useful for Leishmania species identification.

The exocsyt complex is involved in contractile vacuole function in Chlamydomonas reinhardtii

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Chlamydomonas cells contain two contractile vacuoles (CV) involved in osmoregulation. We have isolated osmoregulatory mutants using an insertional mutagenesis approach. Chlamydomonas CC 3395 (cwd, arg 7 – 8) was transformed with a 1.7 kB DNA fragment containing the hygromycin resistance gene (aph7 gene (Berthold et. al., 2002, Protist)). 2585 hygromycin-resistant clones were obtained and screened for failure of growth in hypotonic media. Initially 75 mutants were isolated, which showed differences in growth. Here we characterized one of the mutants, named Osmo75, which cannot grow in hypotonic media at all. The point of insertion of the hygromycin resistance cassette was determined by RESDA PCR (González-Ballester et al 2005, Analytical Biochemistry). Osmo75 showed a huge deletion of 33.641kb in Scaffold 20. Two Genes are affected and four genes are deleted. One of the deleted genes is Cre20.g759900, a gene coding for an exocyst complex subunit (SEC6). As the other genes show no obvious connection to the function of the CVs we focused on CrSEC6. To test whether the phenotype is indeed caused by the deletion of SEC6, we transformed Osmo75 with a sec6 GFP fusion construct. After transformation we obtained 312 clones which grow in hypotonic medium. We analyzed 10 clones microscopically; all showed a normal CV cycle. Both, growth in hypotonic medium and a normal CV cycle, indicate that the deletion of SEC6 caused the phenotype of the osmoregulatory mutant. Overexpression of the sec6-GFP construct in the parental strain led to a shortened CV cycle, suggesting SEC6 as a key component in the control of the CV-cycle in Chlamydomonas reinhardtii. This is the first report of an involvement of the exocyst complex in CV function in green algae or other protists.

Eukaryotic life without haem: The aerobic kinetoplastid flagellate Phytomonas does not require haem for viability

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Haem is an iron-coordinated porphyrin that is universally essential as a cofactor for fundamental cellular processes, such as electron transport in the respiratory chain, oxidative stress response or redox reactions in numerous metabolic pathways. Parasitic kinetoplastids represent a rare example of organisms that depend on oxidative metabolism but are haem auxotrophs. The media for their cultivation have to be supplemented with haem. However, we found that haem is dispensable for the survival of Phytomonas, the serious yet poorly studied parasite of plants. Even one-year long continuous cultivation in a haem-free medium had no impact on cell growth, and we were not able to detect any traces of haem in this culture using very sensitive assays. Together with the lack of haem biosynthesis genes in the genome of Phytomonas, these findings clearly indicate that this flagellate can survive without haem. In order to find out how this is possible, we carefully examined several putative functions of haem in Phytomonas serpens. We found that it does not play any important role in the mitochondrial respiratory chain. Supplementation of the medium with haem does not improve resistance against oxidative stress in Phytomonas, which corresponds with the absence of haem peroxidases in the genome. While it seems that for desaturation of fatty acids, Phytomonas uses an alternative electron donor, haem is required for the 14-demethylation of lanosterol to produce ergosterol. However, we found that Phytomonas grown in the absence of haem can utilize 14-methylsterols in its membranes. It is therefore apparent that this flagellate has unique metabolic adaptations allowing it to bypass all requirements for haem. To our knowledge, this is the first example of a eukaryote totally lacking haem.

Does morphology correlate with molecular data? A case study of the Nebela tincta complex (Arcellinida)

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Nebela tincta-bohemica-collaris (Amoebozoa; Arcellinida) is a species complex of small to medium-sized testate amoebae common in terrestrial ecosystems. The taxonomic validity of characters used to define morphospecies within this group is largely unknown, which is problematic for research in biogeography, bioindication and palaeoecology. The objective of this work is to construct a new phylogeny based on morphological and molecular data, to examine in detail the relationship between morphological and genetic diversity in Nebela tincta and similar taxa in order to clarify the phylogenetic relationships within this clade. The phylogeny of the group was constructed using both the Cytochrome Oxidase Subunit 1 (COI) sequences data and morphometrical data using the Discrete Cosine Transform method. The combined morphological and phylogenetic analyses allowed us to assess if morphological and genetic micro-variations (within a morphospecies) are correlated to each other, how the observed morphologic and genetic diversity is correlated to geographical distance and (micro-) environmental parameters, if the level or morphological and molecular variation is related to the size of the organism, and finally to re-evaluate the position of several putative taxa.

Cyst-forming trypanosomatids and general issues in systematics of Trypanosomatidae family

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At present researchers that study trypanosomatids found themselves in a trap that had been inadvertently set by their predecessors. Description of species from mixed invasions, establishment of cell cultures where only a minor component of the initial invasion survives, incorrect assignment of newly described species to a genus and mistaken specific identification of natural isolates – all these phenomena confuse trypanosomatid systematics. Recently we discovered a lineage of trypanosomatids that are characterized by the presence of cyst-like amastigotes in their life cycle and possessing cryptic or well developed undulating membrane. Depending on the degree of development undul-

ating membrane cyst-forming trypanosomatids had been previously assigned to genera Leptomonas and Blastocrithidia. However there are some cyst-forming species that according to molecular phylogenies are quite distant from this group and belong to clades consisting of species with different morphology. The scrutiny of such conflict cases revealed mistakes made by researchers. There was disparity between cells in the so-called type cultures and those characterized in species description in two instances (B. miridarum and B. gerricola). In the third instance (L. rigidus) the obvious discrepancy was not found but it was notable that cysts were found in natural isolate whereas species description had been made using cell culture. Field studies displayed frequent mixed invasions of insect hosts. Subsequent molecular phylogenetic analysis of pure isolates of trypanosomatids proved that in each case of data conflict two different species had been formerly treated as one. Thus we demonstrated that conflicts between morphology – based systematics and molecular phylogenies of trypanosomatids may be caused by the use of different sources of material.

Where have all the microsatellites gone? A two-method survey in Paramecium caudatum unveils only weak amounts of short tandem repeats

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Microsatellite DNA fingerprinting is an important tool to investigate the genetic diversity of species, to assess intraspecific genetic relationships and to understand population dynamics. Such simple sequence repeats (SSRs) or short tandem repeats (STRs) of 1-6 nucleotide motifs, which show frequent length variations, are thought to be present in just about every known organism. So far, just a few studies used this method for free-living protozoa and only a weak amount of data disclosed STRs for ciliate species. In ciliates, such STRs are supposed to be predominantly located inside the internally eliminated sequences (IESs) of the micronuclei (MIC), while the macronuclear (MAC) genome is almost microsatellite-free. Therefore, we prepared MIC-DNA in a first survey to generate microsatellite-enriched DNA using a modified FIASCO approach (Fast Isolation by AFLP of Sequences COntaining repeats). The resulting library was screened for di- and tetranucleotide repeats, but unveiled telomeric repeat motifs only. Consequently, we considered whether a shotgun high-throughput sequencing strategy could yield a higher amount of potential microsatellite loci. Analysing the resultant ~13,500 sequences reads of a 1/16th 454/Roche GS-FLX run revealed only ~200 sequences with repetitive motifs. Here, just a handful of short di-, tetra- and pentanucleotide STRs (4-8 repeats) could be detected, as well as a number of trinucleotides with few repeats. Longer STRs with higher numbers of motif repeats (up to 32) and therefore potential microsatellite loci were represented by hexanucleotide STRs only. However, due to the limitations of the applied high-throughput sequencing strategy, most of the hexanucleotide containing sequence reads comprised repeat motifs only or featured single 5'- or 3'-flanking non-repetitive sequences. Therefore, it was virtually impossible to design appropriate microsatellite primers. Hence, a further combined analysis using an enrichment-based protocol for hexanucleotide STRs followed by a paired-end short read sequencing strategy might unveil where the microsatellites in Paramecium caudatum hunker down.

Expanding our knowledge of Amoebozoa: Atlantic and Pacific deep-sea amoebae from two expeditions: Phylogenetic and taxonomic implications

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Although it was previously shown that Amoebozoa can be isolated from the deep-sea sediment samples, only one study so far attempted to describe and identify them using all available tools, including electron microscopy and molecular genetic analysis. Consequently, our knowledge of the deep-sea Amoebozoa is very limited compared to other protistan groups. We attempted to fill this gap during two expeditions focused on the deep-sea biodiversity: DIVA3 (RV Meteor cruise 79/1, July-August 2009) to the Western Atlantic, and SoJaBio (RV Akademik Lavrentyev cruise, August 2010) to the Sea of Japan. During both expeditions living, cultivable amoebae could be isolated from the depths of 260 to ca. 5200 meters (in Atlantic Ocean) and ca. 500 to 3600 meters (in the Sea of Japan). A total of ten morphospecies belonging to the families Vannellidae, Paramoebidae, Vexilliferidae and Cochliopodiidae were isolated from the Atlantic Ocean. Based on morphology, ultrastructure and gene sequence data, they all can be recognized as new species; some of them represent new, deeply-

branching, genera of amoebae. Five species were found in the Sea of Japan, one of them could be unambiguously identified as Neoparamoeba aestuarina (Page, 1970), while others can not be assigned to any of the higher order taxa and probably represent the novel phylogenetic lineages of amoebae. We discuss the significance of the presented data for phylogeny, taxonomy and ecology of Amoebozoa. Supported with the DFG grant HA 818/22-1 to KH and the research grant IZLR Z3_128338 from Science and Technology Cooperation Programme Switzerland-Russia to JP.

Genetic structure of amoebozoan morphospecies: A case study on freshwater Cochliopodium spp.

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We make an attempt to estimate the degree of genetic variability within the morphospecies of Amoebozoa using freshwater species of the genus Cochliopodium as an example. The advantage of Cochliopodium is a very convenient definition of the morphospecies: these amoebae have tectum consisting of complex, species-specific scales rich in structural details. A morphospecies in this case can be clearly defined based on the possession of the identical scales. We compared nuclear small-subunit ribosomal RNA and mitochondrial cytochrome oxidase I gene sequences from multiple morphologically and ultrastructurally identical strains of a cosmopolitan species C. actinophorum isolated from various locations. Sequence analysis reveals a considerable genetic diversity within this morphospecies, suggesting cryptic speciation. Supported with the research grant IZLR Z3_128338 from Science and Technology Cooperation Programme Switzerland-Russia to JP.

Foraminifera of the White Sea: Expressing biodiversity through zoological drawings

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Foraminifera are well-known for their incredible appearance. Since 2007, I've been studying benthic foraminiferal fauna of the White Sea, European Arctic. Different ways of imaging of live individuals were performed in this research. On ECOP 2011 I intend to present drawings and light micrographs of foraminifera inhabiting recent sediments of the White Sea. Special attention will be paid to monothalamous foraminifera, since they are frequently called 'simple', 'inconspicuous' and 'easily overlooked'. My aim is to somewhat dispel this common opinion. Also, the presentation/exhibition will contain drawings of multichambered agglutinated and calcareous species. Besides the purpose of artistic impression, zoological drawings are potentially applicable in modern foraminiferal research, especially in creation of databases, atlases and identification guides.

Protelphidium cf. niveum (Lafrenz) from the White Sea: Towards a better view on systematics of primitive elphidiids

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Foraminifera of the family Elphidiidae inhabit shallow waters worldwide. Particularly, they are diverse and abundant on the arctic shelves. We found live representatives of 10 elphidiid morphospecies in near-shore sediments and on subtidal kelp in the western White Sea. One species had not been recorded in the White Sea previously. Based on the examination of shell morphology (light microscopy and SEM), the species was preliminarily identified as Protelphidium cf. niveum (Lafrenz, 1963). We discuss the generic affinities of Protelphidium cf. niveum and functional morphology of its shell. The species in question belongs to the poorly studied basal group of elphidiids (the earliest Protelphidium is known from the Paleocene). Nonion niveum Lafrenz was originally described from the quaternary deposits of Schleswig-Holstein. Since then morphologically very similar specimens have been recorded in Pleistocene and recent deposits and listed under the generic names Protelphidium or Haynesina. A number of similar species ascribed to the genus Porosononion occur in the Red Sea and in the Mediterranean. At present, the taxonomic boundaries between genera Protelphidium, Porosononion and Haynesina are not defined clearly. Unravelling the taxonomy in this group is a valuable investment in reevaluation of elphidiid systematics; also, it helps us to understand better their evolutionary pathways. As many other elphidiid species, Protelphidium cf. niveum feeds on diatom algae, using the shell ornamentation to brake their frustules. Overall shell composition suggests its affinity with the ecological group of elphidiids inhabiting soft sediments.

A hydrogenosome in the free-living excavate Andalucia incarcerata: Common themes in mitochondrial reduction in anaerobic eukaryotes

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Mitochondrion-related organelles (MROs) have arisen independently in a wide range of anaerobic microbial eukaryotes. To date, these organelles have been studied predominantly in parasitic organisms. Here we present the MRO of Andalucia incarcerata, a free-living excavate, and discuss a predicted biochemical map of the pathways believed to be present in this organelle, based on EST data. Localization of key proteins in the iron sulfur cluster assembly and anaerobic energy generation pathways cement the evidence for the mitochondrial origins of the organelle, and support its classification as a hydrogenosome. A. incarcerata's hydrogenosome contains a number of functions that are also found in well-characterized hydrogenosomes from unrelated lineages, but it has also retained mitochondrial functions that are not commonly found in the MROs of parasitic organisms. Investigating MROs in free-living organisms such as A. incarcerata therefore allows us to explore the diversity of these organelles, and to separate adaptations to the anaerobic lifestyle from adaptations to parasitism.

NADP-dependent alcohol dehydrogenase I from Trichomonas vaginalis: An unusual bifunctional enzyme

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Trichomonas vaginalis and other microaerophilic parasites, such as Tritrichomonas foetus and Entamoeba histolytica, possess an NADP-dependent alcohol dehydrogenase (ADH1) which specifically oxidizes secondary alcohols and reduces acetaldehyde and acetone. T. vaginalis produces very high levels of this enzyme, accounting for more than 1 % of total cellular protein visualized in 2D-gels of whole-cell extracts. This is surprising as primary alcohols are not oxidized by ADH1 and secondary alcohols, such as 2-propanol, are not intermediates or products of T. vaginalis metabolism. Also ethanol, which is formed by ADH1 through the reduction of acetaldehyde, is only a minor metabolic product. However, when determining the kinetic parameters of recombinantly expressed ADH1, we also noticed a distinct NADPH-oxidizing activity in the absence of acetaldehyde or acetone. This activity was also observed in the total absence of oxygen. Reduction of acetaldehyde and acetone, as well as the NADPH-oxidizing activity of ADH1 were strongly inhibited by 2-propanol and coenzyme A. Interestingly, 140 mM 2-propanol, but not 140 mM 1-propanol, are rapidly fatal to T. vaginalis when being placed into fresh medium which contains residual quantities of oxygen. When being challenged in anaerobic medium, or after having been grown in the absence of added iron, T. vaginalis are not susceptible to 140 mM 2-propanol. Presumably, 2-propanol not only inhibits the oxidation of NADPH by ADH1, but, conversely, even increases NADPH levels through its oxidation by the enzyme. These observations suggest that ADH1 is an essential enzyme for T. vaginalis in the presence of oxygen by decreasing intracellular NADPH pools. Thereby, it counteracts the generation of reactive oxygen species by NADPH-oxidases. The resistance to 2-propanol in T. vaginalis, grown without added iron, can be explained by the fact that oxidative stress is strongly exacerbated by ferrous iron through the generation of hydroxyl radicals in the Fenton reaction. This scenario is further supported by our observation that T. vaginalis becomes resistant to 140 mM 2-propanol after having been treated with diphenyleneiodonium (DPI), a potent inhibitor of NADPH-oxidases.

The role of parasites in the regulation of small phytoplankton communities in the open ocean

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In the last few years, with the development of molecular techniques, the diversity and spatial distribution of small photosynthetic eukaryotes has been investigated in various studies revealing their key ecological role in global CO_2 fixation and carbon cycling in the ocean. It is therefore of fundamental importance to identify the factors controlling their diversity and distribution. However, factors involved in the regulation of small photosynthetic eukaryote communities remain very poorly known. While the most common consumer strategy, namely parasitism was usually left out of aquatic trophic food web functioning, recent ecological and molecular surveys in aquatic environments have revealed a high occurrence of eukaryotic putative parasitoids, especially in the picoplanktonic size-fraction (López-García et al. 2001, Guillou et al. 2008, Lepère et al. 2008), making them as impor-

tant as the other typical parasitic biological entities such as viruses. All investigations performed so far have shown the importance of environmental DNA sequences affiliated to Syndiniales (Alveolata) which are a group of ubiquitous marine parasitic dinoflagellates infecting a wide range of planktonic species, from phytoplankton to fish larvae. Eukaryotic parasites consist also of fungi (chytrids), Perkinsozoa (Alveolata), oomycetes (Stramenopiles), amoeboa, euglenoids and other flagellates. They are mainly known to infect diatoms and dinoflagellates but also have the capacity to infect others taxa such as cyanobacteria, chrysophytes, cryptophytes, chlorophytes, prymnesiophytes and zooplankton. Double TSA-FISH (Tyramide signal amplification – fluorescent in situ hybridisation) and cloning-sequencing using specific probes and primer sets are used here to characterize the major parasitic groups present in open ocean systems and assess their ecological relevance on small phytoplankton populations. We will also compare the effects of viral infection to the ones caused by eukaryote parasites, using electron microscopy and cloning-sequencing. References: Lòpez-Garcia P, Rodriguez-Valera F, Pedrós-Alió C, Moreira D. (2001) Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature 409: 603 – 607. Guillou L, Viprey M, Chambouvet A, Welsh RM, Kirkham AR, et al. (2008) Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). Environ Microbiol 10: 3349 – 3365. Lepère C, Domaizon I, Debroas D. (2008). Unexpected importance of potential parasites in the composition of the freshwater small-eukaryote community. Appl Environ Microbiol 74: 2940-2949.

The biodiversity of ciliated protozoa in mangrove wetlands of southern China

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The mangrove wetland is generally considered as one of the few ecosystems that with high divergent species. As an important group of the detritus food chain of wetland, the ciliated protozoa in this biotope are seldom investigated worldwide. Therefore during the last three years we investigated the ciliate fauna in mangrove wetlands of southern China, in particular the coastal wetlands of Guangdong province. About 150 free-living species have been isolated and identified, mainly including 35 pleurostomatids, 32 oligotrichs s. l., 25 hypotrichs, 15 hymenostomatids, 10 peritrichs, 9 euplotids, and 9 cyrtophorids. Meanwhile, a DNA bank containing genetic material was established for most isolates. Both morphological and molecular data of them were recorded and analyzed. The biodiversity of ciliates in mangrove wetlands and the taxonomy and phylogeny of some of novel or poorly-known forms will be introduced. Supported by the National Natural Science Foundation of China (project numbers: 30870280, 31030059).

Classification and phylogeny of pleurostomatid ciliates (Ciliophora, Protozoa)

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The taxonomy of pleurostomatid ciliates, especially the marine ones, is extremely confusing with the increasing of new taxa and due to the fact that many "known forms" are mostly insufficiently described before. In last few years, the morphology and infraciliature of 60 plus species belonging to 8 genera of Pleurostomatida have been investigated during our study on the biodiversity of marine ciliates in China seas. Based on the morphology combining molecular data, the classification and phylogeny of pleurostomatids are discussed here. Four families were suggested mainly according to the arrangement of the right somatic kineties (RSK): (1) Apolitonotidae n. fam., the anterior end of RSK gradually shortened along 2-3 rows of leftmost RSK, including Apolitonotus n. gen.; (2) Kentrophyllidae n. fam., the middle RSK gradually shortened and usually forming one anterior and one posterior suture, including Kentrophyllum and Epiphyllum; (3) Amphileptidae Bütschli, 1889, the RSK forming one anterior suture, including Amphileptus, Apoamphileptus et al.; and 4) Litonotidae Kent, 1882, the anterior end of RSK gradually shortened along perioral kineties, including Litonotus, Loxophyllum et al. Molecular information based on the small subunit ribosomal RNA (SSU rRNA) gene sequences supports the monophyly of all these four families, which confirms in great deal the efficiency of our classification. The results indicate also that the arrangement of the right somatic kineties might reflect their evolutional status of pleurostomatid ciliates. In addition, four families branch out in the order of Apolitonotidae – Kentrophyllidae – Amphileptidae – Litonotidae in our phylogenetic trees. Supported by the National Natural Science Foundation of China (project numbers: 30870280, 31030059).

Ultradian clocks (with periods of about one hour) in protists constitute a synchronizing time base and coherence for organized complexities

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In 7 protist and 3 yeast species we have monitored cycles of respiration with periods of about an hour (ie Ultradian rhythms, those that cycle many times every day), In all species these cycles proceed several times during their respective cell division cycles in laboratory experiments using rich growth media. In Acanthamoeba castellanii and Saccharomyces. cerevisiae we have confirmed that these are not merely oscillations or rhythms, but timekeepers: i.e. clocks that provide the time base for synchronization of all cellular activities (metabolism, energetics, biosynthesis, transcription, membrane and organelle assembly, nuclear dynamics and cell division). These Ultradian clocks have temperature-compensated periods that are genetically controlled so that their cycle times are characteristic for different species (or strains, eg in Tetrahymena pyriformis) and can be altered by mutation (eg. in yeasts). These inter-specific differences arise as emergent properties of their differing cellular network structures. However, in all at their cores are redox balances reactions in common (with coupled NAD(P)H, disulphide-dithiol, reactive O2 components). Mitochondrial clocks (t ~ 4 min) work on similar principles, as probably do circadian, and possibly, tidal, and annual cycles. It is likely that these Ultradian cycles represent an ancient basic clock archetype for cellular coherence. Longer cycles are evolutionary icing on the cake, representing rather recent innovations.

Pheromone-mediated cell-cell signaling in Euplotes

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In ciliates, intra-specific cell-cell signalling is functionally associated with the genetic mechanism of the mating types. Based essentially on studies on the "binary" mating-type systems of Paramecium and Blepharisma, this mechanism has traditionally been considered as a sexual mechanism, in which the "mating-type factor" of one type of cells would bind, and stimulate to mate, only cells of the second "complementary" mating type. However, this view does not appear to be supported by genetic and structural studies of families of the water-borne protein mating-type factors (pheromones) that are synthesized by species of Euplotes (i.e., E. raikovi, E. nobilii and E. crassus) characterized by mating-type systems of "high-multiple" type. These studies suggest that the pheromone sexual activity is secondary with respect to an autocrine pheromone activity, which promotes the vegetative (mitotic) growth of the same cells from which these signalling molecules are synthesized and secreted. In accord with the genetic determination of their structural specificities through multiple series of single-locus alleles, Euplotes pheromones have been shown to possess three-dimensional architectures that closely mimic one another. Therefore, they can bind their cell-membrane receptors in a competitive fashion and form either homologous, or heterologous protein-protein complexes. The homologous complexes have been shown to be responsible for signalling a cell growth response. They are internalized through endocytotic vesicles and activate a protein-kinase dependent transduction pathway. In contrast, the heterologous complexes appear destined to remain blocked on the cell surface, where they are probably involved in promoting the cell mating response.

A new genus and species of apostome ciliate infecting the hyperiid amphipod Themisto libellula in the Canadian Beaufort Sea (Arctic Ocean)

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A ciliate was discovered infecting 4.4 % of the hyperiid amphipod Themisto libellula collected with nets and sediment traps in the Canadian Beaufort Sea. The ciliates were found in the haemocoel and associated with tissues surrounding the intestines of the amphipods. Hosts collected in sediment traps were more frequently infected, suggesting that this ciliate may kill its host. Ciliates were isolated from an ethanol-fixed whole amphipod and the DNA was extracted. The small subunit (SSU) rRNA gene was amplified and showed unambiguously that this is an apostome about 2 % divergent from three krill-infesting apostome species assigned to the genus Collinia. Protargol silver impregnation of formaldehyde-fixed ciliates showed a highly unusual infraciliature for an apostome. There are typically 8 (6 – 9) bipolar somatic kineties covering the banana-shaped body. The anterior end of the oral region begins about 1/3 the body

length from the anterior end and is composed of an inpocketing that is lined on its anterior and left wall with an oral field of densely-packed ciliated kinetosomes. A presumed paroral begins at the anterior-left of this field, encircles the anterior of the field, and extends posteriorly for about 1/5 the body length. To the left of the paroral and posterior to the oral field are typically 3 (2 – 5) oral kineties that extend posteriorly parallel to the paroral. Many cells were in division, which appears to be a simple process of transverse fission. Stomatogenesis begins with some dedifferentiation of the parental oral field and elongation of its paroral and oral kineties. A new oral field develops midventrally and the paroral and oral kineties break to form the oral apparatus of the opisthe, which completes development by additional kinetosomal proliferation and migration of the paroral. This morphology is unique among apostomes and justifies the establishment of a new genus and species. Stomatogenesis suggests affinities to the scuticociliates, a group with which the apostomes are genetically related based on SSU rRNA sequences.

Why does picocyanobacteria-feeding Spirostomum teres accompany zoochlorella-bearing Pelagothrix plancticola in the oxycline of a meromictic lake?

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Fine-scale stratification (0.25 m) of microaerophilic to anaerobic ciliate assemblages along with an estimation of grazing rates upon picocyanobacteria (PCY) were studied within the mixolimnion and monimolimnion of the meromictic Lake La Cruz (Cuenca, Spain) during a stratification process. Quantitative Protargol Staining and Fluorescently Labelled Bacteria (FLB) methods were employed, respectively. Scuticociliates dominated the environment: genus Ctedoctema (up 130 cells/ml; feeding upon PCY) was present within a wide range of dissolved oxygen concentrations (DO) while Uronema, Sathrophilus (Sphenostomella) and Cristigera followed strictly DO gradient. Mesodinium sp. (up 20 cells/ml), Monodinium sp. and Askenasia sp. were observed in mixolimnion. Mixotrophic genera Coleps (20 cells/ml) and Pelagothrix (Prorodon; up 28 cells/ml) along with Spirostomum teres (up 12 cells/ml; feeding upon PCY) biomass-dominated the mixolimnion and oxycline, respectively. In the anaerobic monimolimnion, genera Holophrya, Caenomorpha and odontostomatids were present. Ciliates containing vacuoles with PCY also showed feeding upon DTAF pre-stained Aeromonas mimicking the natural PCY size distribution; however, larger, cultured PCY of the lake origin were not ingested; zoochlorella-bearing ciliates did not ingest FLB at all. Microaerophilic scuticociliates were feeding upon FLB even in the assays with untreated water samples but Spirostomum teres was actively feeding only in anaërostat assays (bubbling with helium was used to minimize DO). S. teres feeding rates were not linearly proportional to the incubation time, which was in concordance with observed pattern of vacuole formation. FLB that had been quickly collected into vacuoles during the first 5 to 10 min exposition were moving through the cell and finally joined large vacuoles in the posterior end of the cell. Even though the FLB ingestion dropped during the feeding experiment, FLB persisted in vacuoles more than one hour. Living ciliates were of orange colour with bright red fluorescence of feeding vacuoles upon green excitation light (phycoerythrin rich PCY as known from the isolate used in experiments). Combined use of PCY-photosynthesis products and digestion in the anaerobic conditions with an optimum light for PCY activity is hypothesized, a process similar to described oxygen- and primary production of Pelagothrix plancticola.

Molecular assessment of natural and ship ballast derived Paralia (Bacillariophyta) populations reveals worldwide cryptic diversity

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Paralia Heiberg is arguably one of the most recognizable, widely distributed, and commonly reported diatom genera from contemporary coastal marine environments and ship ballasts. We determined the morphological and genetic profiles of 184 monoclonal cultures of Paralia from 76 sites including 7 from Europe, 33 from Canada and 36 from other sites worldwide. The isolates were sequenced for a fragment of the RuBisCo large subunit (rbcL) gene and the internal transcribed spacer (ITS) and 18S regions of nuclear encoded ribosomal RNA. In total six phylotypes were found with five of these molecular variants corresponding to new Paralia species, most of them cryptic. Species of Paralia resolved included Paralia from "Mexico Pacific", Paralia from the "Caribbean Sea" (Jamaica and Panama), Paralia "European" (formerly P. sulcata sensu Crawford, 1979) which was also present in samples from Uruguay and New Zealand, Paralia

from "north-northwest Pacific" (Vancouver Island and Washington State), and Paralia with "pan-Canadian 1" and "pan-Canadian 2" distributions from both the Atlantic and Pacific coasts of Canada and the United States. P. fenestrata Sawai and Nagumo (only rbcL data), and P. longispina Konno and Jordan (no molecular data available) were also recovered. Paralia species fell into four morphologically distinct groups corresponding to five lineages in rbcL trees. Lineage I included Paralia from "north-northwest Pacific" and Paralia with "pan-Canada 2" distribution and were characterized by the presence of small to large papillae on the surface of separation valves and shallow, cuneate fenestrae. Lineage II was comprised of Paralia with "pan-Canada 1" distribution and featured smooth separation valves and shallow, cuneate fenestrae. Lineage III was comprised of Paralia from "Mexico Pacific", Paralia from the "Caribbean Sea" and P. longispina. All had long tapering spines on separation valves and small, well-defined, ovoid fenestrae. Lineage IV included only P. fenestrata characterized by large, defined, U-shaped fenestrae. Lineage V was composed of Paralia "European". Paralia "European" and Paralia "pan-Canadian 1" were morphologically cryptic, yet molecularly very distinct. Most species had a unique biogeography. Our findings call for the reinterpretation of studies which report all Paralia specimens encountered as P. sulcata.

Myxosporeans species that infect mullets (Teleostei: Mugilidae) of the Catalan coasts (north-eastern Spain)

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During a parasitological survey on mugilid fishes of aquaculture interest in Catalonia (NE Spain), it was detected the presence of five species of myxosporeans infecting two species of mullet, Flathead grey mullet, Mugil cephalus L., 1758, and Golden grey mullet, Liza aurata (Risso, 1810). 210 specimens of Mugil cephalus were captured from a brackish coastal lagoon (l'Encanyissada lagoon) within the Ebro River Delta Natural Park (Western Mediterranean). On the other hand, 36 individuals of Liza aurata specimens were caught off the coast of Barcelona (Zone FAO 37.1). Observed parasites were studied under a compound microscope or processed for ultrastuctural studies, both transmission (TEM) and scanning electron microscopy (SEM). Prevalence of each myxosporean species were determined according Bush et al. (1997). Myxosporeans species found belong to the genus Myxobolus Bütschli, 1882. Members of this genus are important pathogens of fish. Among the determined species of parasites on Mugil cephalus, we found regularly M. ichkeulensis Bahri & Marques, 1996. This parasite forms cysts that appear as elongated whitish opaque masses distributed at the base of arches gills of host (number of cysts were variable: 3 – 11); M. bizerti Bahri & Marques, 1996, forming cysts on the gill filaments, and Myxobolus sp. into the gallbladder. Concerning Liza aurata, we observed the presence of M. spinacurvatura Maeno, Sorimachi, Ogawa and Egusa, 1990, forming whitish oval small cysts on the wall of mesenteric vessels of host. We have also identified the species M. catalaunicus n. sp. that forms spherical plasmodia appearing as a small cysts, located in the connective tissue, between the fins rays of the host. Acknowledgements. The authors would like to thank the Generalitat of Catalonia's Aquaculture R&D and Innovation Reference Network (XRAq). The authors are also grateful for the technical assistance of Scientific and Technical Services (SCT) of Barcelona University.

Family Pyrenomonadaceae (Cryptophyta): Molecular phylogeny of the genera Rhinomonas, Rhodomonas and Storeatula

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The family Pyrenomonadaceae includes genera Rhinomonas, Rhodomonas and Storeatula. The family is a monophyletic taxon and the genera can be separated morphologically. However, in earlier single gene molecular phylogenetic studies two Pyrenomonadaceae genera, Rhinomonas and Storeatula, intervene Rhodomonas branch in phylogenetic trees. Thus, the genus Rhodomonas seems to be non-monophyletic. In this study, we examined molecular phylogenetic relationships of 8 Pyrenomonadaceae strains isolated from Tvärminne, Baltic Sea and 26 strains obtained from culture collections. We used a multi-gene approach that included several protein-coding and ribosomal RNA coding genes from nucleus, nucleomorph, chloroplast and mitochondria, and which enabled a more reliable rendering of the species' phylogenetic relationships. The preliminary results corroborate the earlier single gene finding of Rhodomonas paraphyly. Therefore, a revision of the Pyrenomonadaceae family may be needed. However, the revision requires

electron microscopic examinations of species specific morphological characters, which are in process.

Problemy evolutsii i ekologii: Gause's r-K formulation of competition and the apparent discord between ecology and evolution

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Gause, working on Paramecium among other microorganisms, promoted a reformulation (essentially, a change of variables) of Volterra's competition equations. This resulted in the classical, easy-to-understand r-K formulae found today in most ecology textbooks. Many problems and paradoxes are associated with the r-K logistic formulations, such as Levins' paradox (see Hutchinson 1978). A number of authors have shown, often in rather obscure publications, that returning to a Verhulst/Volterra formulation abolishes these problems. Is it possible that the Gause r-K equations also catalysed an unnecessary divide between ecology and evolution? I believe so. Darwinian natural selection was in fact the topic that Gause's landmark book, "The Struggle for Existence," was designed to investigate. Ecological competition among genotypes is expected to be isomorphic with natural selection. However, classical, constant natural selection modeled by population geneticists is effectively obscured by the r-K formulation of Lotka-Volterra competition in density-regulated populations. The original Volterra formulation, in contrast, makes more sense of natural selection. The Volterra formulation is also helpful in the understanding more complex forms of evolution, such as speciation with gene flow. See also Christiansen, F. B. 2004. Density-dependent selection. Pp. 139-157 in R. S. Singh, and M. K. Uyenoyama eds. The Evolution of Population Biology. Cambridge University Press, Cambridge.

Speciation is easy: It's all around us!

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I present empirical, morphological, and genetic evidence for a continuum of speciation in nature, in a variety of organisms, including protists. Below the species level, there are abundant ecotypes and ecological races that are nearly what we might want to call species, but which we prefer to regard as "races" for one reason or another. Above the species level, hybridization and genetic introgression is a regular occurrence. Hybridization is, of course, very rare on a per-individual basis, but > = 10 % of all taxonomic species seem to hybridize with at least one other species, and not just with sister species. Furthermore, "legitimate" introgression, as well as "illegitimate" horizontal transfer may both have important consequences for evolution in general, especially low down in the tree of life. "Reproductive isolation" among species declines continuously (albeit noisily) with genetic distance. Theoretical analyses, together with recent historical treatments of 20th Century misunderstandings of Darwin's own idea of species, back up the empirical evidence for this continuum, and provide a coherent understanding of species and speciation for the age of genomics. Empirically speaking, speciation is easy!

Giardia intestinalis and regulation of mitotic progression

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Spindle assembly checkpoint (SAC) has been documented across model eukaryotes and represents a regulatory mechanism that maintains genome integrity by preventing chromosome missegregation. Despite its significance little is known about the origin and evolution of this mechanism. Here, we examined SAC and regulation of the mitotic exit (ME) in a binucleate protozoan flagellate Giardia intestinalis. SAC and ME were examined by using different drugs, e.g. albendazole that inhibits mitotic spindle assembly by preventing microtubule polymerization, cytochalasine D, inhibitor of actin polymerization and reversine, Mps1/aurora kinase inhibitor. We endeavoured to HA-tag genes of interest and subsequently the methods of choice were IFAT and qRT PCR. We have shown that only limited regulation of cell division operates in Giardia and that these parasites commit to cell division even in the absence of mitotic spindle and chromosome segregation. Overexpression of the indispenseable SAC component, Mad2, did not lead to metaphase anaphase block, growth retardation or aneuploidies as have been documented in fission yeast or mice. Similarly, we did not observe any accumulation of cyclin B characteristic for cells arrested by SAC when compared to propagating culture. In contrast, an accumulation of doublets of interconnected cells in late cytokinesis in response to albendazole treatment was noted. Slight accu-

mulation was noted also in Giardia population overexpressing GiBub-2 advancing its role in mitotic exit as described for yeast or mammalian Bub-2. Reversine treatment led to slight nuclear fragmentation and again to an accumulation of doublets of cells but moreover with affected symmetry of distribution of nuclei within the cell which is in accordance with proposed function of these kinases. Altogether, we assume that there is a lack of mitotic control of chromosome segregation possibly resulting in frequent variations in karyotypes in Giardia but processes guarding proper exit from mitosis seems to be preserved.

Genetic structure and biogeography of an abundant, widespread, and uncultured marine protist group, the MAST-4

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Recent culture-independent studies of marine planktonic protists have unveiled a large diversity at all phylogenetic scales and the existence of novel groups that had refused scientific examination. MAST-4 is one of these novel uncultured lineages and is formed by small (2 µm) bacterivorous protists that are widely distributed in marine systems (except polar ones) and account for a significant share of heterotrophic flagellates globally (~10 %). In this study we have used group-specific environmental surveys to go further in the knowledge of MAST-4 and assess two critical questions: the extent and limits of its genetic structure and its biogeographical distribution. Using published SSU rDNA sequences and our own data with a large rDNA operon coverage, we show that the MAST-4 group is composed by 5 main clades, all of them well supported in rDNA gene trees (SSU, LSU and 5.8S rDNA) and in crucial conserved regions of the ITS1 and ITS2 secondary structures. This genetic structure is consistent with each clade being a different species (and perhaps additional species within each clade). In a second step, we combine the origin of the sequences together with an ARISA analysis (based on the ITS1 region length) to investigate in more detail the biogeography of each particular MAST-4 clade. This analysis does not detect biogeographical barriers for the distribution of any of the clades, albeit some seem to predominate in particular habitats, suggesting ecotypic differentiation. We conclude that the MAST-4 group comprises at least 5 different species that have a global distribution and perhaps a functional specialization.

Morphological and genetic comparison of the diatom Asterionellopsis glacialis in relation to biogeography

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Asterionellopsis glacialis (Castracane) Round is a marine, basal non-raphid pennate that is planktonic and cosmopolitan in habitat, often occurring as a member of the surf zone diatom assemblage. Earlier, it was included in the freshwater genus Asterionella but was moved due to differences in chain-formation and ecology. Morphologically the diatom's frustules are relatively simple, providing little diagnostic architecture. 22 monoclones of A. glacialis were isolated or purchased through CCMP, representing a widespread geographical region. Clones originated from the North Atlantic (14), South Atlantic (4), Pacific Ocean (2), North Sea (1) and Black Sea (1). All were studied in terms of morphology and genetics. This was done using light microscopy, the scanning electron microscope and molecular tools, specifically studying the nuclear encoded internal transcribed spacer (ITS) and a fragment of the 18S ribosomal gene, a fragment of the plastidal rubisco large subunit (rbcL), and a fragment of mitochondrial gene, cytochrome c oxidase I (coxI). ITS and coxI were chosen for their well documented sequence variability, while the more conserved rbcL and 18S were used for interspecific comparisons. Our results show a higher than expected sequence divergence between geographically separated clones of A. glacialis, with the greatest divergence recovered using the coxI and ITS markers, up to approximately 13 % and 20 % respectively. Morphological analyses of the clones' valve structure mainly divides A. glacialis into two defined clusters, with some clones demonstrating intermediate character groupings. Morphological segregations agree with some markers, but not all. Significance of this will be discussed. Overtime geographically isolated populations can diverge genetically. These genetic differences may coincide with slight phenotypic variation, as well as reproductive incompatibility. Slow changes in morphology may not always result in cryptic speciation, but can still provide insight into the varied diagnostic characteristics existing within a species across a wide range biogeographically. This study is the first to examine and compare the potential for morphological and genetic differences among geographically isolated clones of A. glacialis.

DNA damage in Entamoeba histolytica after metronidazole treatment: Could it be caused by parasite DNases?

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The protozoan parasite Entamoeba histolytica is the causative agent of amoebiasis and affects millions of people worldwide. Metronidazole is the gold standard drug to treat amoebiasis. In amoebae treated with metronidazole in vitro DNA damage can be observed, but it is not known whether DNA damage is the cause or the consequence of the breakdown of cellular processes in E. histolytica. So it is not known if the DNA is damaged chemically by metronidazole metabolites, such as the nitroradical anion, or enzymatically by DNases from the parasite. For a better understanding of this question, different attempts were made to identify nuclease activities. In the E. histolytica genome database 58 nuclease genes were found, but none coding for classical DNases I or II or apoptotic endonucleases (caspase-dependent DNase, endonuclease G). Incubation of E. histolytica lysate with plasmid pUC19 DNA resulted in endonucleolytic digestion of the plasmid DNA, which was shown by agarose gel electrophoresis. The activity was strongly stimulated by Mg++ ions. The DNase activity could also be measured directly in a photometric assay. The activity found in the lysate was different from the DNase activity present in the culture medium originating from the bovine serum component, as shown by an immunodepletion assay using an antiserum against bovine DNase I. Anion exchange chromatography is currently performed to enrich the amoebic enzyme(s) with the aim of mass spectrometric identification. The ultimate aim of the work is to understand the function of the DNase or DNases in E. histolytica and its or their possible role in the mechanism of metronidazole action. This study was supported by Grant P22037 from the Austrian Science Fund (FWF).

Ciliated protozoa from caves, tank bromeliads and springs from Central Mexico: An ecological approximation

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Approximately 990 species of ciliates have been recorded in Mexico but the species with distinct habitats such as cave water bodies, epiphytic bromeliad tank water bodies, and springs of several regions, have not been widely studied, not even under ecological perspectives including similarity degrees of the communities. We present 61 species recorded in a total of 103 samples coming from the states of Michoacán, Puebla, Querétaro, Veracruz and Estado de México. Three classes were the most represented, 17 species correspond to Class Oligohymenophorea, 15 to Spirotrichea and 12 to Colpodea. We found that 13 species were observed only in spring water (21.3 %), eight species only in caves (13.1 %) and 31 in bromeliad tank water (50.8 %). Four species were present in caves as well as in springs, two were shared between bromeliads and springs, and four between bromeliads and caves. Based in the principal component analysis we obtained three clusters (caves, springs and bromeliad tanks) that explained the 84 % of variance; this is confirmed with the Jaccard Index, which maximum value was 0.6 between the Karmidas Cave (Puebla) and Galicia Cave (Veracruz). This low similarity was confirmed when applied the Cody Index. For this reason, the communities are categorized as peculiar and different between them.

Mexican arthropod-ciliated protozoa system: An overview

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Research of protozoa ciliated epibionts has been mainly focused to crustacean hosts, and their specificity degree has not been considered. We present the record of 22 species of epibiotic ciliates of some arthropods (hexapods and crustaceans) collected at several regions of Mexico, which comprises 13 peritrich species and nine of suctorians. From these findings, and taking into account all the previous worldwide records, six species are considered with a low degree of specificity mainly on the basis of their association with a variety of substrates, and one species to several taxa of animals. Our Mexican data show that five species of ciliates only associate to arthropods, and six are symbionts only of crustaceans. Two suctorian species (Periacineta notonectae and P. mexicana) are only attached to aquatic true bug legs, one species (Periacineta laccophili) to coleopterans, and four species of peritrichids (Cothurnia apseudophila, C. ceramicola, Lagenophrys patina, and L. lenticula) to cer-

tain body parts of crustaceans, which denotates a high degree of host specificity.

Systematic analysis of protein trafficking in the apicomplexan parasite Toxoplasma gondii

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In order to invade and survive within host cell apicomplexan parasites evolved specialised secretory organelles (micronemes and rhoptries) that contain important virulence factors. Although trafficking motifs in proteins transported to these organelles have been identified, the machinery involved in protein sorting is only poorly understood. Rab-GTPases are small G-proteins that play a central role in the compartment specific transport of vesicles from a donor to an acceptor membrane. Interestingly the genome of apicomplexan parasites contains a rather reduced set of Rab-GTPases that does not reflect the cellular complexity of these organisms. Using Toxoplasma gondii as a model system, we generated transgenic parasites with tuneable copies of Rab-GTPases for localisation and overexpression studies. We found that most conserved Rab-GTPases localise to the same compartment as their orthologues in other eukaryotes, indicating that the secretory pathway in apicomplexans is conserved. Conditional overexpression of 5 Rab-GTPases results in tight and specific phenotypes and we identified the Rab-GTPases, Rab5A and Rab5C, as important trafficking factors for the transport to the secretory organelles. Surprisingly, only a subset of micronemal proteins requires functional Rab5A and C for their transport to the apical complex. Therefore we conclude that at least two independent trafficking pathways must be present in these parasites that are required for different subsets of micronemal proteins.

Species taxonomy of protists: Morphological and molecular perspectives in testate amoebae

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Species group as well as high level classification of testate amoeba is still mainly based on characters of the test. Among several practical advantages of such an approach there are also serious drawbacks. The building materials have a significant influence on the overall shape of agglutinate tests. The biometry of the test is a very useful tool to distinguish between closely related species, but even clones can have significantly different size classes if the number of nuclei is different and additionally test size varies with food quality and quantity or the water content of the substrate. The perhaps biggest problems are the species complexes where in many cases we have adaptive peaks in a morphological continuum but no clear species boundaries. The question is whether molecular characters can structure the species complexes and can help to evaluate the usefulness of morphological characters for different levels of classification. We have studied species complexes of Euglyphida and Arcellinida morphologically and the molecular markers SSU, partial LSU, internal transcribed spacers (ITS1) and the cytochrome c oxidase gene (CO1). Sequence variation in Arcellinida is usually higher than in Euglyphida and can be as high as 4 % within morphospecies. ITS1 sequences show a large length variation within genera, paralogous sequences are common. These are features that make a reliable alignment impossible. More promising is the CO1 gene. Single cell barcoding is possible. Sequences have an almost equal length and can be aligned easily. Sequence variation within and between species is high enough to analyse difficult species complexes. Molecular methods help to improve the classification of testate amoeba significantly. Unfortunately, there is no simple correspondence between morphological and molecular characters. The biggest problem is that none of the currently used primer combinations is universally applicable! A low level classification of testate amoeba that is useful for ecologists and paleoecologists needs a link to test morphology. Therefore the question cannot be molecular versus morphological taxonomy but a combination of both.

Signaling pathways involved in the dynamic control of the contractile vacuole complex and regulatory volume decrease in trypanosomatid parasites

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Understanding mechanisms involved in osmoregulation control in protozoan parasites has been a challenge for many research groups. Over the past years, a number of key players in cell signaling in trypanosomatid parasites have been identified. Among these, cyclic AMP (cAMP) has been shown to play a key role in osmoregulation, through a

mechanism that involves a cAMP-dependent pathway that leads to the efflux of osmolytes across the parasite surface, and water elimination through a contractile vacuole complex (CVC). In Trypanosoma cruzi, the CVC is formed by a central vacuole surrounded by a collection of interconnected vesicles and tubules that undergo dynamic changes upon osmotic stress. A unique characteristic of this system is the presence of acidic calcium-rich organelles named acidocalcisomes, whose structural organization, chemical properties and physiological activity may vary upon events of osmotic stress. Biochemical and molecular data have shown that the sequence of events that take place in cells submitted to hyposmotic stress leads to an increase in cAMP levels, stimulating the traffic of an aquaporin from acidocalcisomes to the CVC through a fusion mechanism. This has been revealed by electron tomography of cryofixed cells exposed to hyposmotic treatments. Acidocalcisomes contain basic amino acids and high levels of cations and polyphosphate, a content that once released within the contractile vacuole, leads to an increase in the osmotic pressure towards the lumen of the organelle and stimulates water transport across the CVC membrane. Functional analysis of mutant parasites that overexpress enzymes involved in the control of cAMP levels, such as the T. cruzi phosphodiesterase TcPDE C2, showed alterations in the regulatory volume decrease (RVD), when compared to wild type cells. In addition, mutants that overexpress a Class III PI3 Kinase showed a large and functional CVC and were more efficient in volume recovery when submitted to severe hyposmotic stress. Taken together, our data show dynamic changes in the osmoregulatory system of T. cruzi, governed by signaling events that involve a unique mechanism of interaction of the CVC with acidocalcisomal components. Whether or not this mechanism can be extended to other cell models is currently under investigation.

Phylogenetic systematics of the genus Condylostoma (Ciliophora, Heterotrichea) by means of a multidisciplinary analytical study of some marine and brackish morphospecies

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The genus Condylostoma Bory de St. Vincent, 1824 is a well-known taxon of the class Heterotrichea consisting of medium- to large-sized organisms commonly found in various habitats. At present it comprises more than 20 species (Appeltans W, Bouchet P, Boxshall GA, Fauchald K, Gordon DP, Hoeksema BW, Poore GCB, van Soest RWM, Stöhr S, Walter TC, Costello MJ. (eds), 2010, World Register of Marine Species; accessed at www.marinespecies.org on 2011-02-15), but more than half of them are just superficially outlined, i.e. not described with modern morphological methods of investigation (protargol staining, electron microscopy). Species identification is sometimes very difficult because of: 1. unclearness/incompleteness of literature descriptions; 2. lack of easily recognizable diagnostic characters; 3. variability of cell shape (high to impressive tendency to contract); 4. frequent overlapping of diagnostic characters (cell shape and size, infraciliature, and cortical features). Recently, molecular analyses were performed on a few representatives of the genus, namely Condylostoma minutum, Condylostoma spatiosum, Condylostoma curva (by Guo et al. 2008), and Condylostoma sp. strain POE2.2 (by Modeo et al. 2006), which were all characterized based on 18S rRNA gene sequencing; this allowed a preliminary phylogenetic systematics of the genus. In the present research, four marine morphospecies of Condylostoma coming from the Mediterranean Sea and the Atlantic Ocean were isolated, clonally cultivated, and characterized by means of a multidisciplinary analytical study (through optical investigation on in vivo and fixed/stained cells, SEM and TEM, and 18S rRNA gene sequencing). The 18S rRNA gene sequence of a single brackish morphospecies coming from the mouth of the river Serchio (Pisa, Italy) was additionally provided to improve the molecular analysis. New interesting insights into the intrageneric phylogenetic relationships were gained, which also allowed some better understanding of intergeneric relationships within the class Heterotrichea.

Benthic heterotrophic flagellates of the River Rhine: Community structure and seasonal dynamics

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The 'microbial loop' concept was first described from pelagic marine systems, little is known about freshwater benthic microbial communities. Very little is known about betnic heterotrophic flagellate communities of rivers. We studied the structure of the benthic community of heterotrophic flagellates and its seasonal changes in the River Rhine at Co-

logne. Abundance and biomass of heterotrophic flagellates were determined in the upper 3 mm sediment layer and were significantly dependent on water temperature. The abundance of heterotrophic flagellates was lowest at temperatures below 5 °C (5 ind/mm³ sediment). Highest values (20 ind/mm³ sediment) were reported during summer. Though there was no clear seasonal pattern. The community structure of benthic flagellates was surprisingly similar for the whole sampling period. Dominant were kinetoplastids, euglenids and chrysomonads. Thaumatomonads, apusomonads and cercomonads regularly appeared in the samples. Representatives of Protista incertae sedis occurred in moderate numbers, several species were obviously not yet described. The first results indicate that heterotrophic nanoflagellates form a very abundant and productive component of the sediment surface layer in a large river. This has been overlooked in the past and gives new ideas regarding the importance of the microbial food web in river sediments as contributors to the self-purification process of large rivers.

Inferring phylogeny of Amoebozoa from analysis of hsp90 and SSU rDNA concatenated dataset

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Molecular systematics of Amoebozoa has been based so far on SSU rDNA analysis. Other phylogenetic markers are required to verify the reconstructions inferred from SSU rDNA trees. The hsp90 gene is considered to be a good phylogenetic marker as it is relatively long and it has rather uniform rates of evolution in different lineages. To investigate utility of hsp90 for inferring amoebozoan phylogeny we have sequenced this gene from ten amoebozoan species. We found that specific features of hsp90 gene in the target group include: (1) high frequency of gene duplication resulting in appearance of divergent paralogs observed in some species and (2) presence of introns in the representatives of Variosea. Despite these features the hsp90 gene appears to be an informative marker for reconstructing deep- and low-level phylogeny. The trees inferred from analyses of single-gene and concatenated (hsp90 and SSU rDNA) alignments with a high number of taxa from all major eukaryotic lineages showed virtually the same groupings as the recently published multigene trees do. Amoebozoa (excluding breviates) always form a robust monophyletic clade, albeit only Bayesian analysis provides a high support for this clade. In hsp90 trees Amoebozoa and opisthokonts are consistently recovered as the neighboring members of a single superclade (however the support is low to moderate). In the reconstructions inferred from the analyses of concatenated dataset Amoebozoa and opisthokonts are neighbors in ML trees, but not in Bayesian ones. Reconstruction of internal amoebozoan phylogeny based on hsp90 analysis is broadly consistent with the conclusions drawn from SSU rRNA analysis. In the concatenated trees all representatives of Conosa group together, and previously recognized lower-level conosan clades are recovered as the robust highly-supported monophyletic ones (Mycetozoa, Archamoebea, Variosea). Two groups of Discosea (Vannellida and recently established Longamoebia, represented in our trees by Acanthamoeba castellanii and Thecamoeba aesculea) are robust clades recovered with high support, but they do not branch as the sisters in contradiction with SSU rDNA trees. Our data support removal of acanthamoebids from Variosea. Taxon sampling for Tubulinea remains insufficient. Obtained results represent a basis for further hsp90 phylogenetic analysis of Amoebozoa. Supported with RFBR 09-04-01749 grant and research grant from St. Petersburg State University.

Molecular phylogeny of Bertramia asperospora, a protozoan rotifer parasite with obscure taxonomic position: Shuffling cards in favor of the ichthyosporeans

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The protozoan parasites characterized by plasmodial forms developing into the endospore-containing cysts were discovered and described in the rotifers at the end of XIX century. Since then the taxonomic affinity of these organisms has been obscure and remained the matter of discussion. Some authors placed them with microsporidia, while others grouped them either with haplosporidians or with fungi. To reply the question, which puzzled the researchers for over a century, we performed molecular phylogenetic analysis and TEM examination of one of the most abundant and widely distributed representatives of this group – Bertramia asperospora (originally described by Fritsch in 1895 as the microsporidia Glugea asperospora). The specimens were found in the body cavity of the rotifers Brachionus calyciflorus isolated from the Volga Delta. In SSU rDNA trees the parasites

branch together with the members of Ichthyosporea Cavalier-Smith 1998 [Mesomycetozoea Mendoza, Taylor et Ajello 2002] – a unicellular opisthokont lineage near the animal-fungi divergence. The mitochondria with flat cristae, archetypal of the opisthokonts, were observed in ultrathin sections of the parasite cells. No structures that could be interpreted as the polar tube of microsporidia were found. Structure of the cysts and general organization of the cells resemble that of ichthyosporean species. These findings justified the conclusions drawn from the phylogenetic analysis. Supported by RFBR No 10-04-00943.

A new metchnikovellid microsporidium: A hyperparasite of gregarines from the polychaete Pygospio elegans

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Class Rudimicrosporea Sprague 1977, a monotypic taxon with a single family Metchnikovellidae comprised of hyperparasitic microsporidia, has been always treated as a plesiomorphic group basing on fine morphology of spores. Recently a basal position of metchnikovellids within Microsporidia was confirmed by SSU rDNA-based phylogenetic analysis (Simdyanov et al. 2009, Proc. ICOP XIII). Metchnikovellids remain poorly studied due to arduous sampling and lack of economic importance, in spite of significance of this ancestral group for comprehension of evolutionary history of microsporidia. Since mid 1980-ies several metchikovellid species parasitizing aseptate gregarines have been recorded from polychaetes of the White Sea silt littoral zone (Rotari, Paskerova 2007, Proc. ECOP V). We performed TEM examination of one of these species parasitizing in a Lecudina-like gregarina from the gut of the polychaete Pygospio elegans. This microsporidium, a potentially new species, exhibits two sporogonies: sac-bound and free. The sac-bound sporogony results in formation of ellipsoid spore sacs (on average 15 x 7 μm) enclosing about 8 spores. Each spore presumably contains a pair of nuclei. Thick walls of spore sacs are reinforced with characteristic external spiral structures. The second sporogony results in formation of free spores. At the earlier steps of this developmental sequence the products of binary fission of meronts become sporont mother cells, which transform after a series of nuclear divisions into multinuclear sporogonial plasmodia. Its plasmotomy gives rise to numerous (> 20) sporonts, which convert into sporoblasts without further division. Size of free spores is about 1.5 x 2.0 μm. Internal structure of the spores is typical for metchnikovellids. Groups of spores are surrounded by an electron lucid zone matching the contours of the mother cell; however we were unable to distinguished interfacial envelopes. Gross and fine morphology of the novel species suggests its similarity to Metchnikovella spp., though more ultrastructural data are needed for its definite assignment to an already existing or new genus. Supported by RFBR No 11-04-02041.

Genetic differentiation and population structure in desmids (Desmidiales, Zygnematophyceae, Streptophyta)

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The existence of population structure in protists is a hotly debated topic. Protists often form huge populations, and because of their small body size, they are assumed to have high dispersal potential. These two factors led to the „everything is everywhere" hypothesis, which posits that protists have unlimited geographical ranges and therefore lack population structure (Finlay 2002). On the other hand, Weisse (2008) proposed that protists differentiation is based on the microhabitats. Most population studies have focused on heterotrophic protists, while studies on phototrophic protists are limited. These studies, which are based on molecular markers, generally show quite strong population differentiation linked by geographic distance. Desmids could comprise a protists group with differentiated populations as they embody the properties like sexual reproduction, diplontic life cycle and biogeographic pattern. They are a large group of unicellular green algae that occur in mesotrophic and oligotrophic freshwater habitats like peat-bogs. The species Micrasterias rotata and M. thomasiana are well defined morphologically as well as by chloroplast intron sequences of RNA-Gly transfer gene (trnGucc). We have investigated 106 strains of M. rotata and 151 strains of M. thomasiana, obtained from SVCK culture collection and isolated from natural habitats across Europe, mainly from Czech Republic. All strains of M. rotata were identical in trnGucc. Similarly, no sequence divergence was detected in M. thomasiana, except the one strain, diverged by single nucleotide site. How this variability will affect population structure will be seen in more variable nuclear marker. We get cDNA of four nuclear low-copy markers and according to

them we have designed specific primers for these genes. In the actin sequences we have amplified two exons and one intron regions. M. rotata strains were highly variable not only in intron sequences, but also in exon regions. The structure of M. rotata and M. thomasiana populations based on the low-copy nuclear markers (actin, gapC, gapA) will be presented. These preliminary results indicate that the unexpected variability among the populations of broadly distributed species M. rotata and M. thomasiana could be based on ecological parameters of the microhabitats rather than linked to the geographic distance.

Biodiversity and abundance of Phytomyxea (plasmodiophorids) at the Rotmoosferner glacier forefront

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Until now, there are no studies dealing with soil-borne plant pathogens in an alpine ecosystem in general and with phytomyxean plant parasites in particular. Phytomyxea (plasmodiophorids) are a group of eukaryotic micro-organisms belonging to the Rhizaria. Phytomyxea are soil-borne plant-associated organisms with a multi-stage life cycle that is characterised by multiple zoosporic, plasmodial, and resting stages. These organisms cannot be cultured without their hosts, and direct observations of any stage of the plasmodiophorid life cycle are difficult and time-consuming. Plasmodiophorids are obligate biotrophic soil-borne parasites of higher plants, and therefore their distribution is expected to follow that of their hosts. We conducted a first survey of the biodiversity of plasmodiophorids along a gradient of plant succession to answer the following questions. (i) Which plasmodiophorid species are parasitizing which host plants, and (ii) at which stage of plant succession can the first plasmodiophrids be found. At the Rotmoosferner glacier forefront (Austria, Ötztal) we found the plasmodiophorid Ligniera junci to be abundant in Juncus triglumis growing at englacial streams. Using a combination of specific primers and morphological methods, we could identify L. junci associated with Jucus trigulumis along the whole gradient of plant succession, but could not find it in the roots of Jucus jaquinii. Polymyxa graminis, a parasite of Poaceae, was found in the roots of Poa spp. growing in well established soils (> 100 years). With specific primers the parasite could be detected in the roots of Poa alpina (an important pioneer plant) along the succession gradient. In clone libraries from water samples, the clubroot pathogen Plasmodiophora brassicae and Woronina spp., a phytomyxean parasite of oomycetes were detected. These results could not be confirmed morphologically to date, but a forthcoming, more directed sampling should answer these questions. Plant pathogens play a decisive role in the establishment and maintenance of host populations: they generate selective forces influencing frequency and density, but can also affect community diversity and therefore, can influence the community development. Therefore their high abundance and diversity in precious environments raises more questions about this enigmatic group of parasites.

Phylogeny and biodiversity of phytomyxea („plasmodiophorids")

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Phytomyxea (Rhizaria, Endomyxa; common name plasmodiophorids) are an enigmatic group of obligate, endobiotic parasites of higher plants, diatoms, brown algae and oomycetes. The taxonomic position was long debated but recent molecular taxonomic works robustly place them within the eukaryote supergroup Rhizaria as sister group to vampyrellid amoebae. Here we present the most comprehensive phytomyxean phylogeny to date. Currently the group is subdivided into two orders: Phagomyxida – which are parasites of diatoms and brown algae – and Plasmodiophorida, parasites of green plants. An 18S rDNA phylogeny reveals several, well supported clades within Phytomyxea. A "Polymyxa clade" was supported by high Bayesian posterior probabilities. This clade comprises Polymyxa graminis, P. betae (both important vectors of plant viruses), Ligniera junci, Sorosphaera veronicae, and S. viticola. A "Plasmodiophora clade" comprises the clubroot pathogen Plasmodiophora brassicae, Spongospora subterranea (casual agent of powdery scab of potatoes), S. narsturtii (parasite of watercress), and the oomycete parasite Woronina phytii. The position of the oomycete parasitic Woronina suggests that phytomyxid parasitism of stramenopiles developed at two different, independent evolutionary events. Phagomyxida (parasites of brown algae and diatoms) splitted from the plant parasitic Plasmodiophorida, and Woronina which is parasitic in oomycetes is a sister genus to Spongospora and Plasmodiophora and robustly

clusters within the Plasmodiophorida and not with the Phagomyxida. We also reveal a diverse set of well supported clades which very likely represent undescribed species detected in clone libraries from soil samples (vineyard soils, wet meadows, glacier forelands, flood plains), indicating that phytomyxids are ubiquitious in suitable environments worldwide. Although our results indicate a higher biodiversity and ubiquitous distribution, the number of species within each sample was small and strongly influenced by the plants growing at the point of sampling. More concerted and targeted samplings will be necessary in the future to estimate the "real" biodiversity, abundance and consequently the ecological role of these parasites.

Is there a phylogenetic separation of marine and freshwater choanoflagellates?

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Choanoflagellates are supposed to be a sister group to metazoans. Nevertheless not even the phylogeny within this group is well resolved except for the loricated acanthoecids. We examined the rDNA of several freshwater and marine choanoflagellate species regarding the hypothesis whether there is a phylogenetic separation between the two habitats or not. In addition we present for the first time data on one of the earliest described choanoflagellates, Codosiga botrytis and its unexpected phylogenetic position.

The professional phagocyte Dictyostelium discoideum

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The amoeba Dictyostelium discoideum is a useful model for the study of complex biological processes like signal transduction, cytokinesis, chemotaxis, phagocytosis and the medically relevant infection by bacterial pathogens. We are particularly interested in the role of the actin cytoskeleton and in phagocytosis. Phagocytosis is a very complex, evolutionarily highly conserved mechanism that is used by higher eukaryotes to counter the constant threat posed by pathogens. For lower eukaryotes like D. discoideum phagocytosis is a means to ingest bacteria that are then degraded via the phagolysosomal pathway and used as food source. More recently it was found that free living amoebae are probably a reservoir in which bacteria like Legionella survive in the wild. Importantly, the manipulation of host cell processes by Legionella in order to establish a replicative niche, as well as host defense processes to fight infection are similar in macrophages and Dictyostelium. This makes the professional phagocyte D. discoideum an excellent model system for the investigation of bacterial virulence traits and the analysis of the roles of host gene products in phagocytosis and killing. Here I am focusing on RpkA, a 7 transmembrane protein with a GPCR signature and a C-terminal lipid kinase domain predicted as a phosphatidylinositol-4-phosphate 5-kinase. RpkA localises to endosomal membranes and its loss leads to a phagocytosis defect, depletion of phosphoinositides, and affects survival of L. pneumophila making it an important component of the innate defense system of D. discoideum.

Mikro-Dialoge

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The Mikro-Dialoge is a small comic published in the German magazine Mikrokosmos. Started in January 2010, this one page cartoon tells the story of Max, a brilliant protistologist who accidentally discovered that the microcosm is full of chatter. To his amazement, Max experiments the fiery temper of amoebas, how fashion victims euglenas are, the incredible talent of paramecia as ophthalmologists or the neurasthenia of hypotrichs. Encouraged by his lab director, Prof. Osman, Max decides to use his invention to write as much scientific papers as he can rather than sharing it with the world. But his discoveries might be too crazy to be told about.

Roles of symbiotic flagellated protists in the gut of termites in efficient utilization of cellulose

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The relationship between termites and microbial community in their gut is a well-known example of symbiosis, which aids efficient utilization of recalcitrant woody cellulose. The gut community comprises cellulolytic flagellated protists belonging to Parabasalia or Oxymonadida and diverse bacteria. A prominent feature of the gut community is the associations of bacteria with the cells of gut protists as their endo- or ecto-symbionts. The cells of a

large hypermastigid species (Parabasalia) harbor a dense population of endosymbiotic bacteria that represent a major constituent in the gut and have co-speciated with the host protists. The complete genome sequences of two species of these endosymbiotic bacteria were determined without cultivation and disclosed their hitherto unknown roles in supplying essential nitrogenous nutrients poor in the ingested cellulose. The functions and metabolic characters of the gut protists were investigated through their meta-transcriptomic EST (Expressed Sequence Tags) analyses, and we identified diverse genes encoding glycosyl hydrolases obviously responsible for the decomposition of woody cellulose. Their primary energy-generating pathway was also reconstructed, and in Parabasalia whose cells carry anaerobic energy-generating organelles, hydrogenosomes, a differentiation of cytoplasmic primary pathway was detected depending on species. The efficient cellulose fermentation in the cells of a large hypermastigid can be explained by the transfer of more reducing equivalents from cytoplasm to hydrogenosomes where hydrogenases effectively eliminate them as molecular hydrogen to gain more energy. Independent lateral gene transfers of a key enzyme form bacteria have probably caused this metabolic differentiation. These complementary genome approaches gradually unveil the roles of the complex microbial community for efficient utilization of cellulose in the gut of termites.

Updating the microthoracids (Ciliophora, Microthoracida): Four new Leptopharynx species

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We studied the morphology of four new Leptopharynx species, using standard methods. Species 1, which was discovered in tank bromeliads from Jamaica, is minute (~25 × 20 µm) and has a slightly concave preoral region, and a total average of 142 basal bodies. Species 2, which was discovered in jungle soil from Australia, is large (~40 × 25 µm) and has a distinctly oblique preoral region, widely spaced kinetids in kinety 1, a total average of 184 basal bodies, and the oral primordium is inside of a cortical fold thus appearing right of posterior end of kinety 1. Species 3, which was discovered in floodplain soil from the Mato Grosso, Brazil, is large (~60 × 40 µm) and has a distinctly oblique preoral region, widely spaced kinetids in kinety 1, six kinetids in kinety 6, and a total average of 294 basal bodies. Species 4, which was discovered in a soil sample from Florida, USA, is large (~55 × 35 µm) has widely spaced kinetids in kinety 1, a slightly oblique preoral region, and a total average of 256 basal bodies. Species 1 and 4 possibly produce only small-mouthed cells, while species 2 and 3 possibly produce only large-mouthed cells. Eleven features are recognized for distinguishing Leptopharynx species: (1) distinct ridges present vs. absent along the right side ciliary rows; (2) special features, like spines or wings on the body, and of the oral basket; (3) dikinetids present vs. absent from somatic kinety 3; (4) number of kinetids in kinety 6; (5) beginning and structure of kinety 9 as either underneath or far underneath the adoral membranelles and with or without dikinetids; (6) postoral complex present vs. absent; (7) preoral kinety 4 continuous vs. discontinuous; (8) spacing of the kinetids in kinety 1 as either ordinary or wide; (9) shape (flat or concave) and angle (≤ 15° slightly oblique, ≥ 40° distinctly oblique) of the preoral region; (10) total number of basal bodies; and (11) to have a monomorphic (producing either small- or large-mouthed cells) or polymorphic (producing microstomes and macrostomes) life cycle. (Supported by the FWF project P20360-B17.)

Ciliated protist assemblage in two shallow saline-alkaline lakes in Kenya

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Saline-alkaline lakes generally have a limited species inventory and are amongst the most productive ecosystems of the world. We studied the ciliated protist assemblages of two shallow saline-alkaline Rift Valley lakes (Bogoria and Nakuru) in Kenya. The ciliate community composition was similar in both lakes and was represented by fifteen ciliate genera. In both lakes, Cyclidium and Frontonia species dominated numerically. The abundance of bacterivorous ciliates was independent of bacterial abundance, most likely because of the very high and generally satiating bacterial food concentrations in the two lakes. Frontonia abundance was positively related to algal biomass, which mainly comprised of the cyanoprokaryote Arthrospira fusiformis. In terms of biomass, Condylostoma magnum and Frontonia were the most important taxa in L. Bogoria, while Frontonia and Holophrya were the most important taxa in Lake Nakuru. Keywords: Saline-alkaline, Ciliates, Tropical lakes.

Intra-specific variation of Frontonia species in two shallow saline-alkaline lakes in Kenya

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Saline-alkaline lakes in general, have a limited species inventory but are amongst the most productive ecosystems of the world. We studied the ciliated protist assemblages of two shallow saline-alkaline Rift Valley lakes (Bogoria and Nakuru) in Kenya, with a special emphasis on intra-specific variation of Frontonia. Morphometric measurements and molecular analysis of the 18S SSU rRNA revealed that Frontonia morphospecies from both lakes were represented by the same SSU rRNA genotypes. There was size difference between the two populations; probably caused by different environmental conditions like conductivity that was roughly twice as high in Lake Bogoria as in Lake Nakuru with the higher osmotic pressure expected in Lake Bogoria resulting in smaller ciliate cells recorded in the lake. However, the Frontonia species in the lakes differed from other Frontonia taxa in the phylogenetic tree except for Frontonia didieri, and clustered with Apofrontonia and Paramecium. Keywords: Salinity, Morphospecies, Molecular analysis.

The evolution, diversity and biogeography of anaerobic Heterolobosea (Excavata)

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Heterolobosea is a species-poor, but ecologically extremely diverse group of protists. Some heterolobosean flourish in hypersaline or extremely acidic habitats, while some others live in high temperatures. Another few heterolobosean are obligate anaerobes/microaerophiles. Seven anaerobic heterolobosean have been described so far. Four of them constitute the main anaerobic heterolobosean clade, Psalteriomonadidae, while the others are presumably closely related to aerobic taxa. To study the diversity of anaerobic Heterolobosea, we have isolated and cultivated 50 strains of free-living heterolobosean flagellates, amoebae and amoeboflagellates from freshwater, brackish and marine sediments around the world. The phylogenetic analysis of the SSU rRNA gene showed that all but one strains belong to the Psalteriomonadidae. We identify three new genera and four new species. We showed that the anaerobic species Percolomonas descissus is not related to the aerobic P. cosmopolitus, but belongs to Psalteriomonadidae as well. The real diversity of Psalteriomonadidae is thus at least twice as wide as previously assumed. At least one lineage of P. descissus is restricted to the "circumequatorial belt" throughout the world. The remaining isolate of anaerobic Heterolobosea, PC4AM, is rather interesting. Its cell structure is unique and it is impossible to classify the organism PC4AM into any eukaryotic group solely on the basis of morphological features. Its life cycle is also bizarre and consists of more forms, the most prominent being a 1- or 2-flagellated amoeboflagellate and an enigmatic polyflagellate stage. Phylogenetic analysis of three genes showed that PC4AM is a heterolobosean, possibly closely related to Stephanopogon, another enigmatic polyflagellate. It also suggested that PC4AM represents a novel anaerobic lineage of Heterolobosea.

Investigation of the euglenoid ribosomal DNA operon

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In most eukaryotes, the ribosomal RNA genes of the small subunit (18S or SSU) and large subunit (5.8S plus 28S or LSU) are present as tandemly repeated linear cistrons in multiple copies located in the chromosomes. The coding regions for the SSU and LSU rRNA are divided by two internal transcribed spacers, ITS 1 parting 18S from 5.8S and ITS2 separating 5.8S from 28S rDNA. The unusual ribosomal operon of Euglena gracilis (Euglenida) is organized as an 11,056 bp circle with 800 – 4,000 extrachromosomal copies comprising the most highly fragmented LSU rDNA known. An intergenic spacer (IGS) region, dividing SSU from LSU rDNA, has been reported and a read-around transcription hypothesized (Greenwood et al. 2001). Although intensive molecular studies on SSU rRNA genes largely contributed to the understanding of euglenozoan evolution, sister taxa relationships within the Euglenozoa remain inconsistent (Triemer & Farmer 2007) and available data on the phagotrophic euglenids scarse. Like the derived phototrophic Euglena gracilis, phagotrophic euglenids possess a highly fragmented LSU rRNA gene intermitted by additional ITSs. A closer examination of the rDNA operon of taxa as Entosiphon, Petalomonas and Ploeotia could lead to an explanation of these exceptional observed characteristics and

shed new light on the phylogeny of the primordial phagotrophic euglenids.

On the way to gaining plastids in the marine dinoflagellate genus Dinophysis?

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The marine dinoflagellate genus Dinophysis is ideal for investigating plastid evolution, as it includes both phototrophic and heterotrophic species. To survive, the photosynthetic Dinophysis spp. must feed on the plastidic ciliate Mesodinium rubrum (= Myrionecta rubra), itself a consumer of cryptophytes. While ultrastructural and molecular studies and pigment analyses all demonstrate that photosynthetic Dinophysis species contain plastids of cryptophyte origin, whether the plastids are permanent or periodically derived kleptoplastids (stolen plastids) has not been confirmed yet. There has been an obvious contradiction between molecular sequence data and ultrastructural data for the status of the plastid. Phylogenetic studies comparing plastid gene sequences of the three organisms (i.e. Dinophysis, its ciliate prey M. rubrum, and the prey's prey cryptophyte Teleaulax) support the kleptoplastid hypothesis, mostly based on the result of genetically identical plastid gene sequences among them. However, TEM studies support permanent plastid hypothesis, based on the ultrastructure (e.g. plastid surrounded by 2 membranes, terminal position of the pyrenoid, stellate compound structure, and the lack of nucleomorph) different from those in prey. We addressed this issue here using the established D. caudata culture as a model organism, single-cell TEM technique, and time-lapse video microscopy and the results will be discussed in the context of the status of Dinophysis plastids.

Global distribution of deep-sea protists: Insights from Next Generation Sequencing

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Abyssal plains are one of the most diverse and extensive habitats on Earth. Many abyssal taxa are extremely speciose, but their distribution is patchy and their abundance is usually not very high. Some deep-sea species were shown to have very large geographic ranges. However, existing molecular biogeographic data on abyssal protists are sparse. The development of next-generation sequencing (NGS) technologies has opened new avenues for exploring protists diversity in time and space. These techniques seem particularly useful to estimate the diversity in deep-sea benthic environment, which exploration suffers from undersampling, difficult access, and problems involved in culturing of deep-sea organisms. We examined protists richness in the abyss using 454 pyrosequencing to sequence eukaryotic V9 domain of the SSU rDNA and Illumina system to sequence a 37F hypervariable region of the same gene specifically in foraminifera. Our results show that the proportion of the same phylotypes present in different geographic regions is relatively low. In the case of eukaryotes, most of widespread phylotypes were assigned to common planktonic species, which DNA was deposited at the ocean floor. In the case of foraminifera, the majority of "ubiquitous species" were classified among undetermined monothalamous lineages. Our work confirms the usefulness of NGS technologies to studying deep-sea protists diversity, but also pinpoints several pitfalls that have to be overcome to ameliorate interpretation of obtained data.

The impact of creationism and Intelligent Design (ID) in the public's perception of evolution: Strategies adapted to regional, national or international idiosyncrasies

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The antievolution movements are in the rise and not limited to North America. In 2009 The Technische Universität of Dortmund, Germany, hosted the AKESE conference "Attitude and Knowledge concerning Evolution and Science in Europe". Creationism and Intelligent Design (ID) split the public's support to evolution and nourish the controversy between scientific knowledge and popular belief. The United States ranks 33rd in a list of 34 other countries where acceptance of evolution has been polled, contrasting with countries heading the list, i.e. Iceland, Denmark, Sweden, France, Japan and the United Kingdom, in which ≈75 – 85 % of adults accept evolution. Three interacting variables determine an individual's acceptance of evolution: understanding the essence of science (= method to explore reality), familiarity with the processes and forces of change in organisms (= evolution), and personal religious convictions. Recent studies have

confirmed the statistical association of these variables with the understanding of science/evolution (positive association of variables) and religious/political ideology (negative variable association). The involvement of highly educated university professors, as well as specialized scientific societies (e.g. ECOP, ISOP) in the pro-evolution movement is indispensable to increase acceptance of naturalistic rationalism and decrease the negative impact of creationism and ID on "society's evolution literacy."

Identification of the meiotic life cycle stage of Trypanosoma brucei in the tsetse fly

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Elucidating the mechanism of genetic exchange is fundamental for understanding how genes for traits such as virulence, disease phenotype or drug resistance are transferred between pathogen strains. Genetic exchange occurs in the parasitic protists Trypanosoma brucei, T. cruzi and Leishmania major, but the cellular mechanisms are not precisely known as the process has not been directly observed. Here, we have exploited the identification of homologues of meiotic genes in the T. brucei genome and demonstrate that three functionally-distinct, meiosis-specific proteins are expressed in the nucleus of a single specific cell type, defining a new developmental stage occurring within the tsetse fly salivary gland. Expression occurs in clonal and mixed infections indicating that the meiotic programme is an intrinsic but hitherto cryptic part of the developmental cycle of trypanosomes. In experimental crosses, expression of meiosis-specific proteins usually occurred before cell fusion. This is the first evidence of a conventional meiotic division in an excavate protist and the functional conservation of the meiotic machinery in these divergent organisms underlines the ubiquity and basal evolution of meiosis in eukaryotes.

Bacterivory and feeding behaviour of protists from wastewater treatment plants (WWTP)

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The bacterivorous feeding behaviour of three ciliates (Paramecium aurelia, Euplotes eurystomus and Uronema nigricans) and two heterotrophic flagellates (Spumella sp. and Bodo saltans) were evaluated under experimental culture conditions. Specimens were isolated from WWTP with advanced treatment for nutrient removal and the main goal was to investigate both specific growth and clearance rates of these protists isolated or in mixed cultures on nitrificant and enteric bacteria typical from wastewaters. The final objective was to assess the possible effects of this microbial activity on the biological community. Results showed that clearance rate was not a reliable growing parameter, therefore in order to explain further trophic interactions it was necessary to develop long term experiments. These indicated that although bacterial capture could be demonstrated even with high rates, this fact did not always mean a net protist growth. It could be concluded that some bacterivorous protists captured certain types of bacteria but were not able to use them effectively since these were not digested or nutrient content was not appropriate to sustain growth. Initially experimental data were obtained with two enterobacterial strains Enterobacter aerogenes and Escherichia coli. Growth efficiency of Uronema nigricans in mixed culture with flagellates and bacteria was higher than in monoxenic culture, showing flagellates a competitive behaviour in those conditions. Euplotes euryostomus did not grow just on bacteria but it did on nanoflagellates, as shown in the efficient net yield on mixed cultures. Finally, Paramecium aurelia exhibited higher maximal growth and clearance rates in mixed culture experiments, displaying a positive mechanism of coexistence with the heterotrophic nanoflagellates. Flagellates studied did sustain a direct or indirect trophic relationship with the ciliates studied. Two nitrificant bacterial strains were also tested: Nitrosomonas europaea and Nitrobacter winogradskyi. N. europaea was not captured or captured with a low efficiency but even then no protists growth was observed. However, N. winogradskyi was consumed and processed successfully as growth of flagellates and ciliates was observed, except in the case of E. euryostomus. Acknowledgements: This research was financed by the Spanish Ministerio de Ciencia e Innovación project CGL2008-02310.

Using reverse taxonomy approach to identify freshwater foraminifera

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Recent environmental DNA (eDNA) surveys revealed a large hidden diversity of eukaryotic microorganisms. Foraminifera make no exception. The sequences of these well-known marine protists were found in some unexpected places such as freshwater sediment and soil samples. Currently, the only naked freshwater foraminifer described is Reticulomyxa filosa. However, the phylogenetic analyses of environmental DNA sequences indicate the presence of a highly diverse assemblage of freshwater and soil foraminifera. The aim of our project is to determine the diversity and the importance of foraminifera in freshwater environments. To investigate deeper the ecology of this group we amplified partial SSU rDNA sequences of foraminifera from various freshwater ecosystems of Geneva basin. We also plan to amplify and sequence the entire SSU rDNA to improve the phylogenetic position of these novel organisms among foraminifera. Given that the species represented by eDNA sequences are not yet described, we use a reverse taxonomy approach to isolate them and determine their morphology. Based on rDNA sequences, we constructed labelled probes to perform fluorescence in situ hybridization (FISH) to detect specifically foraminifera in environmental samples. We also consider using scanning electron microscopy (SEM) to identify these organisms if they are too tiny for accurate optical microscopy observation. The progress of this study will be reported in our presentation.

Trojan Ciliates: Selected examples of potentially pathogenic bacterial endocytobionts of ciliates

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In the last years, Protists (Protozoa) gained significant attention from the scientific community because of their role as Trojan horses with respect to opportunistic pathogens of humans and animals. Although this is nowadays well established for some Protozoa like amoebas, little is known for other groups of possible hosts. Recently, our group showed that ciliates e.g. Paramecium, Euplotes, Spirostomum, can harbour bacteria with a strong phylogenetic affiliation to human and animal pathogens like Rickettsia and Midichloria (Rickettsiales). More recently, we described a Candidatus new subspecies of Francisella noatunensis, a fish pathogen responsible for massive mortality events in aquaculture plants. These reports raise the question whether ciliates may also represent natural reservoirs for potentially pathogenic bacteria. This point will be specifically addressed by the project: "Ciliates as natural reservoir of potentially pathogenic bacteria: an ecological, functional and evolutionary genomic investigation" financed by the FP7-PEOPLE-IRSES program which involves nine units from eight countries. In the present communication I will show a set of yet unpublished data on novel Rickettsiales and Francisellaceae harboured by ciliate hosts. The implication of these novel data on the evolution of host specificity and horizontal transmission pathways will be discussed. Although intracellular bacteria of ciliates are generally facultative for their hosts and not cultivable on axenic media, we succeeded in stabilizing many of these symbiotic associations. This is the prerequisite for future studies on cross infectivity/host exchange, comparative bacterial genomics and for functional studies on the interaction between hosts and their symbionts.

Molecular characterization of kleptoplastidy in Foraminifera

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Kleptoplastidy is the ability of heterotrophic organisms to preserve chloroplasts of algae they eat and partially digest. As the sequestered chloroplasts can stay functional for months, the "host" becomes photosynthetically active. Although remaining marginal, this process was observed in different protist lineages, including foraminifera. Previous and present studies show that two foraminiferal genera, Haynesina and Elphidium, are particularly apt to perform kleptoplastidy as 12 species of these genera have been shown to bear kleptochloroplasts. Characterizing kleptoplastidy is of the major importance for understanding endosymbiosis and the emergence of photosynthesis among eukaryotes. Most of the hypotheses assume that the acquisition of chloroplasts by the host cell was carried through a predator-prey relationship between a heterotrophic eukaryotic cell feeding on algae. Kleptoplastidy could be assimilated to the first step of this process and therefore it is so important to understand its origin and specificity. Furthermore, the

molecular regulation and maintenance of the acquired chloroplasts is one of the major issues that underlie the understanding of endosymbiosis among eukaryotes. We can speculate that the ancestor of elphidiids and Haynesina probably feed on diatoms and that possibly some of diatoms genes have been transferred to foraminiferal nucleus. This has enabled the functioning of diatom chloroplasts inside foraminiferal cells and gave origin to kleptoplastidy in this group. The objective of our study is to identify these genes in Elphidium ESTs. Preliminary analysis of 17,125 ESTs obtained from Elphidium sp. demonstrated the presence of many genes possibly involved in photosynthesis. Detailed analysis of these genes is in progress.

High diversification of intracellular calcium-release channels in Paramecium cells

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Early on in evolution, Ca^{2+} has become the most important second messenger in eukaryotic cells. In response to widely different stimuli, Ca^{2+} concentration can increase locally, for activating widely different functions, such as secretion (exocytosis), locomotion, gene transcription etc. Ca^{2+} may originate from different sources, i.e., influx from the outside medium or release from a variety of internal stores. In mammalian cells, Ca^{2+}-release channels of the type of the inositol 1,4,5-trisphosphate- and ryanodine-receptor (IP3R, RyR), respectively, are most important. In unicellular organisms (and plants), such channels have not been identified previously (although Ca^{2+} triggers, e.g., host cell penetration by parasitic forms such as Plasmodium and Toxoplasma). We have identified on a molecular level in the closely related ciliated protozoan, Paramecium tetraurelia, six groups of Ca^{2+}-release channels, which in part are genuine IP3Rs and in part RyRs (the first ones identified in protozoa), while some others show one or the other characteristic feature (domain structure, pore domain etc.) of either receptor/release channel type. Localization and gene silencing experiments revealed widely different, but distinct localization, activation, and functional engagement of the different Ca^{2+}-release channel types. Our discovery of a range of such molecules in Paramecium indicates that protozoa already have evolved multiple Ca^{2+}-signaling pathways. Several whole genome duplications may have allowed this unexpected variation, with a total of 34 IP3Rs and RyRs in the Paramecium cell, which – according to gene silencing experiments – can account for the regulation of widely different functions in different cell regions. – Supported by Deutsche Forschungsgemeinschaft, grant PL 78/21.

Do heterotrophic freshwater ciliates acquire UV sunscreen compounds from algal food?

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Small prostomatid ciliates of the genera Urotricha and Balanion belong to the most abundant planktonic ciliates in lakes. Highest numbers coincide with the phytoplankton bloom in spring and they are able to consume a considerable amount of algae. The occurrence of these small ciliates in the uppermost meters of the water column suggests that they have effective strategies to cope with incident UV radiation. Recently, specific UV sunscreen compounds, so-called mycosporine-like amino acids (MAAs) have been detected in several mixotrophic and heterotrophic ciliate species. The source of the MAAs has been proven to be of algal origin such as symbiotic algae and/or algal food. MAAs are a family of secondary metabolites, known as effective UV sunscreen compounds. They are intracellular, colourless and water-soluble with having their maximum absorption between 309 and 362 nm, which is in the range of the damaging UV-B (280 – 315 nm) and UV-A (315 – 400 nm) wavelengths. To test the ciliates ability to uptake and retain MAAs from algal food after being exposed to incident levels of UV radiation, we fed cultivated ciliates either with MAA-rich (Parvodinium inconspicuum) or with MAA-poor (Cryptomonas phaseolus) algal food. Then, we exposed the ciliates to artificial UV radiation (280 – 400 nm) and PAR (400 – 700 nm), extracted the MAAs in aqueous methanol (25 %) and analysed the sunscreens by HPLC. First results show that B. planctonicum was able to uptake MAAs, e.g., palythine (λmax = 320 nm) from their algal food. Supported by the Austrian Science Fund FWF (P21013-B03).

Heterologous expression of C5(6) sterol desaturase from Tetrahymena thermophila restores ergosterol biosynthesis in Saccharomyces cerevisiae ERG3 knockout strain

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Sterol biosynthesis comprises various enzymatic steps, such as squalene cyclization, demethylations, reductions and desaturations. Desaturases are essential enzymes in sterol biosynthesis, such as C-5(6) sterol desaturase that catalyze the introduction of Δ5 double bound. Several studies in plants, mammals and yeasts have shown that desaturation at C-5(6) involves an electron transfer from NADH to the terminal oxidase (the desaturase itself) via a cytochrome b5 reductase and the cytochrome b5. These features are typical of the fatty acid hydroxylase superfamily (FAHS). Even though the ciliate Tetrahymena thermophila can not synthesize sterols, performs several modifications in the sterol moiety. Four activities have been described: C-5(6), C-7(8) and C-22(23) sterol desaturations and removal of C-24 ethyl. We have recently identified and characterized two sterol desaturases of T. thermophila, which revealed the typical features of these enzymes: C-5(6) sterol desaturase, DES5A, and C-24 sterol desaturase-like, DES24. In yeast, the C5(6) sterol desaturase is an endoplasmic reticulum enzyme encoded by the ERG3 gene, its disruption creates an interruption in the synthesis of ergosterol. A heterologous expression of Des5Ap in the ERG3 deficient strain was performed in order to evaluate the functionality of cytochrome b5 dependent enzyme of the ciliate and to confirm its activity. The erg3 mutant was transformed with a p425-C5T plasmid, carrying DES5A gene of Tetrahymena. The sterol profile of the complemented strain was analyzed by HPLC and GC-MS. The results showed that complementation restored ergosterol synthesis. Therefore, this work shows by the first time that a ciliate Cyt b5 dependent enzyme of the FAHS is active in Saccharomyces cerevisiae, indicating that yeast has the requirements to restore the activity with a foreign gene. These data suggest that S. cerevisiae can be used as a good system for both the study of membrane protein complex of the FAHS, and the identification of putative genes of ciliates.

Feeding of ciliates on toxic filamentous cyanobacteria in Lake Zurich (Switzerland)

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Besides its recreational uses Lake Zurich (Switzerland) serves as the major source of drinking water for > 1.5 million people. In the past, the harmful cyanobacterium Planktothrix rubescens showed recurrent mass developments and it gets increasing dominance due to ongoing global warming. The cyanobacterium is generally considered toxic for eukaryotes. Intact cyanobacterial cells contain cyclic heptapeptides (microcystins) and various other cyclic peptides. Although coccoid and filamentous cyanobacteria are often regarded as inappropriate or even toxic food for most consumers, protists and especially ciliates can be efficient predators of cyanobacterial blooms. During the last 3 years, we repeatedly observed pelagic and benthic ciliate species feeding on P. rubescens, and we were successful in the isolation and cultivation of the pelagic Obertrumia aurea and the benthic Trithigmostoma cucullulus. We present a first characterization of the autecology and of population dynamics of these species. Especially T. cucullulus shows a fascinating handling of prey, by taking up intact filaments (up to 15 times longer than the ciliate) or fragments only from distal ends. There are at minimum four ways how the ciliate incorporates cyanobacteria: to roll up filaments resulting in maximal 4 coils, to fold the prey inside the cell with an undefined number of fractures, to ingest a major part of the filament and to break this part mechanically or enzymatically from the remaining rest, and to ingest fragments of filaments which are mostly the same length as the ciliate. In all but the latter, the uptake of long filaments leads to strong deformations of the cell shape. After a successful digestion, the original morphology is regained. Although several authors proofed the uptake of Planktothrix spp. by ciliate species, surprisingly the basic principle of this interaction is not well understood: Why do ciliates feed on toxic filamentous cyanobacteria, and, as eukaryotic organisms, why are they not poisoned by their toxic diet? There is need to study the aut- and synecology of these species for discussing their potential usage as biological control of cyanobacteria.

Reassessing the diversity and taxonomy of the eustigmatophyte algae of the genera Vischeria Pascher and Eustigmatos Hibberd

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Vischeria spp. and Eustigmatos spp. are coccoid algae common in terrestrial habitats. The genus Vischeria, originally described by Pascher as comprising the type species V. stellata and nine other species, was later expanded by a new species described by Vischer (V. punctata) and by one more species (V. helvetica) transferred by Hibberd from the genus Polyedriella. Only the species V. stellata, V. punctata, and V. helvetica have been studied during last decades and shown by Hibberd and Leedale to belong to their newly established class Eustigmatophyceae (Ochrophyta, Stramenopiles). Pleurochloris was another genus to be described by Pascher as a member of his "Heterokonten". Hibberd later recognised that at least two Pleurochloris species (P. magnus and P. polyphem) are eustigmatophytes and placed them to a newly erected genus Eustigmatos together with the newly described species E. vischeri. The genera Vischeria and Eustigmatos are morphologically very similar, distinguished by the cell wall in the former genus occasionally elaborated into projections or ridges. We have initiated a project to reassess the taxonomy and diversity of these two genera by applying molecular methods on an extensive set of ninety Vischeria and Eustigmatos strains from public culture collections or newly isolated from localities worldwide. Sequencing the 18S rRNA gene from a subset of these strains revealed that there is no phylogenetic separation of the genera Vischeria and Eustigmatos and the whole group should be best treated as a single genus. Furthermore, preliminary results from sequencing the ITS2 region show that the current definition of Vischeria/Eustigmatos species may need revision. Most interestingly, two different variants of the ITS2 region were encountered in some strains, raising the question whether they reflect intragenomic heterogeneity or the presence of allelic variants in a diploid genome.

Protein transport to the apicoplast of apicomplexan parasites

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Most apicomplexan parasites, including Plasmodium falciparum, harbour a so-called apicoplast; a complex plastid of red algal origin which was gained by a secondary endosymbiotic event. The exact molecular mechanisms directing the transport of nuclear encoded proteins to the apicoplast of apicomplexans are not well understood. Recently, in silico analyses revealed a second copy of proteins homologuous to components of the ER-associated protein degradation (ERAD) system in organisms with secondary plastids, including the malaria parasite P. falciparum. These proteins are predicted to be endowed with an apicoplast targeting signal, and are suggested to play a role in the transport of nuclear encoded proteins to the apicoplast. Here we have studied components of this ERAD-derived putative pre-protein translocon complex in malaria parasites. Using transfection technology coupled with fluorescence imaging techniques we can demonstrate that the n-terminus of several ERAD-derived components targets GFP to the apicoplast. Furthermore, we confirm that full-length PfsDer1-1 and PfsUba1 (homologues of yeast ERAD components) localise to the apicoplast, where PfsDer1-1 tightly associates with membranes. Conversely, PfhDer1-1 localises to the endoplasmic reticulum. Our data suggests that ERAD components have been "re-wired" to provide a conduit for protein transport to the apicoplast. In further studies, we also reveal a possible molecular identity for the translocon of the third outermost apicoplast membrane. Our results are discussed in relation to the nature and evolution of the apicoplast protein transport machinery.

A two-locus molecular characterization of Paramecium calkinsi

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Paramecium calkinsi (Ciliophora, Protozoa) is known as a euryhaline species which was described at first from freshwater habitat, but afterwards several strains were also collected from brackish water. It is characterized by a clockwise direction of spiral during swimming and a general morphology of the

"bursaria" type. The present paper is the first molecular characterization of P. calkinsi strains recently recorded from distant localities within Russia with the application of ITS1-5.8S- ITS2-5'LSU rDNA (1100 bp) and COI (720 bp) mtDNA sequenced gene fragments. For a comparison we included P. bursaria, exhibiting a similar "bursaria type" morphotype, and species representing the "aurelia type", i.e. P. caudatum, P. multimicronucleatum, P. jenningsi, P. schewiakoffi and some species of the P. aurelia species complex (P. primaurelia, P. tetraurelia, P. tredecaurelia). Trees constructed for both DNA fragments show that P. calkinsi strains are monophyletic and distinct (support for rDNA: 100 NJ, 99 MP, 1.00 BI; for COI: 100 NJ, 100 MP, 0.99 BI) beside a clade which includes species belonging to the "aurelia" subgroup (support for rDNA: 100 NJ, 99 MP, 0.99 BI; for COI: 100 NJ, 100 MP, 1.00 BI). This corresponds with previously presented relationships in the genus Paramecium.

The enigmatic amoeboflagellate Rhizomastix is a member of Archamoebae and a close relative of the genus Entamoeba

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Rhizomastix is a genus of amoeboflagellates with a single anterior flagellum, which live as intestinal commensals of insects and amphibians. A typical feature of Rhizomastix is the so-called rhizostyle, a fiber of unknown structure which originates at the flagellar base and runs deep into the cells along the nucleus. The nucleus possesses a large central nucleolus and peripheral chromatic granules. Although Rhizomastix has been established 100 years ago and six its species have been described, it remains one of the most enigmatic protist genera. It has been hypothesized that Rhizomastix belongs to the group of Archamoebae. However, neither ultrastructural nor sequence data of any Rhizomastix species, which would confirm the hypothesis, have been obtained so far. We have successfully established three strains of the genus Rhizomastix. Two of them were isolated from a millipede and a frog, respectively. The third strain was isolated from fresh-water sediments and represented the first known free-living member of Rhizomastix. We investigated both the phylogenetic position and cell structure of the free-living isolate. The analysis of the SSU rRNA gene confirmed the genus Rhizomastix as a member of Archamoebae. Moreover, it suggested that Rhizomastix is a close relative of the genus Entamoeba which contains the important human pathogen E. histolytica. A TEM study showed that the rhizostyle of Rhizomastix is a modified cone of other archamoebae and forms an internal "skeleton" reminiscent of the axostyle of trichomonads. The flagellum of the strain IND8 bears fin-like lamellae. Our results showed that the genus Rhizomastix is a key organism for studying the evolution of Archamoebae.

The significance of testate amoebae as a biogenic Si pool in soils

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In soils biogenic silicon (Si) pools can be separated into phytogenic, microbial and protozoic pools [1]. While many researchers focus on phytogenic pools [2], less is known about protozoic Si pools [1]. Analyses of AOKI et al. (2007) indicate that testate amoebae (TA) and higher plants play a comparable role in the global silica cycle [3]. To test the significance of TA as biogenic Si pool in soils we conducted a research project on initial ecosystems as well as mature forested ecosystems. Here we intend to quantify the protozoic Si pool and analyse biotic and abiotic drivers affecting the pool size and amoebal communities. In a first step 31 mature forested ecosystems were analysed on TA by a simple counting method (thin sections) [4]. At each site one undisturbed topsoil sample was taken using Kubiena boxes. TA were counted at 12 representative sections (5 mm2 each) per thin section using a light microscope. In a next step a subset of 10 and additional 6 sites of initial ecosystem development were chosen for detailed analysis. At each site 5 field replicates were chosen randomly. TA samples were stored in formalin and stained with aniline blue before classification on species level. Soil samples were air-dried and sieved prior to lab analyses. The fraction < 2 mm was analysed on pH, total soil organic carbon and nitrogen as well as carbonates and labile and pedogenic Si fractions. Our first results confirm the chosen sites to represent a broad range in soil chemical properties. Numbers of TA varied from 0.1 x 108 to 11.5 x 108 TA m-2. If we assume an average Si content of 1400 pg Si in one test we come up with a protozoic Si pool up to 16 kg Si ha-1 confirming the relevance of TA as a biogenic Si pool in soils. References: [1] Sommer et al. (2006) J Plant Nutrition and Soil Science 169:310-329; [2] Cornelis et al. (2011) Biogeosciences 8:89-112; [3] Aoki et al. (2007) Geoderma 142:29-35; [4]

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Revised morphology and phylogenetic position of Microdiaphanosoma arcuatum (Ciliophora, Colpodea)

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The current taxonomic position of Microdiaphanosoma arcuatum Wenzel, 1953, is based on the morphological characters defined by Foissner (1993) to circumscribe the class Colpodea, subclass Bryometopia, order Bryometopida, family Kreyellidae. M. arcuatum shows the kreyellid silverline system characteristic of the order as well as the main features of the family: a paroral membrane distinctly separated from the somatic kineties which does not reach the posterior margin of the vestibulum, slightly spirally coursing right lateral somatic kineties, and a reduced ciliature on the left side. We obtained the first complete small subunit (SSU) rDNA gene sequence of this ciliate, showing that the current taxonomic position of M. arcuatum based on morphological characters is in disagreement with the SSU rDNA topology found in our study. Phylogenetic analysis including available SSU rDNA sequences from another Colpodea species in the GenBank strongly support the position of M. arcuatum within the order Cyrtolophosidida instead of the order Bryometopida. The analysis also suggests a sister relationship between this species and species from the family Cyrtolophosididae. Our observations add to the morphological details of M. arcuatum already described by Foissner (1993) some new information which still had not been recorded on images, revealing the presence of caudal cilia, the excretory pore of the contractile vacuole, and the micronucleus. Moreover, thanks to the impregnation after silver carbonate staining, we add some fine morphological details to the description of the oral apparatus by Foissner (1993). Our results lead clearly to the debate concerning the need for a reevaluation of the main characters used to establish the different groups within the class Colpodea.

Colonization and structure of the hindgut wall of lower termites

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In this study we morphologically examined the hindgut wall of three lower termites: Incisitermes marginipennis, Incisitermes tabogae, and Reticulitermes flavipes. We compared the colonization of the hindgut epithelium with prokaryotes and oxymonad flagellates, and studied the structures of the gut walls by scanning and transmission electron microscopy. Wall structure and colonization of the closely related Incisitermes species have much more characteristics in common than the genera Incisitermes and Reticulitermes. For example, the paunch wall of Reticulitermes possesses cup-like indentations and has a thin cover of horizontally attached bacteria. Instead, the two Incisitermes species have no cups in their hindgut wall and a thick bacterial layer containing long bacterial rods and spirochetes that attach via their cell poles. Further, there were discrepancies between the oxymonad species that have been reported from I. marginipennis and I. tabogae by other authors and by us. For example, the only described Oxymonas species from I. tabogae, i. e. O. clevelandi, could not be found associated to the hindgut epithelium. Instead there were three different morphotypes, including one tiny type with a surprising morphology. It has the typical characteristics described for oxymonads but misses a rostellum (anterior, elongated attachment organelle). Nevertheless it is firmly attached to the wall of the paunch. It has an amoeboid form fitting snugly to the cuticular structures of the hindgut wall. This is the first time that an oxymonad with such an appearance has been described. Molecular studies will prove whether this is a new oxymonad species of I. tabogae or just a life cycle stage of a normal looking species.

Deciphering the effects of space and of environmental heterogeneity on the variation of microbial communities at the global scale

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Understanding the drivers of community changes at different spatial scales is a central objective in ecology. The current revolution in high-throughput sequencing technology has facilitated the production of large biological sequence datasets and there

is now a need by microbial ecologists to interpret community patterns in their ecological context, often over large geographic distances. Here current work with the International Census of Marine Microbe (ICoMM) dataset will be presented, especially the characterization of main patterns of microbial diversity at the global scale and of the effects of major factors of variation. By synthesizing, visualizing and testing hypotheses on such large molecular datasets, novel insights about marine microbial ecology of benthic and pelagic communities may be obtained especially concerning the scales at which ocean realms and ecosystems are structured, the taxonomic scales of relevance to describe microbial diversity patterns, and the types of abiotic and biotic processes being most likely at play.

Intranuclear symbionts of Paramecia: Genus Holospora revisited

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Holospora are obligate intranuclear symbiotic bacteria of Paramecia (Ciliophora, Protozoa). Up to date nine species were described according to their host and nuclear specificity, cell size and morphology. Among them three species are specific to the same host and to the same nucleus, namely H. undulata, H. elegans and H. recta, all three the symbionts of P. caudatum micronucleus. They were described as different species on the basis of morphology of infectious forms (IF): curved or strait. H. undulata is the most common species, about 3 – 5 % of natural populations of P. caudatum are infected by this symbiont; H. elegans is much more rare, we know about only 5 independent isolates described in literature and found by us; H. recta was found only ones. In present work we have sequenced 16S rDNA (PCR-product) of two H. undulata and one H. elegans isolates. Sequences of H. undulata isolates differ in 2 position, H. elegans differ in 1 position from each of them. All three sequences are identical with the partial H. elegans sequence (479bp) deposited in GeneBank. If compare with ssr H. obtusa sequence (X58198,1492b.p. our data 1245b.p.), they are different in 19 positions, that is typical for species of the same genus. H. recta after several yeas of cultivation was gone, it's 16S rDNA was not sequenced. But in the same water pool was found H. undulata isolate, in which the population of infectious forms consisted with both strait and undulate forms. Using reinfection with low concentration of IF, subclones were obtained exclusively with undulate or strait forms. After prolonged cultivation those with the strait forms still produce IF of this morphology, while the clone with undulates became mixed: there were both strait and undulate forms, and the percent of the former gradually increased. According to this observations we propose that H. recta is just morphological mutant of H. undulata. Our results strongly suggest that H. undulata is the only valid species, while H. elegans and H. recta are mutant form of it. 16S rRNA genes of other holosporas were sequenced that allowed validation of this species and construction of their phylogeny.

Properties of species structure in ciliates as a consequence of nuclear organization properties

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In most well studied ciliates each "morphological" species consist of syngens, which are intraspecious reproductively isolated groups. Extensive investigation of species structure in genus Paramecium revealed different degree of divergence: syngens of P. aurelia differ in many aspects: enzymes, molecular characters, even morphology, so they were recognized as sibling species. On the other hand, P. caudatum syngens are much closer to each other there are only minor differences between syngens. Nevertheless, the genetic isolation of syngens is close to 100 % in both morphological species: intersyngen F1 are rare, and F2 do not servive. One may conclude that reproductive isolation does not depend on degree of genetic differences. Another very specific property of ciliates is their nuclear heteromorphism. During macronuclear differentiation, DNA reorganization, elimination, and fragmentation processes occur, resulting in a macronucleus containing short DNA molecules. It is believed that these processes are controlled by small nuclear RNAs and the old macronucleus surves as a template for this rearrangement. Parental noncoding RNA molecules instruct whole-genome reorganization. Here I present a hypothesis that join pecularities of ciliate species structure with nuclear differentiation mechanisms. MA sequences to be removed are transposable elements or their derivatives, able for intranuclear expansion. If new IES sequence appear in the MI, it will be removed during MA differentiation, if both partners have this sequence, or both do not have it, but if only one partner have it, while another do not,

excision of such IES meet problems, that lead to mortality up to 50 % of the progeny. Consequently, strong selective pressure will work against heterologous pares, that must favor all types of mutations preventing heterologous crosses. According to this point of view, genetic isolation is not a result of general genetic differences, but is a prerequesite of it. Secondly, this isolation is supposed to appear in sympatric. Third, in different ciliate groups different genes may be involved in genetic isolation development.

Genetic dissection of the maintenance and replication of the apicoplast genome in Toxoplasma gondii

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The chloroplast-like organelle known as the apicoplast is an essential cellular component of most apicomplexan parasites including Toxoplasma gondii. The evolutionary history of the apicoplast entails two successive endosymbiotic events resulting in a fascinating mix of one prokaryotic and two eukaryotic ancestors. We are interested in the cell biological basis of this union and focus here on the organellar genome. We searched the Toxoplasma genome database for genes encoding proteins homologous to bacterial DNA-binding proteins and have identified a putative apicoplast DNA polymerase I, two gyrase subunits, a recombination helicase, and the HU histone-like protein. We have confirmed through fluorescence microscopy that all of these proteins localize to the apicoplast, and we are establishing the specific function of these proteins in apicoplast DNA replication and maintenance through genetic studies using T. gondii as a model. We have isolated parasite mutants for HU, Pol I, RecG, and GyrB. We have found that the HU protein, a DNA-binding structural protein with implicated roles in transcription, initiation of replication, and DNA repair in bacteria, can successfully complement bacterial HU mutants. The HU mutant parasite, though viable, exhibits significant growth retardation, similar to mutants of the E. coli protein. In addition, this mutant has reduced copy numbers of the apicoplast genome. Ongoing studies will elucidate the impact of HU loss on cell division and apicoplast genome segregation, which will help clarify why HU is required for wildtype DNA levels. Our current work is focused on the phenotypic analysis of the polymerase and gyrase mutants.

High-level interspecific diversity of Euplotes revealed by assessment of mitochondrial cox1 gene for ciliate DNA barcoding

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The gargantuan identity crisis of protists represents a serious pitfall to studying taxonomic, phylogenetic, and biogeographical aspects of these microorganisms. Recent investigations have suggested the feasibility of establishing a species identification system reliant on the analysis of the sequence of the mitochondrial cytochrome c oxidase subunit 1 gene (cox1), (i. e., DNA barcode). The DNA barcode procedure is based on the assumption that the sequence divergence of a small DNA fragment allows species discriminations. We assessed the effectiveness of this procedure in the most cosmopolitan, ubiquitous, and differentiated group of ciliated protists, the genus Euplotes. The availability of the largest collection of living strains of Euplotes currently existing, together with the application of both traditional and innovative methods to ensure the reliability of the identification of each morphospecies, created the necessary conditions to allow this evaluation. Accordingly, we analysed cox1 gene sequences from 47 strains belonging to 13 Euplotes morphospecies representatives from various different habitats. Novel PCR primers for Euplotes were developed to amplify approximately 1100-1600 bp of the cox1 gene and the products obtained were subsequently cloned and sequenced. All Euplotes strains analyzed were partitioned into the 13 morphospecies, whose cox1 sequences diverged of about 60 %. This value is about 5 – 6 times higher than that obtained in the two other ciliate genera and animal taxa recorded in literature, whereas the intraspecific sequence divergence ranging from 0 to 10.5 %. Yet, E. vannus and E. crassus showed high intraspecific sequence divergence values of 35.0 and 33.5 %, respectively, and representative strains of each of these two morphospecies did not cluster together on cox1 phylogenetic trees, suggesting the occurrence of cryptic species. The mean interspecific sequence divergence of Euplotes was about 5 times greater than the mean intraspecific sequence divergence. Overall, our study demonstrates the feasibility of the barcoding procedure to the genus Euplotes.

Polyphasic characterization of protist diversity in the subsurface of a Thuringian aquifer

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Heterotrophic protists play a key role in the microbial food web since they are direct consumers of bacteria and contribute to the flow of energy and organic matter. Knowledge about protist diversity and feeding strategies is essential for understanding the structure of microbial food webs. To further the understanding of protist diversity in aquifers we aimed to compare direct snap-shot techniques without a cultivation bias (microscopic observation after filtration and sequencing of SSU rRNA genes) and cultivation approaches (microscopic observation and sequencing). Groundwater and freshly drilled core material were sampled in 3 to 88 m deep horizons of a Trochitenkalk aquifer in the Thuringian basin (Hainich, Germany). After filtration of 1.5 L groundwater, 3 flagellate and 3 amoeba species were microscopically observed indicating that active protist cells were present in the aquifer. In cultures from groundwater and rock material, a total of 34 protist species were microscopically detected. In groundwater cultures, 11 naked amoeba, 14 flagellate and 2 ciliate species were recorded; whereas, cultures of rock material had 2 naked amoeba, 15 flagellate and 4 ciliate species. As revealed by cultivation and sequencing, groundwater and rock material had about 50 % of the detected protist species in common. Sequencing of groundwater samples (6 L) revealed greater protist diversity than observed after filtration or cultivation. Cell length of flagellate and amoeba species ranged between 6 – 10 µm and 15 – 35 µm (25 – 75 % quartile), respectively. Most of the flagellate species were sessile suspension feeders, e.g., Monosiga spp., Spumella spp., and mobile, surface-associated graspers, e.g., Bodo spp. Hence, we hypothesize that small sized protist species take refuge in the structured surface of pores and fissures of the Trochitenkalk and feed on suspended or biofilm-associated bacteria.

Species boundaries in gregarine apicomplexan parasites: Comparison of morphometric and molecular variability in Lecudina cf. tuzetae (Eugregarinorida, Lecudinidae)

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Trophozoites, the large feeding cells of gregarine apicomplexans with diverse morphologies, have played a prominent role in gregarine systematics. The range of variability in trophozoite cell shape and size can be very high even within a single species depending on developmental stages and host environmental conditions; this makes the definition of different species of gregarines based on morphological criteria alone very difficult. Accordingly, the comparison of morphological variability and molecular variability in gregarines is necessary to provide a pragmatic framework for establishing species boundaries within this diverse and poorly understood group of parasites. We investigated the morphological and molecular variability present in the gregarine Lecudina cf. tuzetae from the intestines of Nereis vexillosa (Polychaeta) collected in two different locations in Canada. Three distinct morphotypes of trophozoites were identified and the small subunit (SSU) rDNA was sequenced either from multi-cell isolates of the same morphotype or from single cells. The aim of this investigation was to determine whether or not the different morphotypes and localities reflected phylogenetic relatedness as inferred from the SSU rDNA sequence data. Phylogenetic analyses of the SSU rDNA demonstrated that the new sequences did not cluster according to morphotype or locality and instead were intermingled within a strongly supported clade. A comparison of 1,657 bp from 45 new sequences demonstrated divergences between 0 % and 3.9 %. These data suggest that it is necessary to acquire both morphological and molecular data in order to effectively delimit the "clouds" of variation associated with each gregarine species and to unambiguously re-identify these species in the future.

Protozoan communities at two stations located in the coastal zone of the Southern Baltic Sea

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Protozoa (ciliates, dinoflagellates, and nanoflagellates; all heterotrophic) were observed at two sta-

tions located in the coastal zone of the southern Baltic Sea. Sampling site inside Gulf of Gdańsk (Sopot, studied between 2003 and 2004) characterises of higher trophic level and stable environmental conditions, whereas sampling site located in Central Pomerania (Ustka, studied between 2007 and 2008) demonstrated unstable conditions with strong benthic resuspension and irregular impacts of fresh water. At both station studied comparable mean annual biomasses of protozoa were observed: 44.1 µgC l-1 in Sopot and 38.6 µgC l-1 in Ustka. However, both communities varied greatly with their composition. In Sopot protozoan community was dominated by ciliates (48 % of biomass), whereas heterotrophic dinoflagellates and nanoflagellates contributed 30 % and 22 % of biomass, respectively. In case of Ustka, the majority of biomass (53 %) was contributed by heterotrophic flagellates, whereas ciliates were less important (35 %) and heterotrophic dinoflagellates contributed only 12 % of biomass. Generally, similar species were observed at both stations studied. In Sopot changes of environmental conditions were gently, what resulted in typical, bimodal seasonal changes in protozoan biomass. In Ustka, due to unstable conditions, seasonal changes in protozoan biomass were atypical with only one peak observed during early summer.

Can actin be involved in contractile vacuole functioning in Paramecium caudatum?

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Actin is generally believed not to be involved in contractile vacuole functioning. Using fluorescent and confocal laser scanning microscopy we demonstrated that polyclonal antibodies against P. aurelia actin 1-1 (a generous gift by Prof. H. Plattner and Dr. I. Sehring, University of Konstanz, Germany) decorated the contractile vacuole, its ampullae and collective channels in P. caudatum. Fixation conditions seem to be crucial for preventing actin cytoskeleton from disassembly. To find out if it is the filamentous form of actin which is associated with the elements of the contractile vacuole the studies using TRITC-phalloidin are under way. Prolonged treatment with the lectin WGA leads to the total block of the cotractile vacuole functioning, which results in the formation of a huge single vacuole occupying most part of the cell. This approach is used to address the question whether increased membrane tension affects actin association with the contractile vacuole. We propose that actin may take part in the contractile vacuole activity, if not in the contraction per se, driven by actin-myosin interaction, but at least in membrane tubulation.

Peritrich community succession in a subtropical lake in Southern Brazil

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Ciliates play different roles in aquatic systems, occupying a wide range of habitats, and being considered good indicators of water quality. Peritrich ciliates are among the most common ciliates, and one of the groups with the largest number of species with over 50 genera and 1000 species described so far. Despite their great diversity, they are poorly studied, especially in South America. Many ciliates and other protists can colonize surfaces of solid objects and artificial submerged substrates. Glass slides may be used as artificial substrates that allow microorganisms to form a periphyton or biofilm, in which periphytic ciliates are usually in high abundance and richness. In the present work we performed a one year baseline survey of peritrich ciliates colonizing glass slides in the Lake Guaíba located in the Rio Grande do Sul state, Brazil. In this period, we analyzed the population dynamics of the species community, and correlate with environmental factors, such as temperature, pH, and chlorophyll a. Results show that the genera Epistylis and Vorticella were the most abundant during sampling. Both seasons presented instability in the number of colonizing species, however, the winter, have shown so far much larger numbers of individuals and zooids, while during the spring there was a growing diversity throughout the season. During the winter, five new species of Epistylis were found, along with many unidentified species of Vorticella and Opercularia.

Changes in the structure of protozoa community in a wastewater treatment plant with advanced biological nutrient removal system

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This study has been proposed with the aim to asses the capability of protists as indicators in activated sludge WWTP for nutrient removal. Two treatment

lines working simultaneously were studied from a WWTP (A2O process) located in Barcelona (Spain): experimental one operating with restricted internal recirculation, and second used as control line. Sampling was carried out during six consecutive months. Results showed that differences of nitrogen removal values between experimental and control lines were statistically significant (97% ammonia-uptake and 76 % NT removal on control line versus 59 % and 56 % on experimental one), but no differences were observed related to organic matter removal (COD, BOD). Concerning to the protozoa community, the experimental line presented a significant increase of the abundance of diplomonadids and higher densities of bodonids and Arcella. In contrast, naked amoeba, euglenoid flagellates and specially Euglypha showed a significant decrease. The ciliated protozoa showed an important decrease of number of species (from 28 to 22) and Shannon diversity index in experimental line, and significantly higher abundance of Aspidisca cicada, Acineria uncinata and Vorticella infusionum, common species reported on conventional activated sludge. On the other hand, control line showed higher abundance and stability of Trochilia minuta, Vorticella aquadulcis, Metacystis spp. and Holophrya discolor, and the exclusively development of species such as Aspidica lynceus, Epistylis plicatilis, Epicharchesium granulatum, Euplotes aediculatus, Gastronauta membranaceus and Thuricola kellicottiana. In summary, results revealed that operational parameters, specifically nitrogen removal due to recirculation characteristics, are directly related to protozoa populations composition. The results reinforce the importance of the study of biological parameters, mainly protozoa, as useful tools to be considered for the management of nutrient removal WWTP. Acknowledgements to the support of the company EMSSA (Barcelona) and to the Spanish Ministerio de Ciencia e Innovación through project n°: CGL2008-02310.

Membrane biogenesis in Toxoplasma gondii: De novo synthesis versus selective scavenging of major phospholipids by the parasite

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Toxoplasma gondii requires membrane biogenesis to assure a faithful obligate intracellular replication within its host cell. The relative contribution of de novo pathways versus scavenging of host-derived phospholipids by the parasite remains unknown. Toxoplasma is capable of synthesizing its major phospholipids (PtdCho, PtdEtn and PtdSer), and de novo synthesis of its most abundant lipid, PtdCho, is vulnerable to an anti-parasitic choline analog, DME. This research reveals that T. gondii can also selectively internalize the tracer analogs of PtdEtn and PtdSer, but not of PtdCho. Unlike Plasmodium, Toxoplasma also lacks a plant-type serine decarboxylase and phospho-ethanolamine methyltransferase (SD-PMT) pathway to produce PtdCho from serine and/or ethanolamine. Together, these results indicate the parasite is a choline auxotroph for PtdCho biogenesis. The endogenous synthesis of PtdCho is catalyzed by TgCK, TgCCT and TgCPT enzymes, which are differentially distributed in T. gondii. The CCT and CPT reside in the nucleus and endoplasmic reticulum, whereas CK, harboring a hydrophobic N-terminal sequence, forms oligomers in the parasite cytosol. Heterologous expression in COS-7 cells confirmed the differential localization of above enzymes in mammalian cells and allows CCT and CPT activity assays. Purified CK can phosphorylate choline with a Km of 0.26 mM and also be inhibited by DME. A conditional knockout of TgCK was generated that confirms the essentiality of this enzyme for T. gondii. Our ongoing work focuses on the characterization of TgCK knockout as well as conditional gene deletion of TgCCT.

Bacterial 'response' to intensive protozoan grazing

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It is known that bacteria have evolved certain defense strategies to prevent grazing of protozoa, e. g. the building of filaments and microcolonies, the release of toxic substances a. s. o. Additionally, it is said that grazing of protozoa enhances the activity of bacteria. The presentation deals with an investigation in a two species system, containing a bacterivorous ciliate (Tetrahymena pyriformis) and a bacterium (Acinetobacter johnsonii). Two different activities were analyzed: respiration (by the use of the substrate analogon CTC (= 5-Cyano-2,3-Ditolyltetrazoliumclorid) and growth (by the use of Fluorescence-in-situ-hybridisation; FISH). We analyzed the bacterial activity as well in a chemostat system as in a batch system. We supposed to find differences in the bacterial activities in the presence and absence of the bacteria, with higher activities under grazing pressure. Moreover, we looked at indi-

vidual differences between single bacteria and compared this to the activity in the whole community. On the poster, first results of the story are shown and the results are discussed.

Ciliate predation mediated phenotypic response and chaotic coexistence in an experimental microbial food web

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The interaction between individuals of species can cause changes in their morphology and behaviour. Especially phenotypic changes triggered by chemical substances of interacting partners can affect the morphological appearance. Phenotypic plasticity is widely distributed within bacterial communities mediated by quorum sensing or kairomones. The ability of forming grazing resistant morphotypes and the underlying mechanisms are well known but the effects on microbial food web dynamics are not well understood. We studied a well defined microbial food web consisting of two prey bacteria and the ciliate Tetrahymena pyriformis as the predator in long-term chemostat experiments with increasing dilution rates as the bifurcation parameter. We could show that phenotypic plasticity of one prey species within our food web can lead the trophic system to a broader range of coexistence along a changing environment and therefore seem to have a stabilizing effect on the predator-prey system. This was supported by a mathematical model. Corresponding analyses of the time series data by calculating Lyapunov exponents indicated chaotic behaviour of the system, which in turn may maintain the coexistence of species.

Identification of potential thioredoxin target proteins in Entamoeba histolytica

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Entamoeba histolytica, an intestinal protozoan that is the causative agent of amoebiasis, possesses a functional NADPH-dependent thioredoxin system comprising the dithiol-containing redox proteins thioredoxin (Trx) and thioredoxin reductase (TrxR). Both proteins were found to be covalently modified by the 5-nitroimidazole drug metronidazole which consequently led to the loss of disulfide reducing activity of the TrxR/Trx system and the covalent modification of only a few defined proteins. The aim of the present study was to search systematically for further interaction partners of thioredoxin in order to extend our understanding of the lethal action of metronidazole in E. histolytica. Based on the Trx reduction mechanism we constructed an active site mutant of Trx lacking the resolving cysteine residue. The recombinant mutant protein (EhTrxC34S) was immobilized on Ni-NTA resin to capture target proteins from E. histolytica cell extracts after formation of intermolecular disulfide bonds. EhTrxC34S and covalently linked proteins were eluted and visualized by two-dimensional gel electrophoresis and Coomassie Blue staining. We could identify more than 40 potential Trx-interacting proteins that will be trypsin-digested and analyzed by liquid chromatography-tandem mass spectrometry. This study was supported by Grant P22037 from the Austrian Science Fund (FWF).

The apicomplexan parasite Eimeria falciformis co-opts host indolamine-2, 3-dioxygenase for its life cycle progression

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Eimeria falciformis is a highly host- and tissue-specific parasite of murine caecum epithelium. Its monoxenous life cycle in a well-investigated host makes it an excellent model to examine in vivo parasite-host interactions during all apicomplexan stages. Our ex vivo gene expression analyses using the parasite-infected intestinal cells revealed the modulation of 1214 up- and 594 down-regulated transcripts during early and late development. The IFNγ-inducible enzyme, Indolamine 2,3-dioxygenase (IDO), which catalyzes the tryptophan catabolism, is induced 51-fold at 144 hrs post-infection, and was identified as one of the most up-regulated proteins. Interestingly, the absence or the biochemical inhibition of IDO as well as the inhibition of two downstream enzymes in mouse is detrimental to parasite development as indicated by total oocyst output. More importantly, the administration of xanthurenic acid (XA) to IDO-/- mice, a downstream metabolite of IDO catalysis and a potent stimulator of gametocyte development in Plasmodium, completely restored the oocyst output. Taken together, these results suggest a previously unknown function of IDO in regulating the development of intracellular parasites within their vertebrate hosts. Briefly, our work demonstrates an elegant parasitic strategy co-opting the host enzyme for progression of its life cycle, and underscore E. falciformis as a

model parasite to investigate the entire development of apicomplexan parasites.

First molecular description of the Paramecium parasite Holospora caryophila

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In 1890, Hafkine described the highly infectious bacterial parasites Holospora, present in the micro- or macronucleus of certain Paramecium strains. Gromov and Ossipov (1981) examined organisms identified as two Holospora species, Holospora undulata Hafkine 1890 and Holospora obtusa Hafkine 1890. They confirmed the original descriptions of Hafkine and validly described this bacterial genus according to the International Code of Nomenclature of Bacteria. Cultivation of Holospora bacteria outside their Paramecium hosts has not yet been accomplished. Thus, images and descriptions of Holospora species serve as representatives of their taxonomic types. Phylogenetic relationships among the different Holospora species are unclear, so far only the 16S rRNA gene sequences of H. obtusa (1,492 bp) and H. elegans (479 bp) have been published in GenBank. In this study we characterized naturally occurring intracellular bacteria of different Paramecium strains. Following the traditional taxonomy based on morphology, host range and infectivity, these bacteria have been identified as Holospora caryophila. Preliminary analyses of the 16S rRNA gene sequences reveal genetic distances with respect to the other molecularly characterized Holospora species are rather large. This finding is in good congruence with the differences between H. caryophila and e.g. H. obtusa in host range and mechanism of horizontal transmission. The molecular characterization of the type strain of H. caryophila (Preer and Preer 1982) and the additional novel strains will allow clarification of the evolutionary relationships among the Holospora species. Re-discovery of this "old" species and its molecular description are important steps to elucidate the phylogenetic diversity of Paramecium intracellular bacteria and might help to address the question concerning the more ancestral traits in this highly specialized obligate parasite.

Structure and function of R-bodies from endosymbiotic Caedibacter in Paramecium

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R-bodies are proteinaceous ribbons produced by bacteria of the genus Caedibacter endosymbiotic in the ciliate species of the genus Paramecium. High resolution transmissionn electron microscopy revealed the composition and surface pattern of this intracellular structure. Its structural characters and function together with the robustness under ex vivo conditions point to its potential as a nanobiotechnological tool.

Remarkable genetic and silica-scale diversity in the colourless chrysomonad Paraphysomonas: Taxonomic and evolutionary implications

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Silica scale morphology of protists has been an anchor-point in the description of new species since the electron microscope first allowed its use. Over the past 60 years protistology has seen an increase in the numbers of described species of silica-scaled organisms, especially from the heterokont genus Paraphysomonas. Many descriptions of new species of Paraphysomonas, a colourless ochrophyte (Chrysophyceae), come from electron microscope surveys of environmental samples (not clones) published in the early 1980s. However, these surveys missed out on molecular data opportunities that we can easily amass today, creating a large gap between the genetic versus morphological data. To date, there are around 56 described species of Paraphysomonas, which, apart from the first established species, are all named according to their scale morphology. Scale structures vary greatly between species, making scales an 'easy' tool for species identification. There is little molecular data available for this large group of organisms despite being a very common protist in both marine and freshwater environments. The most commonly reported species, P. vestita (scales viewed by Manton and Leedale in the 1960s) and P. imperforata, both have a spined scale structure that is not shared with many other named species. I have isolated many clonal isolates of Paraphysomonas to observe their silica-scales, cell dimensions, and 18S phylogeny, so these data together might uncover any hidden diversity beyond the scales. I have observed that there is a common and widespread occurrence of

spine scales within this genus, the genetic diversity of which is notable. Numerous lineages share broadly similar scale morphologies and are genetically distinct. I shall discuss how subtle differences in spined-scale morphology map onto phylogenetic trees within an evolutionary and taxonomical context. My data strongly indicate a far greater diversity within this group of protists than has previously been estimated, and that there are many more species waiting to be described. The commonness of P. vestita and P. imperforata is probably the result of excessive lumping of genetically distinct, species with similar scales, thus indicating a need for further characterisation of species using phylogenetics and morphological data from multiple isolates.

Evolutionary dynamics of inorganic pyrophosphatases in photosynthetic protists: Endosymbiotic replacements, lateral gene transfers and domain fusions

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Inorganic pyrophosphate (PPi) is a ubiquitous product of cell anabolism whose hydrolysis is required for the successful completion of biosynthetic reactions. Several classes of non-homologous proteins called globally pyrophosphatases (PPases) -having different structures, enzymatic mechanisms and subcellular localizations- catalyze this essential reaction for all organisms. Two distinct families (I and II) of soluble PPases (sPPases) located in the cytosol and/or the matrix of energy organelles (mitochondria, plastids) hydrolyze PPi generating heat, while the H+(ion)-translocating PPases (H+-PPases, PF03030), which are integral proteins of prokaryotic cell membrane and eukaryotic endomembranes, couple PPi hydrolysis to the generation of an electrochemical gradient useful in bioenergetics. Protist energy organelles possess nuclear-encoded Family I sPPases (PF00719) that functionally substituted by endosymbiotic replacement the ancestral organelle homolog. A family II sPPase (DHH-DHHA2 phosphoesterase, PF02833) previously described only in some archaea and bacteria is present in a number of marine microalgal groups – prasinophytes, stramenopiles (bacillariophyceae, pelagophyceae), haptophytes – besides an archetypical probably plastidic family I sPPase. The biochemical characterization of the two non-homologous sPPases of the prasinophycean Ostreococcus tauri revealed a comparatively higher catalytic efficiency of the family II counterpart that agrees with the peculiar metabolic features of this tiny photosynthetic protist. These microalgal family II sPPases are closely related phylogenetically to prokaryotic orthologs, and given the intimate coexistence of disparate microbial species in many complex marine communities, they could have been acquired by recent prokaryote-to-eukaryote lateral gene transfers (LGT). Besides that, these marine microalgae possess, like other groups of photosynthetic (Cyanidiales) and non-photosynthetic (Oomycetes) protists, a number of diverse sPPase fusion proteins in which the catalytic PPase domains are integrated in a single polypeptide with other structural domains, either catalytic (ATP-sulfurylase, APS kinase, FBPase) or regulatory (EF-hand, CBS, PH), which could confer novel functional properties (domain accretion). This may explain the striking paralog redundancy within sPPases found in these protists. Moreover, these marine microalgae exhibit several functionally diverse membrane-PPase paralogs; some of which could be Na^+-translocating isoforms that, given their close relation to Na^+-PPases of certain prokaryotes, probably originated via LGT from prokaryotic sources. Supported by BFU2010-15622/BMC and PAIDI-BIO-261 grants (partially founded by EU FEDER program).

A new species of Hemigastrostyla and notes on the non-monophyly of some oxytrichid genera (Ciliophora, Hypotricha)

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We found a new species of the genus Hemigastrostyla Song & Wilbert, 1997 in sandy sediments of the Jiaozhou Bay (Yellow Sea, China). Species of this marine/brackish genus have, like many other hypotrichs, basically an 18 frontal-ventral-transverse cirri pattern. Previously, all 18-cirri hypotrichs have been assigned to the oxytrichids because the characteristic pattern formed by the 18 cirri was interpreted as apomorphy of this group (Kahl 1932, Tierwelt Dtl. 25; Berger 1999, Monographiae biol. 78). Interestingly, the classification based on the ventral ciliature is in conflict with the dorsal kinety pattern and most gene sequence data, indicating that this complex 18-cirri pattern must have evolved much earlier in the Hypotricha tree, very likely already in their streamline (Berger 2008, MB 85). Due to these changes in hypotrich taxonomy, some genera – for example, Oxytricha and Hemigastrostyla – are obviously no longer monophyletic. Oxytricha granulifera Foissner & Adam, 1983 – type of the notoriously difficult genus Oxy-

tricha Bory de Saint-Vincent, 1824 – has a dorsal kinety 3 fragmentation and dorsomarginal kineties. Consequently, Oxytricha species which lack the fragmentation (e.g., Oxytricha islandica Berger & Foissner, 1989; O. lanceolata Shibuya, 1930; O. longa Gelei & Szabados, 1950) have to be transferred to a (new?) genus outside the oxytrichids, but within the Dorsomarginalia Berger, 2006 which unify all hypotrichs having a dorsomarginal kinety. By contrast, Oxytricha species which lack both kinety fragmentation and dorsomarginal rows [e.g., Oxytricha geleii (Wilbert, 1986)] have to be placed outside the Dorsomarginalia, that is, near the base of the Hypotricha tree. Funded by the National Natural Science Foundation of China (Project 40906065; C. Shao) and the Austrian Science Fund (FWF; Project P23415-B17; H. Berger).

Is Prodiscocephalus a hypotrich ciliate? Phylogenetic analyses based on morphogenetic and 18S rRNA gene sequence data (Ciliophora: Spirotrichea)

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The Prodiscocephalus-like ciliates, or discocephalines, are traditionally considered to be euplotid hypotrichs but whose precise systematic position has long been uncertain, mainly because of the paucity of morphogenetic data, with only two discocephalines having been investigated, and a complete absence of molecular data. In the present study the cortical development of Prodiscocephalus borrori was observed during binary division. Five features were observed that are characteristic of stichotrichs: (1) the oral primordium in the opisthe occurs de novo on the cell surface; (2) the undulating membrane in the proter is derived from the parental structure; (3) there are > 5 FTV-cirral anlagen; (4) the two marginal rows form intrakinetally; (5) the dorsal anlagen are formed in two groups. By contrast, only two features are typical of euplotid hypotrichs, i.e. (1) several caudal cirri are formed from the rightmost DK-anlagen; (2) the FVT-cirral anlagen are formed in the primary mode, indicating that the discocephalines are more closely related to the stichotrichs than to the euplotids. Based on a combination of morphological and morphogenetic data, a phylogenetic tree was constructed which also suggests that the discocephalines group within the stichotrichs and separate from the euplotids. In addition, the complete small subunit rRNA (SS rRNA) gene of P. borrori was sequenced and analyzed. In the resulting SS rRNA tree, P. borrori is sister to the Stichotrichia-Oligotrichia-Choreotrichia assemblage, albeit with low bootstrap support, and separate from the euplotids. These data suggest that the discocephalines should be considered as a distinct group at the rank of order, or even subclass, within the class Spirotrichea Bütschli, 1889. Supported by: NSFC (project no. 40676076); Centre of Excellence in Biodiversity Research, King Saud University; Royal Society Joint Projects programme.

A new species candidate of Spirostomum sp. (Ciliophora: Heterotrichea, Spirostomidae) from an estuary in Jeju Island, Korea

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A new species candidate of genus Spirostomum Ehrenberg, 1834 was collected from estuary in Jeju Island, Korea. The morphology of a new heterotrichous ciliate, Spirostomum n. sp. was investigated using live observation and silver impregnation methods. It was distinguished its congeners by the following characteristics: Body size 265 – 410 × 30 – 40 µm in vivo, long and slender but highly contractile. Cortical granules sparsely arranged in 2 rows between somatic kineties, colorless and size about 0.5 µm in diameter. Oral region covered 1/3 – 1/2 of body length. Adoral membranelles 85 – 124 in number. Undulating membrane about 16 µm in length. Somatic kineties arranged longitudinally, 15 – 21 in number with somatic cilia about 10 µm in length. No distinctly long caudal cilia. Macronucleus curved cylindroidal with 3 – 4 button-like micronuclei. The new species is similar to the closely related species of S. yagiui Shigenaka, 1959 and S. teres Claparède and Lachmann, 1858. However, this specis differs from S. yagiui by the body length/width ratio (10/1 vs. 14/1), size of micronuclei (3 µm vs. 1.6 µm in cross), number of adoral membranelles (85 – 124 vs. 130 – 140) and caudal cilia (absent vs. present). In addition, this species differs from S. teres by the shape of macronucleus (cylindroid vs. ellipsoid), number of somatic kineties (15 – 21 vs. 25 – 30), and density and color of cortical granules (loose & colorless vs. dense & yellowish).

Reconsideration of endemic distritution of colpodid ciliate Corticocolpoda kaneshiroae Foissner, 1993 (Colpodea, Colpodida, Colpodidae) and new combinations of names in related species

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The colpodid ciliate Corticocolpoda kaneshiroae Foissner, 1993 proposed as a "Hawaiian endemic species" was collected from the old tree bark of mountainous wetland in Jeju Island, Korea. The Korean population of this species has similar habitat preferences compared to the original Hawaiian population, i.e. the bark of old tree in volcanic island and a low pH (3.8) in rewetted samples. So the endemic distribution of this species needs to be reconsidered. We compared the morphology of these two populations in detail using live observation and silver impregnations. The infraciliature of left oral polykinetids (LOP) of genera Corticocolpoda Foissner, 1993 and Kuehneltiella Foissener, 1990, Bresslaua Kahl, 1931 and all other members of family Colpodidae are also extensively compared with specimens and literatures. The arrangement patterns of LOP could be subdivided into three types, i.e. single-, double- and triple-rows equidistantly spaced arrangements. One species of genus Corticocolpoda and two species of genus Kuehneltiella showed the double-rows equidistantly spaced arrangement type. Therefore, we suggest the new combinations of two species names: Corticocolpoda terricola (Foissner, 1990) and C. muscicola (Foissner, 1993) n. comb.

Influence of pressure on deep-sea heterotrophic flagellates

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The 'microbial loop' was first described from pelagic marine systems, little is known about heterotrophic flagellates from the great abyssal planes. In order to investigate whether marine heterotrophic flagellates can potentially act as important bacterivores in the deep sea, we isolated flagellates of different phyla from the abyssal and determined their growth and survival rates at different temperatures and pressure. Our experimental studies carried out under laboratory conditions revealed that changes of pressure and the changes of temperature had no significant effect on the survival rate of many species, while temperature effects were species specific. The results will be discussed in the context of microbial activities in the deep.

Differential expression of Entamoeba invadens dynamin related proteins in encystation and excystation

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Entamoeba histolytica is a human intestinal parasite that infects approximately 50 million people and causes more than 100 000 deaths annually. E. histolytica has a bipartite life cycle alternating between the oxygen sensitive trophozoites and the robust and infectious cysts. Encystation and excystation play a major role for dispersal and infection. During these processes major cytoskeletal and membrane reorganizations take place. The dynamin protein family is a group of mechanochemical proteins involved in membrane scission. We have used the closely related reptilian parasite E. invadens as a more tractable model to study encystations processes. Here, we demonstrate that the E. invadens dynamin related proteins are differentially expressed during the life cycle stages of this parasite suggesting a role in the production of infectious cysts.

Evolution of Rhizaria: Comparing transcriptomes of Foraminifera and Acantharea

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To date, Rhizaria are the super-group of eukaryotes, for which the least genomic data are available. A few efforts have been made towards the sequencing of Expressed Sequence Tag (EST) libraries for some rhizarian species. Recent phylogenomic study revealed strongly supported relationships between Foraminifera and Acantharea. As both groups of protists are uncultivable, a special cDNA library construction method requiring very small amounts of starting material was applied and coupled with massively parallel pyrosequencing. To complete this study and search for genes shared by both groups, we sequenced cDNA libraries from two additional foraminiferal species (Elphidium sp. and Globobulimina turgida) and compared them to previously obtained cDNA librairies of two foraminiferans (Reticulomyxa filosa, and Quinqueloculina sp.) and two acathareans (Phyllostaurus siculus and Astrolonche serrata). Specific gene

identification has been performed through sequence similarity searches against publicly available databases for general annotation of the obtained sequences and against manually curated databases. According to preliminary results, approximately 30 % of the resulting sequences in every library have homology to other sequences in public databases while almost 70 % remain unclassified. The genes have been classified according to their molecular function and biological process as described in the Gene Ontology (GO) consortium. Different set of genes participating in specific processes such as motor activity, membrane transport, cell projection organization and skeletonization were identified. Further analyses of these data will aim on finding new phylogenetic markers for elucidating the evolutionary history of Rhizaria and will provide new insights into the foraminiferan and acantharean biology.

Epigenetic regulation of the surface protein coat of Paramecium tetraurelia

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Antigenic variation was described to be a general mechanism in parasitic as well as in free-living protists and expression of variable surface antigen coats is widely used as a masking mechanism but can also be used for detection of special ligands of the cell's environment. The cell surface of the ciliate Paramecium is covered by a variety of GPI-anchored proteins consisting of the high molecular surface antigen family and several smaller proteins presumably representing receptors. Basically, these two groups of GPI-proteins show differences in their expression and also in release into the medium. Expression of most of the smaller proteins seems independent of the expressed surface antigen and I will describe how transcriptional silencing and epigenetic mechanisms are involved in this special expression mechanism. Our data implicates that this involves RNAi mediated reversible chromatin modifications to define active and silent states of the antigen genes in the vegetative macronucleus. Obviously, this mechanism acts exclusively on the surface antigen genes. However, nothing conclusive has been elucidated about the homology dependency of this siRNA mediated mechanism – why does one gene of the surface antigens escape silencing and why does the mechanism not affect other (similar) surface proteins? Characterization of the involved small RNAs gives (not only) the opportunity to understand the organization of the surface protein coat but will moreover results in an entirely new point of view to homology dependent regulation of gene expression.

The diversity and evolution of obligately halophilic protozoa

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Microbiologists tend to assume that extremely saline habitats (> 25 % salinity) such as solar salterns are populated exclusively by prokaryotes (principally Haloarchaea and Salinibacter) and the extraordinarily halotolerant chlorophyte alga Dunaliella. Nonetheless, a diverse selection of protozoa have been recorded as active in extremely saline environments. Recently a range of protozoa that are obligate halophiles/extreme halophiles have been cultured from these environments. The inventory of obligately halophilic protozoa cultured from solar salterns and similar natural environments overlaps substantially or entirely with that associated with salt mines. By contrast we see minimal (but non-zero) overlap with published libraries of rRNA genes/sequences from extremely hypersaline basins on the Mediterranean seafloor. The obligately halophilic protozoa cultured to date are mostly distinct at the genus level from marine taxa, and represent several different major groups of eukaryotes – Stramenopiles, Alveolates, and Heterolobosea. Those within Heterolobosea are especially diverse, including amoebae and flagellates, as well as true amoeboflagellates, and they represent at least three independent clades within Heterolobosea. This underscores the fact that obligate halophily has evolved many times amongst protozoan eukaryotes. One possible case exists of a clade of obligate halophilic protozoa that has undergone modest adaptive radiation. In a couple of other instances close relationships are seen between obligate halophiles and unusually halotolerant (but not obligately halophilic) protozoa, possibly tracing evolutionary progressions. Almost nothing is known at present about how obligately halophilic protozoa cope with very high salinity, and this area is ripe for comparative study.

Novel biodiversity pattern: A protistan species maximum in the horohalinicum of the Baltic Sea

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We re-assessed the plankton diversity in the brackish-water Baltic Sea, which was previously considered a species-poor basin, and discovered the unexpectedly high species richness (4056 taxa), with dominance by protists (up to 85 %). Results show that species number of unicellular organisms in the salinity gradient of the Baltic Sea follow the binomial distribution mode, while the metazooplankton diversity decreases exponentially with salinity growth; however species richness of both groups peaks within the horohalinicum (5 – 8 psu). This newly discovered brackish-water biodiversity pattern was outlined by the novel 'protistan species maximum concept' (Telesh, Schubert, Skarlato, 2011. MEPS 421:1 – 11). Our results agree with the hypothesis that the horohalinicum zone presumably supports protistan species with a broad range of environmental tolerance. This study challenges the established Remane's Artenminimum (species minimum) concept, originally developed for macrozoobenthos in large water bodies with relatively stable salinity gradient, and substantiates a new ecological perspective of the previously overlooked exciting protistan diversity in brackish waters. We assume that pronounced adaptability and advanced osmoregulation strategies of protists allowed these small-sized fast evolvers developing considerable species richness and filling in biodiversity gap in a large brackish-water basin. Moreover, drifting within large water masses, planktonic protists are affected by only moderate salinity fluctuations (if compared to benthos), and therefore they can prosper in brackish environment. The Baltic Sea thus represents a clear example of how pelagic biodiversity in a large, osmotically stressed though relatively stable ecosystem is promoted when fast-growing eukaryotic unicellular organisms are abundant. This new knowledge is transforming our view of biodiversity in transition areas by refining the Remane's model via discriminating between the salinity effects on diversity of large sessile versus small motile aquatic species in the fluctuating environment. Grants: RFBR 10-04-00943, LSS 3276.2010.4, IB/BMBF RUS 09/038 and Program "Biodiversity" of the Presidium RAS.

Colony formation in a freshwater bacterial strain (Acinetobacter johnsonii) as a result of predation pressure: The role of micro-evolution and phenotypic plasticity

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The formation of grazing resistant bacterial aggregates mediated by a high grazing pressure of protists is a well known phenomenon. The scope of our study focuses on the outcome of adaptive phenotypic plasticity and micro-evolution on the dynamic behaviour of a simple predator-prey system. We studied the morphological variability of the bacteria Acinetobacter johnsonii (gamma-proteobacteria) under grazing pressure by the ciliate Tetrahymena pyriformis in short-term batch experiments and in long-term chemostat experiments. We investigated these strains regarding the changes in the phenotypic composition of bacterial morphotypes in the presence and absence of predation and select for the best adapted (grazing resistant) strains after exposition to extreme predation pressure. Previous experiments showed that predation pressure induces shifts towards extreme morphotypes, such as bacterial chains or extremely large filaments which are inedible for the ciliate. The reduction or even the total loss of small sized bacteria could therefore be explained by the feeding preference of the ciliate with regard to single and small cells. Experiments were carried out which displayed shifts in morphotypes and demonstrated that changes in genotypes towards grazing resistant forms – presumably genetically fixed – may occur.

Diversity and host specificity of intestinal trichomonads of non-human primates

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Despite the fact that the non-human primates are our closest relatives, represent a species-rich mammalian group, and many of them are seriously endangered, little is known about their intestinal parasites. Particularly their intestinal trichomonads are considerably understudied. We have established 30 trichomonad strains isolated from feces of 11 primate species kept in four Czech zoos, and performed an analysis of their SSU rDNA and ITS1-5.8S rDNA-ITS2. The prevalence of intestinal trichomonads in the examined hosts reached 85 %

indicating that they are common at least in captive non-human primates. Phylogenetic and morphological analyses showed that our isolates are unexpectedly diversified, belonging to eight or nine distinct species including at least four so-far undescribed ones. Interestingly, the vast majority of the strains from non-human primates belonged to the genus Tetratrichomonas while no member of this genus has been found in the human intestine so far. In addition, hominoid and non-hominoid primates differ in their intestinal trichomonads. Importantly, our results also showed that at least some primate species may be probably infected by intestinal trichomonads of other vertebrates such as pigs, cattle, birds, tortoises, and lizards. The host range of many trichomonad species is thus far wider than previously expected.

Lobose amoebae: Diversity, phylogeny and species problem

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Lobose amoebae (amoeboid protists forming lobose pseudopodia and moving by mean of actomyosin cytoskeleton) do not form a monophyletic group and are distributed among several phylogenetic branches of Amoebozoa. Those are probably monophyletic Tubulinea; paraphyletic (or polyphyletic) Discosea, and Variosea – a clade that includes a number of rather distinct amoeboid moprhotypes and few flagellated species. Species diversity of amoebae remains very poorly studied; recent studies bring many new species (sometimes representing distinct new phylogenetic lineages) from virtually all kinds of habitats. The main reason for this is the difficulty of amoebae morphospecies distinction. Molecular studies show relatively high level of genetic variability even within properly identified "species", suggesting a kind of a cryptic speciation. Sequence comparison often helps to differentiate morphologically similar species, but the high level of gene polymorphism in amoebae does not allow us to identify species by sequence identity; until now few distant isolates assigned to the same morphospecies show complete identity of SSU sequences, and this happens only among members of the order Leptomyxida. This embarasses DNA barcoding of amoebae and virtually blocks molecular ecological studies of these organisms. Primary targets in amoebae studies remain (1) isolation, identification and sequencing of amoebae species belonging to the clades, poorly sampled or non represented in the phylogenetic trees; (2) studies of the genetic structure of amoebae morphospecies and related problem of DNA barcoding of amoeba species and (3) obtaining reliable data on the amoebae species distribution patterns in local- and global- scale. Studies supported by IZLR Z3_128338 grant from STCP, Switzerland. The author acknowledges St. Petersburg State University and RFBR for research and travel grants.

Diversity of free-living marine ciliates in Chinese coastal waters, with notes on new taxa from the South China Sea

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Over the past two decades the Protozoology Group, Ocean University of China, and collaborators have investigated marine ciliates in Chinese coastal waters, focusing primarily on their morphology, morphogenesis and molecular phylogeny. Long-term surveys of the coastal waters of northern China have resulted in detailed studies of about 600 free-living species including 134 new, and numerous poorly known, forms. Taxonomic information on 320 species is summarized in the dual-language monograph Free-living Ciliates in the Bohai and Yellow Seas, China (eds Song, Warren and Hu, Science Press, Beijing, 2009). Compared to temperate regions, knowledge of tropical and sub-tropical ciliate faunas is scant. Therefore in recent years we have been investigating the ciliates of sub-tropical areas in southern China, in particular the coastal region of the South China Sea near Guangzhou. Our initial studies indicate that there is a huge undiscovered ciliate diversity in this region. To date, three new families (Lynnellidae Liu et al., 2011; Eurystomatellidae Miao et al., 2010; Bergeriellidae Liu et al., 2010) and 13 new genera (Williophrya Liu et al., 2010; Bergeriella Liu et al., 2010; Eurystomatella Miao et al., 2010; Wilbertia Fan et al., 2009; Lynnella Liu et al., 2011; Heterocyclidium Fan et al., 2011; Apodiophrys Jiang et al., 2010; Heterodiophrys Jiang et al., 2010; Pseudodiophrys Jiang et al., 2011; Apocoleps Chen et al., 2009; Apobakuella Jiang et al., in press; Heterohartmannula Pan et al., in press; Aporthotrochilia Pan et al., in press) have been established and over 30 new species have been described. The phylogenetic relationships of many of these taxa have been determined following analyses of their morphogenetic and/or gene sequence data. Other outputs of this

work include: (1) the establishment of microscope slide reference collections at OUC, Qingdao, and SCNU, Guangzhou; (2) the deposition of type and voucher slides in international collections such as the NHM, London; (3) the establishment of a DNA bank at OUC/SCNU which currently contains genetic material from 600 isolates representing over 500 identified morphotypes. Supported by: National Natural Science Foundation of China; Center of Excellence in Biodiversity Research, King Saud University; Darwin Initiative programme; Royal Society Joint Projects scheme.

Ciliate biodiversity patterns in alpine and subalpine lakes

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Remote alpine lakes are exposed to extreme environmental conditions such as low temperatures, low nutrient availability or low coloured dissolved organic matter (CDOM). In such oligotrophic lakes above the treeline, the water is highly transparent to ultraviolet radiation and even the potentially harmful UV-B wavelengths (280 – 315 nm) penetrate the water column down to the lake bottom. From investigations of ciliate morphotypes in these lakes we generally find that the abundance and diversity is very low with less than five predominant planktonic species in numbers < 10 ind. ml-1. For example, we compared the planktonic ciliate assemblages of three alpine lakes that are approximately 30 km apart from each other. Molecular analyses of the cytochrome c oxidase of a yet undescribed Bursaridium species show that two populations are identical and the third one differs by 24.6 % although morphological characteristics reveal that they are the same. Differences between populations can also be observed in cultivated individuals concerning their ecological demands such as light or food conditions. In contrast, in subalpine lakes that are surrounded by vegetation, morphological species diversity is much higher than in alpine lakes. Usually around 50 to 150 planktonic morphospecies are found in the course of a year. For example, common euplanktonic ciliates like Balanion planctonicum are frequently found near the surface, however, their distribution pattern in alpine lakes is different as the species prefers to remain in the greatest depths. In my talk, I will discuss ciliate biodiversity and biogeography in alpine and subalpine lakes in respect to the ongoing debate about climate change. As climatic warming may alter the input of allochthonous CDOM into lakes, large changes will occur, for example, in the attenuation of ultraviolet radiation. However, a prerequisite to follow changes in microbial food webs is to have a good knowledge on the autecology of single species. This requires the assessment of morphologic and molecular characteristics as well as their ecological demands. Supported by the Austrian Science Fund FWF (P21013-B03).

Diversity of the genus Monocercomonoides and its genetic codes

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Oxymonads are heterotrofic protozoa living in anaerobic environment. They occur mainly in the gut of insects, especially cockroaches and termites. The exception is the genus Monocercomonoides, which also inhabits in the intestine of vertebrates. The cells usually carry four flagella arranged in two separate pairs. These organisms lack a cytostome, mitochondria, peroxisome and Golgi apparatus. Oxymonads reproduce by binary division and have a closed type mitosis with intranuclear spindle. Trophozoites are the dominant life stage of their cell cycle. Taxonomically oxymonads belongs to the kingdom Excavata. We focused on the morphologically simplest genus Monocercomonoides and we want to clarify the phylogenetic relationships both within the genus and towards other genera of oxymonads. Using PCR, we obtained 14 sequences of small subunit ribosomal RNA (SSU rRNA) from different strains of Monocercomonoides. Our phylogenetic analysis showed that the representatives of Monocercomonoides form a monophyletic group (bootstrap 56) sister to Streblomastix (bootstrap 45). The relationships within Monocercomonoides clade reflect the host origin of strains. We also investigated the genetic code of some strains of Monocercomonoides. Non-canonical genetic codes evolved in several eukaryotic groups of eukaryotes, including oxymonads. Oxymonad genus Streblomastix uses non-canonical genetic code in which TAA and TAG codons code amino acid glutamine. This code was found also in an environmental sequence of α-tubulin from the gut of wood roach Cryptocercus punctulatus, which was ascribed to Monocercomonoides. We have investigated the cDNA sequences of the Monocercomonoides strain PA203 isolated from Chinchilla and we can conclude that this strain uses a canonical genetic code, because all three canonical stop codons are present. Furthermore, we have obtained a partial se-

quence of α-tubulin gene from the strain POTFIB isolated from beetle Potosia fieberi. In this sequence are all glutamine codons coded canonically, which suggests that also this strain uses the canonical code.

IFN-γ and its receptor are essential for the suppression of pathological Th17 responses during infections with an intracellular intestinal parasite

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The contributions of Th1 and Th17 responses to intestinal pathology are the subject of intense investigation. Here we utilise the apicomplexan parasite Eimeria falciformis, which infects epithelial cells of the murine caecum, as a model system. To assess the role of IFN-γ in this system, we investigated mice deficient in this cytokine and its receptor (IFN-γR). In contrast to wild type mice, IFN-γ -/- and IFN-γR -/- mice showed dramatically exacerbated body weight loss. Surprisingly, this pathology was not caused by an increased replication of the parasite, as significantly fewer parasites developed in IFN-γR -/- mice compared to wild type mice and development of immunity against challenge infections was not impaired. Instead, we observed a striking increase in the production of antigen-specific IL-17A and IL-22 in mesenteric lymph node and spleen cultures of IFN-γR -/- mice. CD4+ T cells were found to be the cellular source of IL-17A and IL-22 and cell numbers were dramatically increased in IFN-γR -/- and IFN-γ -/- mice. Our data show, that deficiency in IFN-γ and its receptor in context of an intracellular intestinal parasite infection leads to a dysregulated immune response, which is characterised by a switch towards a pathogenic Th17 response. These data reveal important information concerning the interplay between Th1 and Th17 responses and the development of intestinal pathology.

Exploring micro-eukaryotic life in the deep hypersaline anoxic lake Thetis

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Driven by previous assumptions that extreme hypersaline environments do not allow eukaryotic life, we explored the micro-eukaryotic diversity using morphology and molecules in the recently discovered deep hypersaline anoxic lake (DHAL) Thetis in the Eastern Mediterranean Sea. This lake is 3,258 m in depth, and, with a salinity of 348 ‰, it belongs to the most saline habitats known on Earth to date. Cell counts were obtained with a eukaryote-specific probe and fluorescence in situ hybridization (FISH), resulting in 0.6x104 micro-eukaryotes per litre in the anoxic brine layer. The amplification of SSU rRNA from cDNA showed that the most diverse group in the brine is the fungi (37 %). Further diverse taxon groups are ciliates and stramenopiles, each about 1/5 of all analyzed phylotypes. These same ciliates also were found in other Mediterranean DHALs; presumably they have specific adaptations to hypersaline habitats. Additional phylotypes were found that branch in the marine stramenopiles (MAST), dinoflagellates, haptophytes, choanoflagellates and jakobids. The micro-eukaryotic communities detected in the brine layer and the overlaying seawater-brine interface differ from each other as indicated by beta diversity analyses. Distinct morphotypes that were labeled via FISH probe witness to the viability of micro-eukaryotes in the DHALs. With these results, we suggest that brine lakes are valuable environments to discover unknown micro-eukaryotic communities, with potential for biotechnology and medical treatment. Furthermore, our study shows that the salinity frontiers of eukaryotic life have not been defined yet.

Diversity and localization of bacterial symbionts associated with Trichonympha flagellates in lower termites

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Cellulolytic flagellates are the key players in the digestion of lignocellulose in the hindgut of lower termites. The flagellates are typically colonized by host-specific lineages of ectosymbiotic and endo-

symbiotic bacteria. Previous studies have shown that Trichonympha flagellates, a parabasalid genus that dominates the gut microbiota of many termites, harbor more than one type of symbiont. Using an rRNA-based approach, we comprehensively investigated the phylogeny and subcellular locations of the symbionts. Endomicrobia were present only in Trichonympha Cluster I, which comprises gut flagellates from the termite families Rhinotermitidae and Termopsidae. In contrast, the bacterial assemblage associated with Trichonympha Cluster II was dominated by a novel, hitherto undetected lineage of Actinobacteria. With a few exceptions, each flagellate species contained also a second population of bacterial symbionts, whose phylogeny was much more variable. Depending on the flagellate lineage, they represented uncultivated phylotypes, e. g., Deltaproteobacteria or Verrucomicrobia. These phylotypes are closely related to bacteria previously detected in termite hindguts, but their association with flagellates was so far undocumented. In one flagellate species, we detected a novel lineage of the genus Prevotella (Bacteroidales), whose presence in insect guts has not been observed before. Using fluorescent in situ hybridization, we localized the different phylotypes in specific subcellular compartments of their Trichonympha hosts. The identical location of phylogenetically distinct lineages of bacterial symbionts in Trichonympha species from several termite species suggests a specific interaction with their host flagellates.

Polyphyly and paraphyly within the order tintinnida: How can we solve the dilemma?

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The prominent defining character of tintinnid ciliates is the lorica. It exhibits an incredible amount of variation and, not surprisingly, was the main feature used for species identification and tintinnid systematics. However, in the 1980s, studies revealed the intraspecific plasticity of the lorica, and infraciliary patterns were first described. With the acquisition and analysis of tintinnid SSU rRNA gene sequences over the last 10 years, we now have a tool to study the phylogenetic relationships and understand tintinnid evolution. The phylogenetic tree reveals paraphyly of the genus Tintinnopsis and polyphyly of the genus Favella, and shows that substantial revisions of the order Tintinnida are necessary. In our study we acquired 16 more tintinnid sequences – 5 Favella spp., 4 Tintinnopsis spp., 6 isolates of specimens with agglomerated loricas and not assigned to a specific genus at the time of sampling, and 2 Eutintinnus species (E. angustatus, E. pectinis). Phylogenetic analyses were run with the complete data set of available tintinnid sequences. The results show that: (1) the new sequences increase support for the 2 Favella clades; (2) the Tintinnopsis species group in different clusters, confirming the assumed paraphyly of the genus; and (3) some of the SSU rRNA gene sequences, collected separately as different species, are identical, affirming the high intraspecific plasticity of the lorica. How can we use the molecular data to help us revise the systematics of the order Tintinnida? Gene sequences of specimens identified by lorica morphology only are not sufficient for a revision; they need to be accompanied by reliable morphological (i. e., infraciliature, ultrastructure) data. The major problem in the Tintinnida is the lack of these morphological data for most type species. Additionally, the infraciliary patterns of 'problematic' genera like Codonellopsis, Tintinnopsis, Codonella, and Stenosemella are all of the most complex type and show only minor deviations. Further collaborative studies of tintinnid morphology, ultrastructure, and molecular sequences, focusing on type species and analyzing the minor differences in the infraciliature are needed to understand tintinnid phylogeny and evolution.

The evolution of MAT and MATX genes in euglenids

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Methionine adenosyl transferase (MAT) is an essential enzyme producing S-adenosylmethionine (SAM), which is one of the most important cellular metabolites required for methylation reactions. MAT is sequentially and structurally highly conserved enzyme that occurs in nature in two relatively divergent paralogs – MAT and MATX. MATX differs from MAT sequentially and includes four unique insertions of several amino acids. With one exception (diatom Thalassiosira pseudonana), MAT and MATX do not occur simultaneously in one organism. Very strange is the phylogenetic distribution of MATX, as it was found in four unrelated groups of secondary algae – dinoflagellates, haptophytes, Stramenopiles and photosynthetic euglenids. Such patchy distribution suggests that the evolutionary history of MAT and MATX was com-

plicated. We focus on clarifying the origin and evolution of the MATX gene in euglenids. From previous findings is known that Peranema trichophorum and kinetoplastids contains MAT, Euglena gracilis and Euglena longa contains MATX. Using PCR with specific primers, we have amplified MAT from cDNA of heterotrophic euglenid Distigma sp. Using PCR and EST sequencing we have obtained MATX from cDNA of photosynthetic euglenid Eutreptiella gymnastica. We have also examined prasinomonad alga Pyramimonas parkae, which is the closest known relative of euglenid plastid, and found that it contains MAT. These results support the hypothesis that the ancestors of euglenids contained MAT and MATX appeared at the base of the photosynthetic clade after the split off Peranema and before the split off E. gymnastica, i. e. approximately at the same time when probably appeared the secondary plastid of euglenids. However, there is no indication that euglenids acquired MATX from the plastid endosymbiont, because Pyramimonas, relative of euglenid plastid, contains MAT. The previous fact and the fact that MATX of photosynthetic euglenids do not branch at the base of MATX clade indicate that euglenids are not the group of eukaryotes, in which the MATX evolved, and they probably acquired this gene by horizontal transfer from other group of eukaryotes

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Planktonic ciliates in the Baltic Sea: Species diversity, community structure and seasonal succession

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The available data on ciliates' diversity in the Baltic Sea was recently revised and the annotated checklist was produced which comprises 814 species of ciliates currently known from different Baltic Sea regions (Telesh et al., 2009, www.io-warnemuende.de/marine-science-reports.html). However, even the most recent investigations (e. g., in the Neva Estuary) allowed discovering a good number of species that are new records for the Baltic Sea, which indicates that the present-day knowledge on the diversity of Baltic ciliates is still far incomplete. The important role of ciliates in aquatic environments is commonly accepted, although the majority of studies are usually focused on the dominant species only, and little is known about diversity and structure of the whole microzooplankton community. In our model study area, the Neva Estuary (the Gulf of Finland, the Baltic Sea), we investigated the taxonomic, size and trophic structure of the entire ciliates community, including rare species, which are often ignored. Special attention was given to nanociliates (< 20 μm). Ciliates from 111 taxa were detected during 2007 – 2009, including 25 new records for the Baltic Sea. Significant seasonal changes in ciliates community structure occurred at water temperatures 5 – 12 °C. By ordination of samples (MDS) and analysis of similarity of the ciliates' communities (ANOSIM), two distinct species associations were revealed that replaced each other during seasonal succession. In late April through October, algophagous, mixotrophic and algo-bacteriovorous ciliates dominated; their numbers decreased in cold season. Species composition of bacteriovorous and predatory ciliates was specific for each association. Small ciliates (< 30 μm) formed the most abundant size group. Proportion of larger ciliates (> 60 μm) increased in cold season due to appearance of benthic species in plankton. Total ciliates' abundance and biomass ranged 0.12 – -10.3 × 103 ind L-1 and 2.8 – 622.2 μg L-1, correspondingly. The atypical winter peak of ciliates was registered although generally the overall ciliates numbers decreased in cold season. Grants: RFBR 10-04-00943, LSS 3276.2010.4, IB/BMBF RUS 09/038 and Program "Biodiversity" of the Presidium RAS.

Localization and density of amoeba-resisting bacteria in Arcella spp.

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The intracellular distribution and density of amoeba-resisting bacteria was studied by different optical sectioning techniques with fluorescent microscopy (confocal laser microscopy and ApoTome from Carl Zeiss MicroImaging) in Arcella spp. The living bacteria were scattered all over the cell without any preferential localization, as demonstrated by FISH, using labeled oligonucleotides against general Eubacteria and different proteobacteria subgroups, chosen in view of several bacterial strains obtained formerly from the Arcella host by traditional microbiological methods. Simultaneously we applied simple DAPI staining to visualize all bacterium content of fixed Arcella cells. Completing the optical sectioning processes we evaluated the images of DAPI stained and FISH labeled Eubacteria by AxioVision software and detected no significant differences among the host cells' bacterial contents originating from the same sample. These results expand our previous knowledge suggesting that at a certain moment the whole

Arcella population in a culture vessel is inhabited at the same rate by the amoeba-resisting bacteria.

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Amoeba-resisting bacteria in testate amoebae: Insights from the long term investigation of an Arcella strain and fresh environmental samples

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Clonal cultures of Arcella were established in order to study amoeba-resisting bacteria. Following isolation from the environment, cultures were set up after washing cells in sterile water drops and then grown axenically for eukaryotes in natural mineral water and fed with Enterobacter aerogenes. The oldest strain, persisting since more than five years, is an Arcella intermedia, exhibiting a rich endobiont assemblage of prokaryotes. Five distinct investigations in approx. one year intervals were performed to monitor the bacterial spectrum over a five year period applying molecular biological methods. The first insight revealed a rich set of taxa, comprising the phylogenetically most diverse assemblage including endobiont species known from other protists. Later on the phylum level diversity decreased but the dominance of Proteobacteria especially that of the Alpha-group was conspicuous. A tendency of forming a quasi oligotrophic intracellular bacterial assemblage could be observed. Certain bacteria were detected several times, suggesting that in the stable lab environment a specific prokaryotic community was selected. Following the experimental eradication of intracellular bacteria, a sudden outburst of fungi destroyed all the host organisms. While we tend to agree that the general role of the bacterial assemlage, as a whole, might be to counterbalance against devastating fungal growth – as emphasized by certain authors –, we estimated the putative role of each individual species of bacteria according to the literature. Both in the lab clone and in environmental Arcella specimens bacteria liable to survive in intracellular space – either as mutualist or parasite – were a clear majority. Although the partial change in the bacterial assemblage over time suggests that culture conditions might influence the microbiota, the permanent occurrence of certain taxa, e. g. Variovorax paradoxus or Sphingomonas sp. implies the possibility of a tight relationship with the host. Our study contributes to generalize the view on the amoeba-resisting bacteria and host relationship by extending the range of host organisms so far mostly confined to pathogenic gymnamoebae. The re-

Searching for mitochondria of Monocercomonoides

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Oxymonads are one of the poorly studied groups of eukaryotes and so far any mitochondrial organelle has not been found in their cell. Organelles potentially with two membranes have been found by electron microscopy in oxymonad Saccinobaculus doroaxostilus, but there are no biochemical or molecular data about this organism. We tried to uncover mitochondrial organelles in another member of oxymonad group Monocercomonoides spp., strain Pa 203 isolated from Chinchilla. We have studied this strain using electron microscopy, but have not detected any potential organelles. We also searched for mitochondrial specific genes. We prepared two cDNA libraries and for each we used different protocol. Then we performed an EST sequencing of both libraries using 454 technology. Among sequences we found only four proteins commonly associated with mitochondrial organelles, namely, pyruvate ferredoxin oxidoreductase, [FeFe] hydrogenase, and two subunits of pyridine nucleotide transhydrogenase (PNT). Out of these proteins only the PNT is almost strictly mitochondrial with one known exception for Entamoeba. PNT transports protons over the inner mitochondrial membrane and at the same time transfers hydrogen between NADPH and NAD^+. To confirm the evolutionary origin of PNT in Monocercomonoides we obtained the whole sequence and performed phylogenetic analysis. In the tree, the sequence of Monocercomonoides PNT formed a clade with sequences of other mitochondrial PNTs. This clade was closest sister of α-proteobacteria. From this we conclude that PNT of Monocercomonoides had a common ancestor with PNTs of eukaryotic organisms and α-proteobacteria, indicating that Monocercomonoides once possessed a mitochondrial organelle. Localization of PNT in Monocercomonoides is unknown. Currently we are trying to localize it with fluorescent microscopy using two different antibodies.

Structure and expression of Euplotes pheromone genes

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In some species of Euplotes (i.e., E. crassus, E. nobilii, E. octocarinatus and E. raikovi), substantial information has been obtained on the structure of the cell type-specific, water-borne signaling proteins (pheromones) that regulate the cell switching between the mating and growth stages of the life cycle. However, little is known about the organization and expression of the genes that encode pheromones at level of the transcriptionally active macronuclear genome. Based on the determination of the pheromone amino acid sequences, we used PCR approaches to clone a significant number of full-length (from telomere to telomere) pheromone genes in E. raikovi and E. nobilii. The comparative analysis of these genes showed that they form species-specific gene families in which each member is structurally closely related to all the other members by high levels of sequence identity. Marked variations were observed only at level of the sequences coding for the secreted pheromones. The intra-family sequence conservation of the 5′ and 3′ sub-telomeric (non-coding) regions (the 5′ regions are apparently conserved at a higher level than the 3′ regions) thus suggested a functional association between these regions and the mechanism of pheromone-gene expression. The study of this mechanism has consistently revealed not only that each gene synthesizes multiple mRNA's starting from different sites of initiation of the transcription, but also that this synthesis requires the removal of introns from the 5′ non-coding gene regions.

Symbiosis evolution: Establishment, replacements and re-replacements in the association between Polynucleobacter-like bacteria and the ciliate Euplotes

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Polynucleobacter necessarius (Betaproteobacteria) was first described as endosymbiont of strain E24 of the ciliate Euplotes aediculatus. Further studies showed that the Polynucleobacter-Euplotes association is an obligatory symbiotic system between a monophyletic group of Euplotes species and bacteria belonging to the genus Polynucleobacter. In this system, neither the hosts nor their symbionts are able to survive independently. Recent studies revealed the existence of free-living populations of Polynucleobacter-like bacteria which are phylogenetically closely related to the endosymbiotic ones, but never share associations with Euplotes in the natural environment. In former studies it was hypothesized that the symbiosis between Polynucleobacter and the monophyletic group of Euplotes, once established, was maintained by the hosts as a synapomorphic character. Phylogenetic analyses based on 16S/18S rRNA data from twelve new characterized strains of Euplotes showed that the evolutionary path of this association could have been more complex than previously supposed. First of all, in three cases, we observed different bacteria as obligate symbionts. These bacteria are the first characterized representatives of a phylogenetic lineage branching in a basal position with respect to the genus Polynucleobacter and to the related genera Cupriavidus and Ralstonia. Hence, they constitute a new genus for which no free-living relatives have been described so far. These results suggest that the original obligate symbionts belonged to this newly discovered genus, and that, only subsequently, in most cases they have been replaced by Polynucleobacter bacteria recruited from the environment. Genome-size data also support this hypothesis. Furthermore, phylogenetic reconstructions suggest that Euplotes species, during their evolution, recruited Polynucleobacter bacteria as symbionts more than once and that, at least in some cases, even further substitutions by different strains of Polynucleobacter could have taken place. Once more, the study of microbial symbiosis contributed to the understanding of the complex evolutionary path leading to stable associations.

Species taxonomy of protists: Morphological and molecular perspectives in ciliates

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From the nomenclatural point of view, the species is the principal taxonomic unit, ranking below a genus and denoted by a Latin binomial. However, the matter is much more complex in taxonomical approaches, where as many as 27 species concepts have been proposed. According to Mayr's biological concept, a species is an aggregation of populations whose interbreeding produces fertile offspring. However, this can be hardly applied for protists because of methodological problems, ranging from cultivation difficulties to the rarity of some

species. Thus, it is problematic to test whether two protistan populations can reproduce sexually and to what extent this is influenced by laboratory conditions. Furthermore, some protists reproduce only asexually or by inbreeding. Therefore, protistologists usually use the morphospecies concept, in which a species is an aggregation of populations that share a strong and stable morphological similarity. Although there is a distinct general trend of decreasing genetic similarity with decreasing morphological similarity, some exceptions occur. This stimulates taxonomists to search for "good" species and generic characters. For instance, 18S rRNA gene sequences confirmed the nuclear pattern as an important species discriminator within the ciliate genera Stentor and Blepharisma. On the other hand, body size alone is seldom sufficient to distinguish a species within dileptid ciliates because transitions in this character occur. Further, taxonomists face another problem, i.e., which characters are appropriate to diagnose species and genera? Some reasons for erecting new genera will be presented using the example of trachelophyllid ciliates. Supported by the Austrian Science Foundation (FWF projects 19699-B17, 20360-B17, and 22846-B17).

Monograph of the dileptids (Ciliophora, Litostomatea)

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Dileptids are holotrichously ciliated, rapacious ciliates with a conspicuous proboscis carrying a complex ciliary pattern. Most species have a wide or global distribution, occurring in limnetic, marine, and terrestrial environments as well as in benthic and planktonic habitats. Light- and electronmicroscopical investigations suggested the dileptids as strongly derived crown Litostomatea because of their complex morphology and ontogenesis. However, recent molecular studies show the opposite, i.e., the dileptids form a distinct clade at the base of the litostomateans, supporting the subclass Rhynchostomatia established by Jankowski (1980). The genus Dileptus was established by Dujardin (1841). Although afterwards some interesting studies on dileptid biology and diversity were performed, progress was slow during the next ninety years. In 1931, Kahl revised the dileptids recognizing three genera (Dileptus, Paradileptus, Trachelius) and 25 nominal species, including eight new ones. After Kahl's classic study, more protozoologists became interested in the biology and taxonomy of the dileptids, which culminated in the monograph of Dragesco (1963). He revised the genus Dileptus and recognized about 50 species, showing that dileptid diversity doubled between 1931 and 1963. In the following decades, the number of genera and species increased slowly but steadily. In our monograph, we recognize 11 genera and 133 nominal species, of which 65 are possibly reliable taxa; however, only 44 species are so well described that their identity is not threatened. The overall synonymy rate is comparatively high (~ 28 %) because a few common species have been described several times. Our monograph commences with a detailed general section, explaining the dileptid morphology, ultrastructure, resting cysts, ontogenesis, conjugation, ecology, and phylogeny. In the main portion of the monograph, we provide, if available, for each species the following data: author, data, and journal page of the original description; a list of synonyms; nomenclatural matters; a morphological treatise including the original description, redescriptions, and all figures published; morphometric data; details on ontogenesis and resting cysts; a comparison with related species; and a detailed compilation of ecological and faunistic data. Supported by the Austrian Science Foundation (FWF projects 19699-B17, 20360-B17, and 22846-B17).

Abundance and size structure of testate amoebae community (Arcellinida and Euglyphida) in different biotopes from a floodplain lake

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The present study quantified and tested the relationships of abundance, size structure and morphological types of testate amoebae, among the communities present at different biotopes (plankton, aquatic macrophytes and sediment) from a lake in the Upper Paraná River floodplain. Samplings were undertaken monthly, at triplicates for each biotope, from April 2007 to March 2008. We identified 89 infrageneric taxa, belonging to 10 families, and Difflugiidae, Arcellidae, Centropyxidae and Lesquereusiidae were the most representative regarding the number of taxa. The most abundant species in plankton was Difflugia gramen, whereas in aquatic macrophytes and sediment, Centropyxis aculeata. The scores from DCA, classified according to biotope and shell morphology, evidenced the predominance of spherical and hemispherical species in plankton, and flattened and elongated in macro-

phytes and sediment. Testate amoebae were represented by individuals from < 50 and 400 µm. The smaller individuals predominated in plankton samples (50 ≤ T < 100 µm) while the bigger ones were most representative in the sediment (100 ≤ T < 150 µm). The predictions that would explain the occasional presence of testate amoebae in plankton from a lentic environment, driven by random events, as the resuspension from sediment and displacement from marginal vegetation, were rejected. Moreover, the results evidenced significant differences between hydrological periods, suggesting the environmental stability as one of the main factors driving the temporal variation in size structure of these organisms. Financial support: CNPq/Peld and CAPES.

Plankton ciliates community (Protozoa: Ciliophora) in the Upper Paraná River floodplain, Brazil: Patterns of composition, species richness and abundance

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Considering the high level of spatial and temporal heterogeneity, floodplains have one of the highest levels of biodiversity among the known aquatic environments. Spatial and temporal patterns of the composition, species richness and abundance of plankton ciliates communities were investigated in the Paraná River floodplain. Samplings were carried out in 36 environments, including open and closed lakes, channels and rivers, in two distinct hydrological periods: limnophase and potamophase. A total of 108 species of ciliates were recorded, belonging to thirteen orders. Among them, 59 species were recorded in the limnophase and 93 in the potamophase. Hymenostomatida was the most specious with 18 species followed by Haptorida (15) and Hypotrichida (14). Peritrichida and Pleurostomatida also contributed substantially to the total number of species, with eleven species each. Higher values of richness and abundance were found in lentic environments, especially in the limnophase, whereas in the potamophase the environments presented similar values of these attributes. Ciliates density ranged from 81 cels.L-1, in a channel, during the potamophase, to 21,955 cels.L-1, in a closed lake, during the limnophase. Regarding to the species richness, the values fluctuated from four species in rivers and in two open lakes, to 31 species in a closed lake. Among the more frequent species, stand out Rimostrombidium humile Petz and Foissner, 1992; Balanion planctonicum Foissner, Berger and Kohmann, 1994 and Halteria grandinella Dujardin, 1841 in the lakes, Urotricha farcta Claparède & Lachmann,1859 and Tintinopsis sp. in the channels, and Tintinidium sp. and B. planctonicum in the rivers. On the other hand, Tintinidium sp., Codonella cratera Imhof, 1885 and Calyptotricha lanuginose Wilbert and Foissner, 1980 were the most abundant in the lakes, U. farcta and Charchesium polypinum Ehrenberg, 1830 predominated in the channels, whereas Tintinidium sp. and Mesodinium pulex Stein, 1867 dominated in the rivers. Our results support the idea of flood pulse as manly factor driving planktonic ciliates community structure, determining changes in species composition, as well as homogenizing the abundance and diversity of plankton ciliates communities among the environments in the Paraná River floodplain. Financial support by CNPq/PELD and CAPES.

Mitosomal fusion induced by SNARE protein in Giardia intestinalis

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Giardia intestinalis contains reduced mitochondria called mitosomes. It is the simplest known mitochondrion-like compartment containing only 20 different proteins. It has two membranes and similar protein import machinery like mitochondria but it has no DNA and no ATP production. Fe-S cluster assembly is the only known mitosomal function. Elias et al (2008) proposed mitosomal distribution of Sec20 homologue which is a prototypical SNARE protein, that in other cells, mediates vesicular fusion between ER and cis Golgi network. It is a tail-anchored membrane protein exposed on the organelle surface and thus suited for the interactions between individual organelles and/or compartments. Like Elias et al., 2008 using overexpression of HA-tagged protein we were able to detect Sec20 in the mitosomes. In additon, the overexpression induced apparent mitosomal fusion and organelle redistribution with highly reduced growth rate of the transformed trophozoites. In order to characterize targeting signal in Sec20 we have isolated its C-terminal transmembrane domain which is sufficient in targeting reporter protein into mitosomes. This C terminal targeting signal can also be recognized in different cellular system of Saccharomyces

cerevisiae that efficiently transports Giardia Sec20 into mitochondria. Surprisingly, using specific polyclonal antibody on Giardia trophozoites we were able to detect solely the endoplasmic reticulum distribution of the native form of Sec20 with no signal in the mitosomes. In order to resolve this, we are currently working on two scenarios: (i) Sec20 is a protein with dual (the ER and mitosomal) distribution, cellular distribution of which is carefully balanced or (ii) it is a resident ER protein which under certain experimental conditions may induce mitosomal fusion. In any case, Sec20 seems to be short but invaluable peek into the biogenesis of the simplest known mitochondria.

Putative peptidase in the mitosomes of Giardia intestinalis

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The proteome of Giardia mitosomes is represented by twenty proteins, which are imported from the cytoplasm, as mitochondrial genome is missing. The N-terminal sequences, which target proteins to mitosomes are removed upon translocation and the remaining protein is folded into its native conformation. Out of three types of mitochondrial processing peptidases only functional half of dominant matrix peptidase is present in the mitosomes of G. intestinalis – feature unique among all eukaryotes. Based on the proteomic analyses, we identified novel putative mitochondrial peptidase (peptidase X) in G. intestinalis mitosomes. Overexpressing the protein with hemagglutinin tag we confirmed its mitosomal localization. Using specific polyclonal antibody combined with carbonate extraction and protease protection assay we have specified the distribution of the protein in the mitosomal subcompartments. Based on these results, peptidase X is anchored in the inner mitosomal membrane via its C-terminus facing the mitosomal matrix. Peptidase X has mitochondrial targeting sequence on the N-terminus, which is also capable of targeting protein into yeast mitochondria. By using bioinformatic tools we are trying to detect structural similarities of peptidase X to other proteins in order to identify its substrate specificity and thus its role in the metabolism of extremely reduced mitochondria of Giardia intestinalis.

Encystation in Acanthamoeba: Dynamics on the protein level

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Acanthamoebae are ubiquitously occurring potentially pathogenic protozoa. Encystation is their main survival strategy in the environment and also plays an important role in pathogenesis, because cysts surviving treatment within the tissue can lead to re-infection. The aim of this study was to elucidate the main factors triggering encystation in Acanthamoeba on the protein level. Firstly, it was shown that the encystment potential is lost during long-term axenic culture. Moreover, this loss was shown to occur shortly after transfer into axenic culture and to be reversible by passage through human cell monolayers and also by treatment with AzaC, a methyltransferase inhibitor, and TSA, a histone deacetylase inhibitor, indicating a participation of epigenetic mechanisms. The onset of encystation was blocked by the protease inhibitors PMSF and E64d, however not by the protein synthesis inhibitor cycloheximide. Proteases, including the encystment mediating serine proteinase (EMSP), have been demonstrated to be involved in encystation. Interestingly, we detected the gene coding for EMSP and also its expression in all investigated strains of morphological group II and III, but not in morphological group I. Moreover, it was shown that the encystment process in Acanthamoeba is of a bipartite nature, most changes in protein content, characterized by autolysis and protein degradation, occurring very early in the process. In this stage levels of translation elongation factor 2 (EF2) are sharply decreased, indicating a low rate of protein synthesis. The most prominent proteins in this stage are truncated actin isoforms. In the advanced stage, EF2 levels and the trophozoite proteome are partly restored accompanied by the expression of encystation-specific genes.

Testate amoebae spatiotemporal dynamics of biogenic silica pools and their relevance for desilication

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Biogenic Si pools in soils can be separated into phytogenic, microbial and protozoic pools [1]. Only

few papers focused on protozoic Si pool in soil, revealing that its size appears to be comparable to the phytogenic Si pool [2]. The protozoic Si pool is represented by testate amoebae, unicellular eukaroytes covered with agglutinated exogenous mineral material (xenosomes) or endogenous material (idiosomes) such as SiO2. Our objective is to clarify the interactions between dynamics of biogenic Si pools and desilication rates in transient state soil systems. The dynamics of the zoogenic Si pool (testate amoebae) in soil will be quantified as a function of plant pattern dynamics. An artificial water catchment built as a terrestrial hillside and located in a reclaimed lignite mining area in Lower Lusatia (Germany) is used as investigation site. First results [3] revealed that substrate removed from small vegetation patches (containing organic matter) revealed tenfold higher amoebal densities compared to uncovered, sandy sites which were only a few cm apart. A cluster analysis separated these colonization patterns into covered and uncovered microsites. However, larger-scaled, consistent processes dependent on environmental parameters or gradients were not visible, as demonstrated by redundancy analysis. These preliminary results demonstrated that the immigration and colonization process of testate amoebae on newly exposed soil surfaces seems to be highly dependent on plant pattern dynamics. As a next step, silica contents of tests from different testate amoebae species will be analyzed using energy-dispersive X-ray spectroscopy combined with scanning electron microscopy (SEM-EDX). The parameters affecting testate amoebae densities will be identified in plot and lab experiments. The presumed carbon, water, nutrient and Si limitation on amoebal growth will be tested using a randomized block design established on an experimental site nearby the catchment area. The influence of silica supply on testate amoebae (idiosome growth) will be clarified in lab experiments under controlled conditions using clonal cultures. [1] Sommer et al. (2006) J Plant Nutrition and Soil Science 169: 310-329; [2] Aoki et al. (2007) Geoderma 142: 29-35; [3] Wanner & Elmer (2009) Acta Protozool 48: 281–289.

Documenting the marine free-living ciliate diversity of northern China

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Over the past 20 years about 510 species of marine free-living ciliates have been recorded in the coastal waters of northern China, including ~150 new species, 36 new genera, 8 new families and > 100 new combinations. According to Finlay et al. (1998) the global number of marine free-living ciliate species is ~1590. Therefore, based on this estimate, ~30 % of all marine free-living ciliate species have been recorded in this one relatively small region. However, the true size of global marine ciliate species diversity is open to debate. For example, it has been estimated that there are over 1230 benthic species, and over 1200 (mostly planktonic) tintinnid species, alone (Azovsky and Mazei, 2007; Lynn, 2008). Furthermore, the World Register of Marine Species lists 2,615 (free-living and non-free-living) species of marine ciliates. In order to document the marine free-living ciliate diversity of northern China, a website has been established (www2.ouc.edu.cn/akfs/ciliate/asp/). This site includes descriptions, line diagrams and, in most cases, photomicrographs of 320 selected species. There is also an extensive bibliography and glossary. All text (apart from the glossary) is in both English and Chinese languages. It is intended that the website will be updated periodically when additional taxa are recorded. In this talk I will introduce the website with examples of some of the species included. Estimates of global marine ciliate species diversity will also be discussed. This work was funded by the Darwin Initiative, the Royal Society Joints Projects programme and the NNFC, China. References: Azovsky AI & Mazei YA (2007). Protistology 5: 13 Lynn DH (2008). The Ciliated Protozoa. Springer, 605 pp. Finlay BJ, Esteban GF & Fenchel T (1998). Protist 149: 29-37.

Taxonomic novelty of heterotrophic protists revealed by unamended brackish water incubations from the Baltic Sea

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Currently, our knowledge of the protistan inventory of freshwater and marine systems is increasing very fast driven by molecular surveys and next generation sequencing approaches. This has lead to a considerable discrepancy between the taxa known from cultures and those known from environmental studies. Therefore, it is often difficult to assign an ecological function to new taxa detected by culture-independent molecular approaches. The aim of our study was to link molecular data on the basis of 18S rRNA sequences with a heterotrophic life style.

Therefore, we performed incubation experiments from a brackish coastal site in the South Western Baltic Sea. The incubation conditions (in situ temperature, filtration, darkness and no nutrient addition) were chosen to promote the growth of heterotrophic protists while preserving their community structure. By daily monitoring, a general succession could be detected which was characterized by a numerical decrease of phototrophic organisms, a peak of bacteria after 3 days and a tenfold enrichment of heterotrophic flagellates (HF) after 5 to 6 days. The construction of clone libraries from enriched HF samples revealed both sequences which are related to cultured representatives like Paraphysomonas sp. and a larger proportion of yet uncultured protists which in some cases have seldom been found in environmental surveys. Most of our sequences affiliated to chrysophytes and choanoflagellates with a surprisingly high novelty degree. Moreover, we found many cercozoans, putatively mixotrophic "novel photosynthetic stramenopiles" and picobiliphytes. We assume most of the found taxa to be heterotrophic or mixotrophic protists. Nevertheless, their in situ relevance and ecological features have to be resolved in the future by newly designed probes for fluorescent in situ hybridisation combined with targeted grazing experiments.

The diversity of naked amoebae in soil along a gradient of forest management intensities

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Protozoa have been shown to exert significant effects on bacterial turnover, diversity and function in soil, being responsible for high levels of nutrient release from consumed bacterial biomass and subsequent positive effects on plant growth. Among soil protozoa, naked amoebae are considered as the most important grazers of the bacterial biofilms and colonies attached to soil and root surfaces, which make up to the majority of bacteria in soil, because due to their tiny pseudopodia, amoebae can reach bacterial colonies in soil pores inaccessible to most other bacterivores. However, despite laboratory experiments and food-web models repeatedly confirmed the functional importance of amoebae in soil systems, we have only a vague idea about the abundance and diversity of amoebae in the field. Within the DFG-Project "Biodiversity Exploratories" we investigated the influence of different levels of land use, and management intensity on the biodiversity of naked amoebae in forest soils. Soil samples were taken from the upper 10 cm of mineral soil at different forest management intensities, ranging from intensive monocultures to unmanaged "natural" forests. The biodiversity of naked amoebae was investigated both by cultivation methods and molecular techniques. We will present our first results, documenting a highly diverse community of naked amoebae and their dependence on the levels of land use.

Temperature dependent resistance to starvation of the freshwater ciliate Meseres corlissi Petz and Foissner, 1992 (Ciliophora, Spirotrichea)

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Aquatic protists are generally subject to large fluctuations in their food supply and are adapted to a 'feast and famine existence'. The nutritional status of protists is affected by temperature, i. e. an interactive effect of temperature and food concentration had been demonstrated for freshwater ciliates. The threshold food concentration needed to sustain a population increases over-proportionally at high temperatures. Accordingly, if food is scarce, high temperature should impose a stronger stress than low temperature. The present study investigates the temperature effect on the fitness and survival of the freshwater ciliate Meseres corlissi under food deplete conditions. We compared a M. corlissi clone isolated from warm temperate Australia to two clones isolated from cold temperate Austria over a temperature range from 15 to 27.5 °C. In particular, we wanted to determine the temperature dependence of the point of no return, at which cell division and thus survival has become impossible. Since M. corlissi, like many other ciliates, is able to form resting cysts, we also monitored the production of cysts in the course of our starvation experiments. Our results demonstrate (i) a general, non-linear temperature effect on survival of M. corlissi under food deplete conditions and (ii) significant differences between the warm temperate and cold temperate clones.

Life cycle and ecology of Bromeliothrix metopoides Foissner, 2010, an enigmatic ciliate (Protista, Colpodea) from tank bromeliads (Bromeliaceae)

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Bromeliothrix metopoides was discovered in tank bromeliads from Central and South America and recently been described as a new species (Foissner, 2010). This ciliate has a complex life cycle with Metopus-shaped, bacterivorous theronts and trophonts (microstomes) and obovate, flagellate-feeding macrostomes with a large, triangular oral apparatus. Two types of resting cysts are frequently formed. Reproduction occurs either by binary fission or polytomy, producing a motile "division chain" composed of four globular cells. Although the species is wide-spread in various tank bromeliads, can be easily cultivated on a variety of freshwater and terrestrial media, and should be able to sustain periods of adverse environmental conditions, it did not occur in ~2,000 soil and freshwater samples investigated globally. We performed ecophysiological laboratory experiments to investigate its peculiar ecological role. Our results demonstrate that B. metopoides shows characteristics of a typical r-strategist under optimal food conditions, reaching several doublings per day. With bacteria as the sole food, the ciliate does not form macrostomes and can be maintained easily at high bacterial levels. However, populations declined if bacterial numbers fell below 10*8 cells/ml. In the presence of flagellates of the genus Polytomella, macrostomes are always present but rarely exceed 10 % of the total ciliate population. Our results suggest mutual interactions between the ciliates and the flagellates. Another reason that may restrict its occurrence is that B. metopoides prefers moderately acidic to alkaline conditions (pH 5.0 to > 9.0), but does not tolerate pH ≤ 4.0.

Simultaneous effect of two predators on prey abundance: Laboratory-microcosm studies with protists

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Predation is one of the most important factors affecting structure and functioning of ecosystems. The effects of predators are not limited to direct consumption of their prey. Various non-consumptive effects, like induced defences, may indirectly affect other members of a community. In natural communities, prey are usually exposed to many predator species. However, their joint effect is difficult to pre-dict from single-predator studies. Interactions among predators may either increase or reduce their joint predation rate. Laboratory microcosms with protists are well suited to study such phenomena. A simultaneous effect of two predatory ciliates with different feeding strategies: Urostyla grandis and Homaloozoon vermiculare, on the abundance of the ciliate Euplotes daidaleos has been studied. The prey species is known for its induced morphological defence capability. In the presence of U. grandis, the prey strongly increased cell width, significantly reducing predation rate. The other predator, H. vermiculare, preyed much more effectively on E. daidaleos inducing only slight morphological response of the prey. However, the efficacy of H. vermiculare was significantly reduced due to the prey transformation induced by the presence U. grandis. Two different experimental approaches: substitutive and additive models produced different results. Their comparison demonstrates that the predation rate may be significantly reduced as a result of indirect trait-mediated effects when the two predators are simultaneously present.

Explaining a paradox: Response of ciliates and microbes to nutrient addition in a highly oligotrophic system

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The Gulf of Aqaba is characterized by very high ciliate diversity despite extremely low productivity. At times when chlorophyll levels were ≤ 0.3 µg/L, 30 – 40 ciliate species could be found, although total ciliate abundance was usually ≤1 ind/mL. In order to explore the mechanisms which allow high diversity in a low productivity system, experiments were conducted that examined both the roles of nutrient limitation and predation in structuring the protist and microbial community. Size-fractionated samples (< 64 µm, < 6 µm, < 0.8 µm) were cross-classified with the addition of nutrients (3 µM N, 0.19 µM P) in experiments that were run during summer stratification, winter mixing and during the spring Synechococcus bloom. The effects of adding small nutrients pulses resulted within 24 h in not only higher algal biomass, but also 2 to 10 times higher ciliate biomass. Apparent ciliate species richness also increased with the addition of

nutrients, as species beneath the counting threshold in treatments without nutrients also increased in abundance. The increase in ciliate biomass, however, was not reflected in a measurable increase in grazing pressure on autotrophs, indicating the extreme bottom-up limitation of this system. Adding nutrients not only increased ciliate biomass and species richness, but also increased the dissimilarity between replicate bottles. While the total increase in biomass between replicates was quite similar between replicates when nutrients were added, the community composition was highly variable. The high dissimilarity between added-nutrient replicates could explain the unexpectedly high diversity in the water column. If nutrient release in the water column occurs at a fine spatial scale, the extremely high potential growth rates of ciliates would ensure a rapid increase in ciliate abundance, with a random assortment of the species present in the area of the nutrient release. Thus, the high diversity of this highly oligotrophic system could be the result of metacommunity dynamics operating at a small scale and without defined boundaries between local communities.

Effects of inhibitors on the antioxidant system of Spironucleus vortens

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Redox balance is essential in all living organisms. It is especially important in metabolic processes, whereby substrates are sequentially oxidised to yield energy, and reactive intermediates, particularly one electron reduction products of oxygen, are further reduced to a non-toxic product. Antioxidants, reactive oxygen species (ROS) and the redox coupler NAD(P)+/NAD(P)H are the main components of this process. Disruption in the delicate balance of these oxidised and reduced intracellular components can have devastating consequences on key cellular processes and lead to apoptosis. As a result, this process is conducively attractive to chemotherapeutic challenge. We investigated the redox system of the protozoan fish parasite Spironucleus vortens by fluorimetry and two-photon laser-scanning microscopy using fluorescent probes targeted to key intracellular redox components. In addition, the major antioxidant thiols present in S. vortens were characterised by HPLC. The effects of novel and traditional chemotherapeutics, auranofin (AF), garlic-derived compounds and metronidazole (MTZ), respectively, were investigated by these methods. Results gained provide insight into the sophisticated antioxidant system of S. vortens, whereby glutathione (GSH) is the major component (0.77 ±0.39 µmol/107 cells). MTZ-treatment (50 µM) oxidised the NAD(P)H pool, whereas AF (50 µM) oxidised the GSH pool and evoked a burst in H2O2 production by the parasite. Garlic-derived compounds (1 mM) had a long-term effect on the antioxidant system of S. vortens. Continuous monitoring during live cell imaging using two-photon laser-scanning microscopy provided spectacular insights into the dynamics and mechanisms of maintenance of normal intracellular redox balance within S. vortens.

Spironucleus vortens: A proteomic study of novel and traditional chemotherapeutic agents

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The flagellated diplomonad, Spironucleus vortens, is an economically important parasite of ornamental fish. Systemic infection can result in 100 % mortality of fish stocks and is associated with the characteristic condition of 'Hole-in-the-head' disease. As the drug of choice for most anaerobic microbial infections, metronidazole (MTZ) is traditionally employed in the treatment of Spironucleosis. However, its ban from use on outdoor European fish farms, due to its persistent and putatively carcinogenic nature, means that this drug becomes an inappropriate choice amongst ornamental aquaculturists. Allium sativum (garlic)-derived compounds, have previously been shown to inhibit S. vortens growth in vitro. As a result, we investigated the combinatorial effect of ajoene oil, extracted from garlic, with MTZ, in an attempt to decrease the minimum inhibitory concentration (MIC) of this drug against S. vortens. The contents of the oil were analysed by GC-MS and the most potent compounds present, ajoene, allyl alcohol (AA) and diallyl disulphide (DADS), were used to determine their effects on S. vortens morphology by SEM. Proteomic analyses were employed to uncover the mode of action of AA, DADS, Ajoene and the nitroimidazoles MTZ and tinidazole (TNZ) against the parasite. Proteomic methods include 2D gel electrophoresis (2DE) for analysis of the parasite proteome, and quantification of total intracellular thiol content by Ellman's assay. Ajoene oil and MTZ displayed synergistic activity against S. vortens, with a fractional inhibitory concentration (FIC) value of 0.6 reducing individual MIC values by 70 – 80 %. Allyl alcohol had the greatest effect on parasite morphology over

time, with most cells unrecognisable after 24 h treatment with 700 µM by SEM. 2DE revealed enolase to be one of the MTZ-shifted proteins, which is consistent with previous findings for Trichomonas vaginalis, a related microaerophilic flagellate. Furthermore, TNZ depleted intracellular thiols. Shifts observed for garlic-treated cells were not reproducible; however DADS also depleted intracellular thiols as observed previously in Candida albicans. These data demonstrate the potential application of ajoene oil in combination with attenuated concentrations of MTZ as a treatment of Spironucleosis in fish.

Direct and indirect competition among heterotrophic nanoflagellates

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Understanding the mechanisms of species coexistence and hence the maintenance of diversity is a central theme of ecology and evolution. The most important processes influencing the distribution and abundance of species are competition and predation. Many field experiments have shown that interspecific competition effects abundance and reproduction of species and predation has been shown to either promote, inhibit, or have no effect on competitive interactions. In this study we wanted to determine the direct and indirect competition between four species of marine heterotrophic nanoflagellates differing in their feeding behaviour and therefore are expected to occupy different ecological niches. We hypothesised that the two species feeding on benthic bacteria („benthic" species) and the two species ingesting bacteria from the pelagial („pelagic" species) compete with each other for resources. Both pairs of species and all four species together were kept in 8-well-slides for 10 days. The results revealed a dominance of one „benthic" and one „pelagic" species in each case, being also the highest abundant species in the trial with all four types of flagellates. It might be that the competitive advantage for these species lies in a more effective mechanism of food uptake and thus leads to higher growth rates compared to the others. To test whether apparent competition could be detected all trials were repeated adding a common predator to the system. As a result still the same species dominated the community but displayed much higher growth rates than in the absence of a predator. A possible explanation for this phenomenon could lie in a better access to resources for the superior species due to the reduction of competitors by the predator.

An in vitro model of neonatal porcine coccidiosis: Isospora suis in an epithelial cell culture system

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To gain knowledge about the interaction between parasites and their host cells animal models and experimental infections may not always be sufficient. A reproducible in vitro cultivation system in representative cell lines offers the possibility of research on cell-cell interactions – like invasion, evasion and defence mechanisms of the host cell – and also on mechanisms of pathogenesis. Moreover, highly purified parasitic material can be obtained from such a system and new drugs can be tested in advance to animal testing. To benefit from those advantages an in vitro system in a porcine epithelial cell line from the neonatal jejunum (IPEC-J2) was established for Isospora suis, an intracellular protozoan parasite causing neonatal porcine coccidiosis. This disease is distributed worldwide and causes significant losses in pig production, mainly by severely reduced weaning weights of affected piglets. The establishment of the in vitro system included the setup of optimal purification procedures for the oocysts, of an excystation protocol and culture conditions. Cells were infected with a ratio of cells : sporozoites of 10:1 in DMEM medium with 1.25 % FCS. All developmental stages described for in vivo infections could be detected in the cell culture (meronts and merozoites of type I and II; micro- and macrogamonts and -gametes; oocysts). Merozoites harvested from the culture are used as antigen for the detection of specific antibodies in piglets and sows and for restimulation experiments with lymphocytes from infected pigs. Highest numbers of oocysts were harvested at day 12 post infection with a sporulation rate of 15 – 57 %. Jejunal epithelial cells of neonatal piglets are the target cells of I. suis. Therefore, this system provides an appropriate in vitro model of neonatal coccidiosis, a disease with significant economic impact in swine production. At the moment the establishment of life imaging is in progress to investigate invasion mechanisms of the parasite and the modification of the host cell in detail. Moreover, a co-cultivation system with various leukocytes is under development to monitor the interaction between infected host cells and the adaptive as well as the innate immune system of the pig.

Protist diversity in suboxic and sulfidic waters of the Black Sea

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The oxic-anoxic transition zone of the Black Sea comprises a large suboxic zone as well as anoxic and sulfidic waters. While prokaryotes and biogeochemical cycles that characterize this zone have been frequently studied, little is known about the diversity or ecology of its microbial eukaryotes. This study presents the first qualitative report of the protist species composition in the Black Sea redoxcline. Fingerprint analysis from the whole redoxcline revealed a complex community structure of metabolically active protists with distinct shifts along the redox gradient. Additionally, 18S rRNA clone libraries were used to compare protist species composition of suboxic and sulfidic water layers. Among the ciliates, sequences related to Pleuronema and Strombidium were dominant in both water layers whereas sequences affiliated with anaerobic plagiopylids and Cyclidium were detected only in the sulfidic zone. Among the flagellates, mainly stramenopiles (mostly bicosoecids and chrysophytes) occurred throughout the redoxcline. In the sulfidic zone we found stramenopilan sequences but also euglenids, jakobids and choanoflagellates which were related to clonal sequences from other anoxic marine habitats, thus indicating the existence of globally distributed groups of anoxic flagellates. Higher species richness in the sulfidic zone and about twice as many new sequence types of ciliates and stramenopiles compared to the suboxic layer emphasizes the importance of anoxic, sulfidic waters as habitat for high protist diversity although with yet unknown functions.

Redescription of two marine planktonic tintinnids, Eutintinnus apertus (Kofoid & Campbell, 1929) Kofoid & Campbell, 1939 and Favella campanula (Schmidt, 1901) Jörgensen, 1934

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Historically, identification and systematics schemes for tintinnid ciliates have largely been based on lorica features and virtually ignored zooid morphology. However, the polymorphism of this structure due to the life cycle and environmental conditions is known which makes the identification of tintinnid ciliates problematic. In the 1,200 previously reported tintinnid species, only 20 species are described based on the cytological features, including the ciliary pattern revealed by protargol staining. We here redescribe two further tintinnid species, Eutintinnus apertus (Kofoid & Campbell, 1929) Kofoid & Campbell, 1939 and Favella campanula (Schmidt, 1901) Jörgensen, 1934 based on the lorica as well as cell features. Eutintinnus apertus can be differentiated from its congeners by having only two macronuclear, one dorsal kinety and two posterior kineties. Favella campanula can be separated from Favella ehrenbergii (Claparède and Lachmann, 1858) Jörgensen, 1924 whose infraciliature was recently reported by the numbers of somatic kineties as well as collar membranelles.

Comparative ultrastructure of Fornicata excavate, Kipferlia bialata

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The Fornicata is a group of excavate microeukaryotes consisting of free-living (e.g. Carpediemonas) and parasitic (e.g. Giardia and Retortamonas) lineages that share several molecular and ultrastructural characteristics. Fornicates tend to live in low oxygen environments and contain reduced mitochondria in the form of hydrogenosomes or mitosomes. Up until 2007, Carpediemonas membranifera was the only free-living sister lineage of diplomonads and retortamonds; four additional Carpediemonas-like organisms (CLOs) have been described since then: Dysnectes brevis, Hicanonectes teleskopos, Ergobibamus cyprinoides and Kipferlia bialata. Molecular phylogenetic analyses of small subunit (SSU) rDNA sequences from free living fornicates and environmental sequences demonstrated that CLOs comprise six different lineages that diverge early within the Fornicata. Improved understanding of this group is expected to elucidate the early evolution of fornicates and the highly modified mitochondria found within this group. Although C. membranifera, D. brevis, H. teleskopos, E. cyprinoides have all been studied at the ultrastructural level, K. bialata has not. Only light micrographs and a single transmission electron micrograph have been reported from this taxon so far. K. bialata is part of a diverse subclade of CLOs that is represented mostly by environmental SSU rDNA sequences. We characterized K. bialata in detail in order to understand the ultrastructural features associated with this clade, paying particular attention to the organization of the microtubular cytoskeleton (e.g., flagellar apparatus). The combination of

ultrastructural characteristics in this taxon was distinctive among CLOs and provided insights into the evolutionary history of fornicates as a whole.

Dissecting biomineralization by mining the transcriptome of two closely related coccolithophorids, Emiliania huxleyi and Isochrysis galbana

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Coccolithophores are one of the most spectacular calcifying microalgae. While the calcareous skeletons otherwise known as coccoliths, have attracted the attention of scientists from diverse fields, information relating to the function and molecular complexity of the underlying biomineralization processes is lacking. The manner in which macromolecules, in particular proteins, orchestrate the crystal growth processes and dictate the nanoscale architecture of the coccoliths is not known. Hence, the broad and long-term objective of our laboratory is to understand the molecular underpinnings of biomineralization and the nanoscale shape and patterning of the calcite plates characteristic of marine coccolithophores. The major hypothesis underlying this research is that the design principles governing the synthesis of coccoliths can only be determined by identifying and characterizing the genes and gene products involved in their synthesis and assembly. Emiliania huxleyi is recognized as the model coccolithophore because of its abundance, cosmopolitan distribution, and the ease with which it can be cultured. Its genome was recently sequenced in a collaborative effort between our laboratory and the U.S. Department of Energy, making it feasible to apply various global approaches to explore biomineralization. We have been dissecting biomineralization and the regulatory mechanisms required to coordinate this complex process by applying a comparative transcriptomics approach. To this end, we used high-throughput Solexa sequencing to interrogate the transcriptome of E. huxleyi and its non-calcifying sister species, Isochrysis galbana under nutrient conditions known to affect biomineralization. Cells were grown in artificial seawater media containing 1) 0 mM Ca^{2+}, 2) 9 mM Ca^{2+}, 3) 0 mM Ca^{2+} spiked with 25 mM NaH_{CO3}, and 3) 9 mM Ca^{2+} spiked with 25 mM NaH_{CO3}. Rates of photosynthesis and calcification were monitored every other day for 7 days at which time total RNA was extracted and prepared for sequencing. Transcriptional programs driving biomineralization were revealed by examining changes in gene expression patterns under the different growth conditions, and by comparing profiles across species. Independent validation of candidate biomineralization transcripts was obtained using real-time PCR analysis, and an attempt to identify cis-regulatory elements was made by examining the promoter sequences of similarly expressed sets of genes.

Phylogenetic analyses of trichodinids (Ciliophora, Oligohymenophora, Mobilia) inferred from 18S rRNA gene sequence data

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A total of 24 complete or partial 18S rRNA gene sequences, including our three newly sequenced trichodinid species, were used to construct the phylogenetic trees by Maximum likelihood (ML) and Bayesian Inference analyses conducted respectively by PAUP*4.0b10 and MrBayes 3.1.2 softwares, the result has revealed that: 1) The GC content of 18S rRNA gene plays an important role in the Mobilia ciliates phylogeny and it can be concluded that the lower the GC content was, the earlier the species may differentiate; moreover, in the Mobilia species, the Urceolariid species were first markedly divergent from other Mobilia species, and then so were trichodinid species. 2) After the main clades of phylogenetic trees determined by the GC content have been formed, the second important factor affecting the structure of trees is the denticle morphology especially the blade morphology which is involved with the phenomena that those Trichodina species whose blades are similar would be clustered together in the phylogenetic analyses, namely blade morphology dominance. 3) The present research result has proved the genus Trichodina paraphyletic and suggests that the validity of the genus Trichodinella should be re-evaluated, because the Trichodinella species was always nested within the Trichodina assemblage rather than clustered respectively forming the trichodinella-clade and trichodina-clade. 4) With regard to central granules in the adhesive disc and the relationship between trichodinids and their hosts, we agree to the suggestion that central granules could be not a generic character and also agree on the trichodinids' co-evolution with their host for those trichodinids depending on the phylogenetic analysis. Supported by the National Natural Science Foundation of China (No. 30970329) and the Science Research Foundation of the Education Committee of Chongqing (No. KJ090814).

Taxonomic study on three trichodinids (Ciliophora, Oligohymenophora, Mobilia) isolated from Siluriformes fishes in China

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During the parasitological survey in China, three species of trichodinid ectoparasites (Ciliophora, Oligohymenophora, Mobilia) subjected to the three genera, Paratrichodina Lom, 1963, Tripartiella Lom, 1959 and Trichodina Ehrenberg, 1830, respectively were isolated from the Siluriformes fishes collected from China. One species from Ietalurus punetaus was believed to be new species, named as Paratrichodina rotundiformis sp. nov. and Paratrichodina rotundiformis sp. nov. is characterized by the undeveloped and round blade with round tangent point, undeveloped triangular central part, slim ray with inclining backward and the whole fitting loosely denticle. The other two species were Tripartiella orthodens Basson & Van As, 1987 and Trichodina matsu Basson & Van As, 1994. Tripartiella orthodens Basson & Van As, 1987 from Pelteobagrus nitidus is redescribed as a first record in Asia and Trichodina matsu Basson & Van As, 1994 from Pelteobagrus vachelli is also established as a first record in Chinese Mainland. Three populations of Trichodina matsu fall within the range of morphometry and agree closely in the overall appearance of the adhesive disc. The population comparative study was presented detailedly in the present work for Trichodina matsu. Descriptions presented here were obtained by examinations of specimens prepared using the dry silver nitrate impregnation and the methyl green-pyronin staining. Comparisons with closely related species are provided for the new species. Supported by the National Natural Science Foundation of China (No. 30970329) and the Science Research Foundation of the Education Committee of Chongqing (No. KJ090814).

Biodiversity, taxonomy and evolution of "Heliozoa"

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Heliozoa is a group of predatory axopodiate protists which are phylogenetically not related, but for many years were treated as a natural taxon. The presence of axopodia gives cells a characteristic sun-like shape, and microtubules in their axopodia normally are arranged in a three-dimensional lattice. Heliozoans are both marine and freshwater inhabitors and have no skeleton at all, or their skeleton elements (scales, spicules or organic capsule) is external (this basically differ them from Radiolaria). Skeleton elements are very diverse; shape of scales may be a good species marker. As were recently shown naked forms are secondary in evolution of centrohelid heliozoans and switches from siliceous scales to organic spicules occurred at least twice. It was long believed that heliozoan skeleton consists either of siliceous or organic elements, but recently I have described the species Raphidiophrys heterophryoidea combining siliceous scales and organic spicules, as shown by energy dispersive X-ray microanalysis. It is evident that the biodiversity of heliozoans is heavily undersampled; for example, even our brief examination of heliozoan fauna of Valamo island discovered three new species of centrohelid heliozoa; all on the basis of scale structure. Unknown diversity could be represented with small species (about 5 – 10 μm), hardly identifiable with routine optics; for example, recently I have described the smallest known heliozoan – Choanocystis minima, which is only 3 μm in diameter. "Heliozoans" are now spread all over eukaryotic tree in different supergroups: actinophryid heliozoans (in Chromealveolates), desmothoracid heliozoans (in Rhizaria), centrohelid heliozons (in Chromalveolates, close to Haptophyta and Cryptophyta), rotosphaerids (in Opistokontha). Purely studied gymnosphaerids are supposed to be in Rhizaria, but never were molecularly studied. So, the presence of axopodia is not a solid base for establishing of higher taxa. Pseudopodia of rotosphaerids are filopodia and have no internal microtubules. From the other hand axopodia were found in some alveolates (in sporozoan piroplasmid gametes and in suctorian ciliates). Evidently, "Heliozoa" is a group, which exists only for historical reasons. Those organisms are not evolutionary related.

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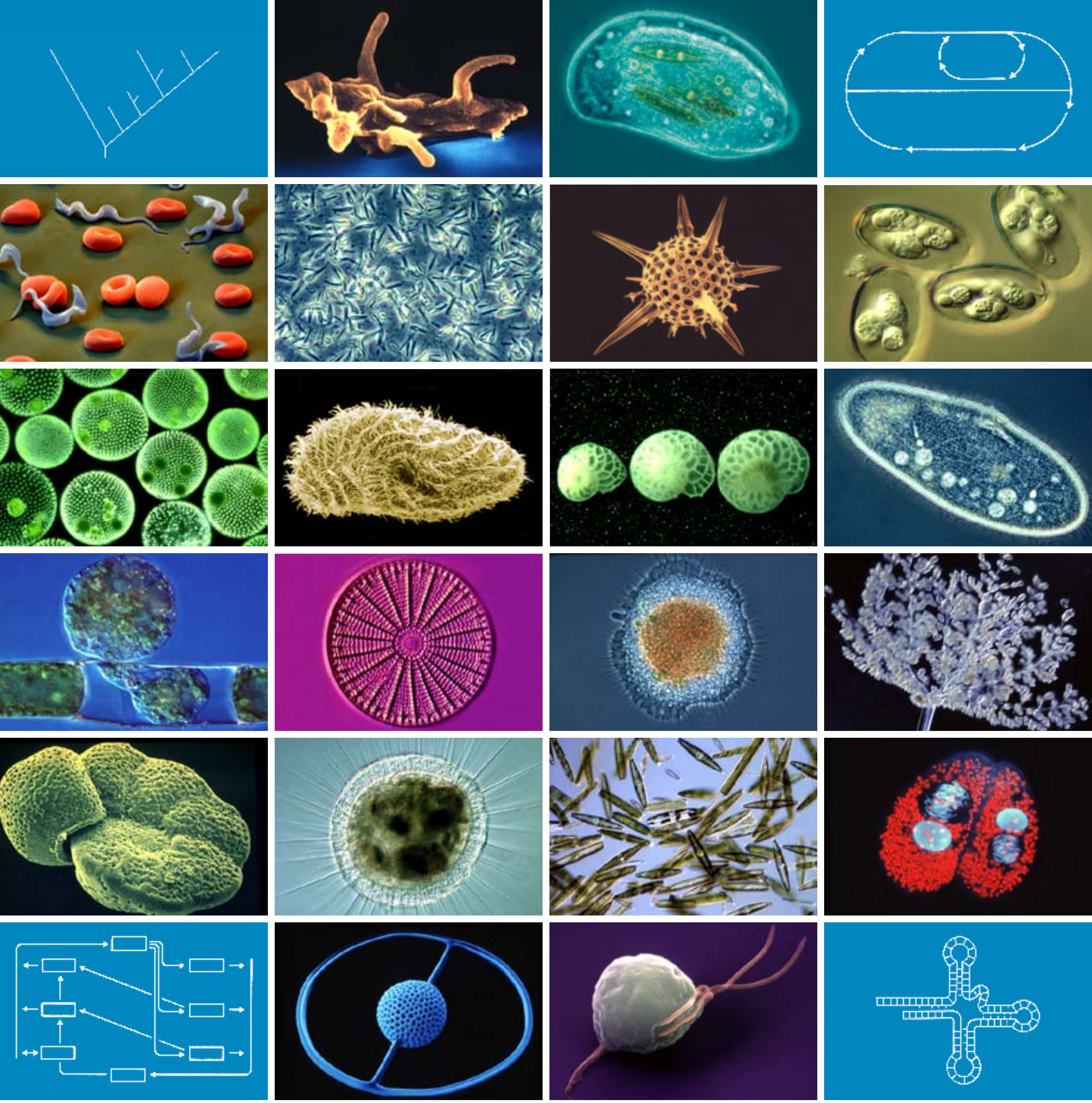

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