Protist2012

OSLO 29 July - 3 August

INTERNATIONAL SOCIETY FOR EVOLUTIONARY PROTISTOLOGY

UNIVERSITAS OSLOENSIS MDCCCXI

MLS UiO
Molecular Life Science

UiO : Department of Biology
University of Oslo

UiO : Faculty of Mathematics and Natural Sciences Library
University of Oslo

UiO : Museum of Cultural History
University of Oslo

Roche

BIO-RAD

Conference venues

Protist2012 will take place in three different buildings.

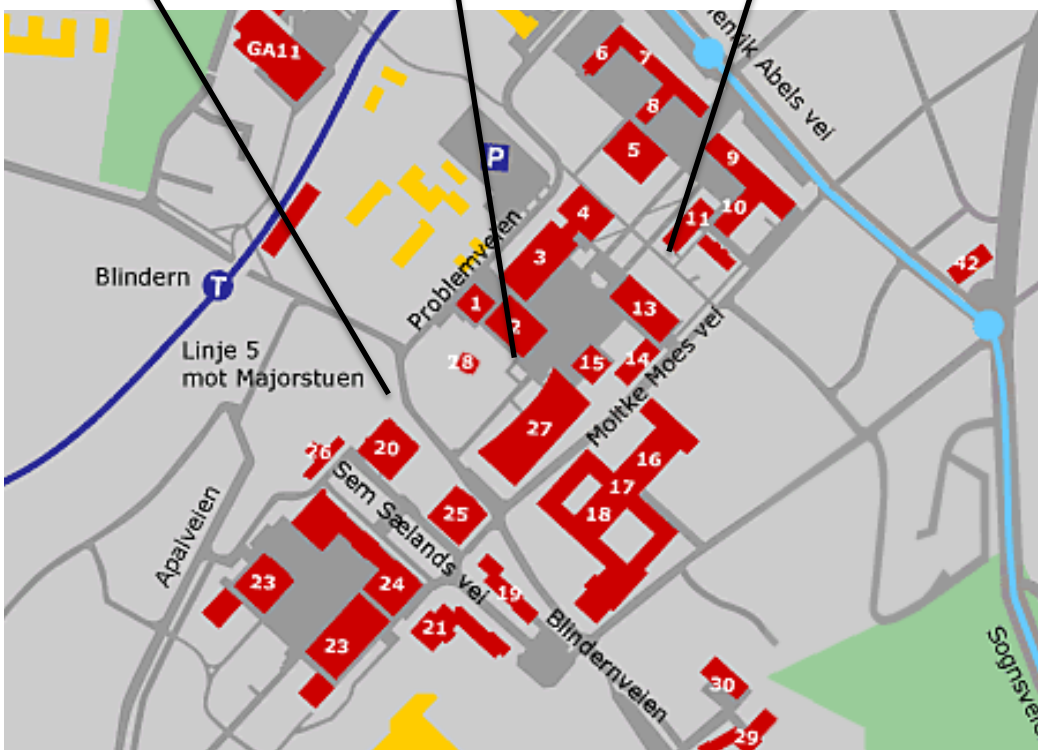
The main location is Vilhelm Bjerknes (building 13 on the map). This is the Life Science library at the campus and is where registration will take place at July 29. This is also the location for the posters and where lunches will be served. In the library there is access to computers and internet for all conferences participants.

The presentations will be held in Georg Sverdrups (building 27) and Helga Engs (building 20)

Helga Engs

Georg Sverdrups

Vilhelm Bjerknes



Map and all photos: UiO

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ISOP – International Society of Protistologists

The Society is an international association of scientists devoted to research on single-celled eukaryotes, or protists. The ISOP promotes the presentation and discussion of new or important facts and problems in protistology, and works to provide resources for the promotion and advancement of this science. We are scientists from all over the world who perform research on protists, single-celled eukaryotic organisms. Individual areas of research involving protists encompass ecology, parasitology, biochemistry, physiology, genetics, evolution and many others. Our Society thus helps bring together researchers with different research foci and training. This multidisciplinary attitude is rather unique among scientific societies, and it results in an unparalleled forum for sharing and integrating a wide spectrum of scientific information on these fascinating and important organisms.

ISOP executive meeting Sunday, July 29, 13:00 – 17:00
ISOP business meeting Tuesday, July 31, 17:30
Both meeting will be held in Vilhelm Bjerknes (building 13) room 209.

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ISEP – International Society of Evolutionary Protistology

ISEP is a division of ISOP, and focuses on functional genomic, evolution and development of protists, and addresses the origin and evolution of the eukaryotic cell and the history of the eukaryote life.

ISEP meeting executive Sunday, July 29, 11:00 – 13:00
ISEP business meeting Monday, July 30, 17:30

Both meeting will be held in Vilhelm Bjerknes (building 13) room 209.

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Local organizing committee

The Microbial Evolution Research Group (MERG) is the local organizing committee for the Protist2012 meeting.

MERG is an interdisciplinary research group focusing on the ecology and evolution of microorganisms, including protists and fungi. MERG started in 2007 as a strategic initiative by the University of Oslo

MERG has scientific staff of 12 (8 professors, 4 associate professors), 6 postdocs, 4 engineers and 1 administrative leader. Our research projects span a wide range of disciplines, including bioinformatics, genomics, statistics and field work. All ongoing projects are listed in our Annual plan for 2010 (http://www.merg.uio.no/).

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General information

Registration will take place at Vilhelm Bjerknes (building 13) on Sunday, July 29 between 17:00 and 19:00.

Oral presentations
Each speaker has been allocated the time indicated in the program. This INCLUDES question time – you should allow 5 minutes for question.

You will ***not*** be able to use your own computer for the presentation. All presentations will be run on a windows or a mac present in the auditorium. Make sure that your presentation is in a PowerPoint or PDF format.

Please name your file as follows: *SessionNumber.LASTNAME.ppt/pptx/pdf.* (e.g. 13D.Krabberod.pptx). The session number is available in program. Please do not use any special symbols (#&%$*@ etc.) when naming your file. You will need to save a copy of your presentation on a USB drive/memory stick.

If your presentation needs to call up additional files (e.g. videos), please ensure that these files work properly and are available for transfer as well. However, we strongly advice against using any files in addition to the presentation as experience has proven that this can be a source of trouble.

Note: Due to a tight schedule please bring your presentation to the information desk in Vilhelm Bjerknes (building 13) ***during lunch the day before*** your presentation. Those of you presenting on Monday should bring the presentation to the information desk on Sunday, July 29 during the reception.

Poster sessions
The poster sessions will be in the lobby of Vilhelm Bjerknes (building 13) on Wednesday, August 1, 12:50-14:10 for those with family names A-M; and Thursday, August 2, 11:50-12:50 for those with family names N-Z. Presenters are expected to remain with the poster for the duration of the poster session.

Posters must be placed prior to the first poster session – no later than 09:00 on August 1. Please notice that the posters may be placed on 2 floors. There are no pre-assigned poster positions: First come – first served!

Access to buildings
Due to student holidays there will be limited access to the buildings after 16:00. Please leave the buildings when the presentations are finished for the day and make sure that you have your name badge with you at all times – especially outside the opening hours of the University.

Wireless networking on Campus
There are several wireless networks at the University:

- conference– accessible to all conference participants. An unencrypted network for short term users. Network key:.
- eduroam – Access given to eduroam users and users with UiO access credentials. Visitors can log in with their username and password from their institution. The username must be written in the form username@institution.domain. Further information about eduroam as well as an overview of the institutions that support it, can be found at www.eduroam.org and http://www.uio.no/english/services/it/network/wireless/help/eduroam/

Lunch
Lunch will be served at noon each day in Vilhelm Bjerknes (building 13) Special dietary needs will be marked with name.

Banquet
The Banquet will take place in *Gamle Logen* Thursday, August 2 from 19:00, More info on page 74

Social Program
Sightseeing will be arranged on Wednesday, August 1 at 15:45, more info page 75

Conference facilities

The main building for the conference is Vilhelm Bjerknes (building13). This is where all lunches will be served and the posters will hang for the duration of the conference. The building houses the Library of Mathematics and Life Sciences. All conference participants are free to use the facilities offered by the library including reading rooms and computers. There are also several electrical outlets where it is possible to charge laptops, cell phones etc.

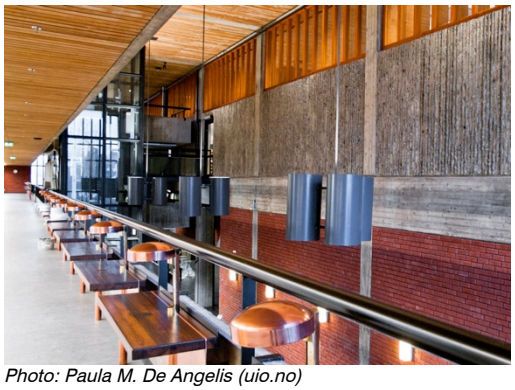


Photo: Paula M. De Angelis (uio.no)

Photo: Universitats

Photo: Anders K. Krabberød

Information boards

In Vilhelm Bjerknes (building 13) there are two information video boards - please check the boards for program updates and other important information.

Photo: Anders K. Krabberød

Computer access

In the library you can get access to the university computers. Please request a temporary login at the reception in the library.

Photo: Anders K. Krabberød

Visitor information

A tourist information guide is provided in the conference bag.

Public transport
The public transport in Oslo is quite efficient with busses, trams and subway (called T-bane) using the same ticket system. If you are using public transport the cheapest solution is to use the electronic smart ticket called Flexus.

You can either buy:

- 1 hour travel NOK 27
- 7 day pass for NOK 220

A single ticket bought on the tram or metro without using the Flexus card costs NOK 50 for one hour. More information including where to buy tickets can be found here http://ruter.no/en/

Parking
Vehicles must be parked in the designated car parks. You will need a parking permit to park at the University grounds (please contact the Protist2012-committee).

Emergency procedures
In case of an emergency an alarm siren is operated. Proceed immediately out of building. You will be advised when re-entry is permissible.

Please familiarize yourself with emergency exits.

Emergency numbers
Fire: 110
Police: 112
Ambulance: 113
University of Oslo Security: (+47)22 85 66 66

The Emergency Ward
Is open 24 hours a day.
Oslo Emergency Ward
Storgata 40
Tel. (+47) 22 93 22 93

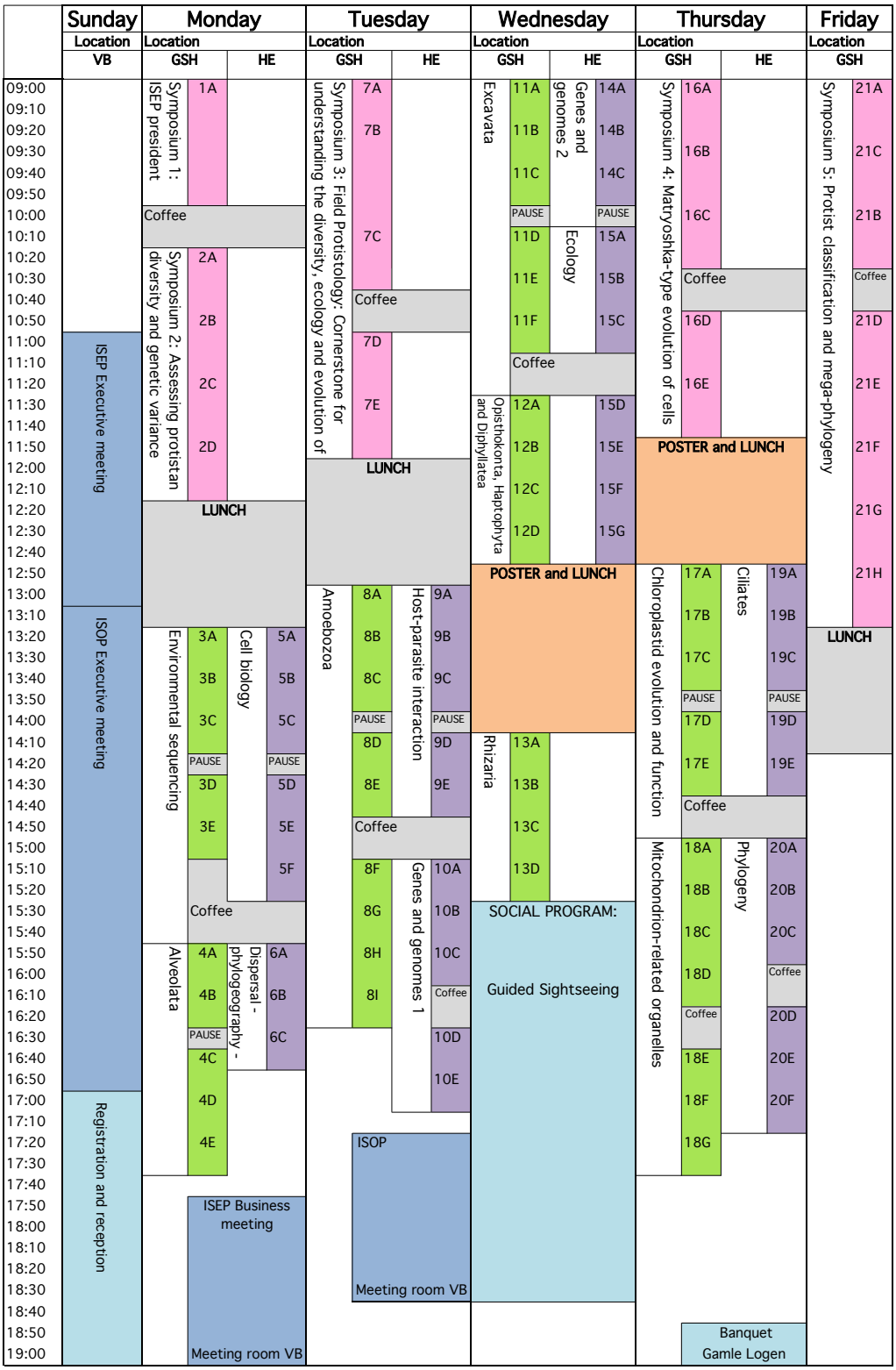
Oslo Akutten casualty clinic (private)
Emergency medical service. Open for everyone. X-ray. Closed on Sundays and public holidays.
Rosenkranzgate 9
Tel. (+47) 22 00 81 60

Pharmacies
Pharmacies (in Norwegian: Apotek) can be found all over Oslo, including campus. Jernbanetorvets Apotek across the street from Oslo Central Station as well as the Apotek 1 at the emergency ward are both open 24 hours a day.

Emergency dental care
Oslo public emergency dental clinic is located on the third floor of Galleriet (next to Oslo Bus Terminal). Pre-scheduled appointments not possible; you must show up in person. For everyone - tourists, children, adults. Payment by bank card only.
Schweigaardsgate 6, 3rd Floor
Tel. (+47) 22 67 30 00

Tourist information center

- Oslo S, Trafikanten, Jernbanetorget 1
- Town Hall, Fridjof Nansens plass 5
- Aker Brygge, Akershusstranda, Skur 35



Meeting room 209 Vilhelm Bjerknes (VB), Building 13

Symposia, Auditorium, Georg Sverderups (GSH), Building 27

Parallel session, Auditorium, Georg Sverderups (GSH), Building 27

Parallel session, Auditorium, Helga Engs (HE), Building 20

Social program

Program summary

Sunday, July 29

11:00 – 13:00 ISEP executive meeting
13:00 – 17:00 ISOP executive meeting
17:00 – 19:00 Registration and reception

Monday, July 30

---------- *GSH/Building 27*

09:00 – 10:00 Symposium 1,session 1:
ISOP president

10:20 – 12:20 Symposium 2, session 2:
Assessing protistan diversity and genetic variance

12:20 – 13.20 LUNCH

----------*GSH/Building 27*

13:20 – 15:30 Parallel session 3:
Environmental sequencing

15:50 – 17:30 Parallel session 4:
Alveolata

----------*HE/Building 20*

13:20 – 15:30 Parallel session 5:
Cell Biology

15:50 – 17:30 Parallel session 6:
Dispersal-phylogeography-diversification

Tuesday, July 31

----------*GSH/Building 27*

09:00 – 12:00 Symposium 3, session 7:
Field Protistology: Cornerstone for understanding the diversity, ecology and evolution of microbial eukaryotes

12:00 – 13:00 LUNCH

----------*GSH/Building 27*

13:00 – 16:30 Parallel session 8:
Amoebozoa

----------*HE/Building 20*

13:00 – 14:50 Parallel session 9:
Host-parasite interaction

15:10 – 17:30 Parallel session 10:
Genes and genomes 1

Wednesday, August 1

----------*GSH/Building 27*

09:00 – 11:10 Parallel session 11:
Excavata

11:20 – 12:40 Parallel session 12:
Opisthokonta, Haptophyta, Diphyllatea

12:50 – 14:10 POSTER SESSION (A-M) and LUNCH

14:30 – 15:30 Parallel session13:
Rhizaria

----------*HE/Building 20*

09:10 – 10:00 Parallel session 14:
Genome evolution

10:20 – 12:50 Parallel session 15:
Ecology

12:50 – 14:10 POSTER SESSION (A-M) and LUNCH

Thursday, August 2

----------*GSH/Building 27*

09:00 – 10:30 Symposium 4, session 16:
Matryoshka-type evolution of cells

11:50 – 12:50 POSTER SESSION (N-Z) and LUNCH

----------*GSH/Building 27*

12:50 – 14:40 Parallel session 17:
Chloroplastid evolution and function

15:00 – 17:40 Parallel session 18:
Mitochondrion-related organelles

----------*HE/Building 20*

12:50 – 15:00 Parallel session 19:
Ciliates

15:20 – 17:40 Parallel session 20:
Phylogeny and classification

Friday, August 3

----------*GSH/Building 27*

09:00 – 13:20 Symposium 5, session 21:
Phylogeny and classification

13:20 – 14:20 LUNCH

Program

Sunday, July 29

11:00 – 13:00 ISEP executive meeting
13:00 – 17:00 ISOP executive meeting
17:00 – 19:00 Registration and reception

Monday, July 30

1

Symposium 1: ISOP president (Session 1)

9:00 – 10:00 Endosymbiosis, plastid acquisition and the chlorarachniophytes
Ken Ishida (1A)

10:00 – 10:20 Coffee break

2

Symposium 2: Assessing protistan diversity and genetic variance (Session 2)

Chair: Vera Tai and Noriko Okamoto
Sponsored by: International society of Protistologists (ISOP)

10:20 – 10:50 Towards a molecular taxonomy: benefits, risks and applications in plankton ecology.
David A. Caron (2A)

10:50 – 11:20 Has there been an 'inordinate fondness for diatoms' and if so why? Insights into diatom speciation and diversification from genetic, mating, and morphological data.
David G. Mann and Pieter Vanormelingen (2B)

11:20 – 11:50 Assessing spatial variation in ecophysiological diversity - does selection shape free-living protist populations?
Chris Lowe (2C)

11:50 – 12:20 From single cells to communities - deciphering the ecology and evolution of microbial symbionts in termite/cockroach hindguts using multiple sequencing approaches.
Vera Tai and Patrick J. Keeling (2D)

12:20 – 13.20 LUNCH

3

Parallel session 3: Environmental sequencing

Chair: Jan Andersson and Alexandra Stock

13:20 – 13:40 Comparison of eukaryotic microbial communities from a broad range of free-living and host-associated environments.
Laura Wegener Parfrey and Rob Knight (3A)

13:40 – 14:00 Global analysis of plastid diversity reveals new lineages of apicomplexan related protists associated with coral reef environments.
Patrick J. Keeling (3B)

14:00 – 14:20 Large scale DNA-based characterization of eukaryotic communities from bromeliad tank waters.
Laura R. P. Utz, Adriana Giongo, Eric Triplett, Raquel Dias, César A. F. De Rose, Claudio A. Mondin, Renata Medina da Silva, Leandro V. Astarita and Eduardo Eizirik (3C)

14:20 – 14:30 10 min break

14:30 – 14:50 The coupling and succession of protists in the seaice and water column in an Arctic fjord.
A.R. Meshram, L. Kuckero, A. Vader, K. S.Jacobsen and T.M. Gabrielsen (3D)

14:50 – 15:10 Phylogeny and biodiversity of Phytomyxea (“Plasmodiophorids”).
Sigrid Neuhauser, David Bass and Martin Kirchmair (3E)

15:10 – 15:50 Coffee break

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Parallel session 4: Alveolata

Chair: Øjvind Moestrup and Rosa I. Figueroa

15:50 – 16:10 A calcified cyst-producing dinoflagellate from freshwater: fine-structure characterization and phylogeny.
Sandra C. Craveiro, Mariana S. Pandeirada, Niels Daugbjerg, Øjvind Moestrup and António J. Calado (4A)

16:10 – 16:30 Molecular characterization of *Ichthyophthirius multifiliis*.
Matthew Therkelsen and Wei-Jen Chang (4B)

16:30 – 16:40 10 min break

16:40 – 17:00 *Neospora* spp. and *Toxoplasma gondii* antibodies in donkeys from Southern Italy.
E. Bartova, T. Machacova, K. Sedlak, G. Fusco, U. Mariani, and V. Veneziano (4C)

17:00 – 17:20 New species of gregarine parasites from invertebrates in Japan and the UK.
Sonja Rueckert (4D)

17:20 – 17:40 Species discovery and evolutionary history of marine gregarine Apicomplexans.
Kevin C. Wakeman and Brian S. Leander (4E)

5

Parallel session 5: Cell Biology

Chair: Wenche Eikrem and Birger Skjelbred

13:20 – 13:40 Lectin binding patterns in marine raphidophytes.
Anette Engesmo, Richard Dillaman, Carmelo Tomas and Wenche Eikrem (5A)

13:40 – 14:00 Fine structural observation of shell formation in a rhizarian testate amoeba *Paulinella chromatophora.*
Mami Nomura, Takuro Nakayama, Taizo Motomura, Chikako Nagasato, Ken-ichiro Ishida (5B)

14:00 – 14:20 Evolutionary significance of chlorophyll metabolisms of herbivorous protists in aquatic ecosystems.
Yuichiro Kashiyama, Akiko Yokoyama, Takashi Shiratori, Isao Inouye, Ken-ichiro Ishida and Hitoshi Tamiaki (5C)

14:20 – 14:30 10 min break

14:30 – 14:50 Three-dimensional ultra-structural study of encystment and astaxanthin accumulation in the green alga, *Haematococcus pluvialis.*
Shuhei Ota, Marina Wayama, Nobuhito Nango, Aiko Hirata and Shigeyuki Kawano (5D)

14:50 – 15:10 Different types of locomotive activity in heliozoans.
V. V. Zlatogursky (5E)

15:10 – 15:30 Sex or no sex: The probe for sexual reproduction in fish parasite. *Ichthyophthirius multifiliis.*
Wei-Jen Chang, Ke Xu, Yonghyun Song, Matthew Therkelsen, Donna Cassidy-Hanley and T Clark (5F)

15:30 – 15:50 Coffee break

6

Parallel session 6: Dispersal - phylogeography - diversification

Chair: Tove Gabrielsen

15:50 – 16:10 Intermediate fragmentation per se provides stable predator-prey metapopulation dynamics.
Jen Cooper, Jiqiu Li, David Montagnes (6A)

16:10 – 16:30 The genetic structure of amoeba morphospecies.
A. Smirnov, E. Nassonova, A. Glotova, A. Kudryavtsev, V. Zlatogursky and J. Pawlowski (6B)

16:30 – 16:50 Ecological and biological researches on *Mesodinium rubrum*, one of the major red tide protists in Korea.
Wonho Yih, Hyung Seop Kim, Jong Woo Park, Myung Gil Park, Hae Jin Jeong (6C)

18:00 ISEP business meeting Vilhelm Bjerknes (build. 13) meeting room 209

Tuesday, July 31

7

Symposium 3: Field Protistology: Cornerstone for understanding the diversity, ecology and evolution of microbial eukaryotes (7)

Chair: Naoji Yubuki and Thierry Heger

Sponsored by: International society of Protistologists

09:00 – 09:20 Field Protistology: Importance and challenges.
Thierry J. Heger, Naoji Yubuki and Brian S. Leander (7A)

09:20 – 10:10 Insights provided by recent field studies of protists in low-oxygen/anoxic marine environments.
Virginia Edgcomb and Joan Bernhard (7B)

10:10 – 10:40 Origin of photosynthetic eukaryotes: Inferring traits of pre-green ancestors.
Eunsoo Kim (7C)

10:40 – 11:00 Coffee break

11:00 – 11:30 Meta-protistomics
Colomban de Vargas (7D)

11:30 – 12:00 Evolutionary morphology of uncultivated euglenozoans living in low-oxygen marine environments.
Naoji Yubuki and Brian S. Leander (7E)

12:00 – 13:00 LUNCH

8

Parallel session 8: Amoebozoa

Chair: Smirnov Alexey and Cédric Berney

13:00 – 13:20 Cox I and 18S rDNA genes as barcodes for species differentiation and study of population structure in two lineages of lobose amoebae.
A. Glotova, V. Zlatogursky, A. Kudryavtsev and A.Smirnov, J Pawlowski (8A)

13:20 – 13:40 Exploring slime moulds biodiversity with environmental RNA analysis in high-altitude forests and grasslands.
A. M. Fiore-Donno, A. Kamono, M. Meyer, M. Fukui and T. Cavalier-Smith (8B)

13:40 – 14:00 Entamoeba diversity and prevalence in cockroaches.
Mustafa H. Fakhri, Courtney A. Cagle, and Jeffrey D. Silberman (8C)

14:00 – 14:10 10 min break

14:10 – 14:30 Taxonomical enigma - genus Kelleromyxa (Myxogastria): evidence from the SSU rDNA gene.
Daria A. Erastova, Mikchail V. Okun, Anna Maria Fiore-Donno, Yuri K. Novozhilov and Martin Schnittler
(8D)

14:30 – 14:50 Phylogeny and DNA barcoding of the Himatismenida based on three genes.
Alexander Kudryavtsev, Alexey Smirnov and Jan Pawlowski (8E)

14:50 – 15:10 Coffee break

15:10 – 15:30 Phylogeny and origin of parasitism in Archamoebae.
Eliska Ptackova, Lukas Falteisek, Alexei Y. Kostygov, Lyudmila V. Chistyakova, Alexander O. Frolov, Giselle Walker, and Ivan Čepička (8F)

15:30 – 15:50 Polyphyly of the genus Pelomyxa and general issues in the systematics of Archamoebae.
Alexei Y. Kostygov, Lyudmila V. Chistyakova and Alexander O. Frolov (8G)

15:50 – 16:10 Using behavioral and chemical cues to resolve crypticity in free-living, commensal, and parasitic Entamoeba lineages.
Avelina Espinosa and Guillermo Paz-y-Miño-C (8H)

16:10 – 16:30 Biogeography and diversity of dictyostelid social amoebae in high-latitude environments.
A. L. Perrigo, M Romeralo and S. L. Baldauf (8I)

9

Parallel session 9: Host-parasite interaction

Chair: Sonja Rueckert and Chris Lane

13:00 – 13:20 GTPases of the ROCO family: a component of an ancient eukaryotic immune system?
Marek Elias (9A)

13:20 – 13:40 Host genotype by parasite genotype interactions underlying the resistance of toxic microalgae species to the protist parasite *Parvilucifera sinerae*.
R. I. Figueroa, L. Råberg, E. Alacid, M. Turon and E. Garcés (9B)

13:40 – 14:00 Organellar interactions in red algal host/parasite heterokaryon cells.
Nicolas A. Blouin and Christopher E. Lane (9C)

14:00 – 14:10 10 min break

14:10 – 14:30 Good families gone bad: The origin and evolution of pathogenic genes in the Oomycete secretome.
Ian Misner, Guy Leonard, Eric Bapteste, Philippe Lopez, Thomas Richards and Christopher Lane (9D)

14:30 – 14:50 The molecular machinery behind Trichomonas morphogenesis during host cell infection.
Gary Kusdian, William F. Martin and Sven B. Gould (9E)

14:50 – 15:10 Coffee break

10

Parallel session 10: Genes and genomes 1

Chair: Marek Elias and Yuji Inagaki

15:10 – 15:30 Distribution of conventional and non-conventional introns in tubA and tubB genes of euglenoids.
Rafał Milanowski, Anna Karnkowska-Ishikawa, Abiba Boulahdjel, Takao Ishikawa and Bożena Zakryś (10A)

15:30 – 15:50 Diversification of cytoskeletal genes following multiple whole genome duplications in Paramecium species.
Yana Eglit, Casey L. McGrath, Lydia J. Bright, Thomas G. Doak and Michael Lynch (10B)

15:50 – 16:10 Evolutionary questions raised by RNAi studies of *Paramecium parafusin.*
Birgit H. Satir, Peter Satir, Søren T. Christensen, Johan Kolstrup and Elzbieta Wyroba (10C)

16:10 – 16:30 Coffee break

16:30 – 16:50 Neofunctionalization of rab7 duplicated genes studied by molecular biology tools and high resolution imaging techniques.
Elzbieta Wyroba, Rafał Bartosiewicz and Magdalena Osińska (10D)

16:50 – 17:10 BLASTGrabber - visualization and taxonomic analysis of BLAST output files.
Ralf Neumann, Surendra Kumar and Kamran Shalchian Tabrizi (10E)

17:30 ISOP business meeting
Vilhelm Bjerknes (building 13) room 209

Wednesday, August 1

Parallel session 11: Excavata

Chair: Martin Kolisko and Vladimir Hampl

09:00 – 09:20 The *Spironucleus salmonicida* genome
Feifei Xu, Jon Jerlström-Hultqvist, Elin Einarsson, Staffan G. Svärd and Jan O. Andersson (11A)

09:20 – 09:40 Combining molecular data with classical morphology for uncultured phagotrophic euglenids.
Gordon Lax and Alastair Simpson (11B)

09:40 – 10:00 Diverse Trichomonas lineages associated with columbids.
A. Peters and S. R. Raidal (11C)

10:00 – 10:10 10 min break

10:10 – 10:30 Diversity of insect trypanosomatids: all that is hidden shall be revealed.
Julius Lukeš, Jan Votýpka, Vyacheslav Yurchenko and Dmitri A. Maslov (11D)

10:30 – 10:50 Unexpected diversity of free-living trichomonads.
Ivan Čepička, Vít Céza, Jeffrey D. Silberman and František Šťáhlavský (11E)

10:50 – 11:10 Ultrastructural and phylogenetic evidence for the polyphyly of retortamonads.
P. Smejkalová, J. Kulda and I. Čepička (11F)

11:10 – 11:30 Coffee break

Parallel session 12: Opisthokonta, Haptophyta, Diphyllatea

Chair: Akinori Yabuki and Sigrid Neuhauser

11:30 – 11:50 Unicellular opisthokonts diversity and distribution in the European coast.
Javier del Campo, Ramon Massana, Colomban de Vargas and Iñaki Ruiz-Trillo (12A)

11:50 – 12:10 Diversity and seasonal dynamics of haptophytes in outer Oslofjorden revealed by 454 pyrosequencing.
Elianne Egge, Wenche Eikrem, Vladyslava Hostyeva, Tom Andersen, Lucie Bittner, Torill Johannesen, Ruth-Anne Sandaa and Bente Edvardsen (12B)

12:10 – 12:30 A strategy for 454-pyrosequencing of ribosomal SSU DNA and RNA to more accurately assess haptophyte diversity and relative abundance.
Elianne Egge, Lucie Bittner, Stéphane Audic, Colomban de Vargas, Hervè Moreau and Bente Edvardsen (12C)

12:30 – 12:50 Diversity, evolution and systematics of the Diphyllatea: understanding by combining conventional culture and environmental survey.
Sen Zhao, Kamran Shalchian-Tabrizi, Akinori Yabuki, Makoto M. Watanabe and Dag Klaveness (12D)

12:50 – 14:10 POSTER SESSION (A-M) and LUNCH

Parallel session 13: Rhizaria

Chair: Jan Pawlowski and David Bass

14:10 – 14:30 Benthic foraminifera in plankton samples: the illusion of environmental DNA study.
Jan Pawlowski, Emanuela Reo and Franck Lejzerowicz (13A)

14:30 – 14:50 Environmental diversity and lineage discovery in Haplosporidia.
Hanna Hartikainen, Cédric Berney, Beth Okamura, Stephen Feist, Grant Stentiford, Craig Austin Baker and David Bass (13B)

14:50 – 15:10 Phylogeny and ecology of Endomyxa, a bewilderingly diverse assemblage of lineages within Cercozoa (Rhizaria).
Cédric Berney, Patricia Dyal and David Bass (13C)

15:10 – 15:30 18S + 28S rDNA phylogeny divides Radiolaria into Polycystina and Spasmaria and supports the Retaria hypothesis
Anders K. Krabberød, Jon Bråte, Jane K. Dolven, Randi F. Ose, Dag Klaveness, Tom A. Kristensen, Kjell R. Bjørklund and Kamran Shalchian-Tabrizi (13D)

15:45 – 18:45 SOCIAL PROGRAM: SIGHTSEEING

14

Parallel session 14: Genes and genomes 2

Chair: Bente Edvardsen

09:00 – 09:20 Unravelling the evolutionary forces that shape the B12 requirements of algae.
K. E. Helliwell, G. L. Wheeler, K. C. Leptos, R .E. Goldstein and A. G. Smith (14A)

09:20 – 09:40 Trans-splicing and expression of nucleus-encoded genes for chloroplast proteins in *Euglena gracilis.*
Juraj Krajčovič, Bianka Mateášiková-Kováčová, Kristína Záhonová and Matej Vesteg (14B)

09:40 – 10:00 Diversity of marine Prasinophyceae and associated viruses.
Evelyne Derelle, Camille Clérissi, Yves Desdevises, Gwenael Piganeau, Nigel Grimsley, Bente Edvardsen and Hervé Moreau (14C)

10:00 – 10:10 10 min break

15

Parallel session 15: Ecology

Chair: Juan Saldarriaga and Jon Bråte

10:10 – 10:30 Nutritional history matters to predator-prey population dynamics: the effect of past food experience on Didinium eating Paramecium, a model system.
David J.S. Montagnes and Jiqiu Li (15A)

10:30 – 10:50 SEM, TEM, and nanosims imaging of microbes from the hindgut of a lower termite: Evidence for carbon transfer between protists and their bacterial symbionts.
Kevin J. Carpenter, Peter K. Weber, M. Lee Davisson, Jennifer Pett-Ridge, Michael I. Haverty and Patrick J. Keeling (15B)

10:50 – 11:10 Soil C & N nutrient transfers through food web functional groups using stable isotope methods.
Sina Adl and Felicity Crotty (15C)

11:10 – 11:30 Coffee break

11:30 – 11:50 Effects of oil pollution on attached microbial communities in short term indoor microcosms.
Young-Ok Kim, Seung-Won Jeong and Eun-Sun Lee (15D)

11:50 – 12:10 Investigating the effect of light on the flagellated form of *Chromera velia.*
Kate Weatherby, Jan Slapeta and Dee Carter (15E)

12:10 – 12:30 Temperature-driven changes in growth and bacterivory in a common mixotrophic chrysophyte.
Robert W. Sanders, Sarah B. DeVaul and Adam W. Heinze (15F)

12:30 – 12:50 Responses of an intertidal microbial community to petroleum hydrocarbons at a bioremediation site on Prudence Island in Narragansett Bay.
Gaytha A. Langlois (15G)

12:50 – 14:10 POSTER SESSION (A-M) and LUNCH

15:45 – 18:45 SOCIAL PROGRAM: Sightseeing

Thursday, August 2

16

Symposium 4: Matryoshka-type evolution of cells (16)
Chair: Ken-ichiro Ishida and Jun-ichi Obokata
Sponsored by: MTE Project

09:00 – 09:30 Mitosomal transport system in *Entamoeba histolytica*.
Tomoyoshi Nozaki, Takashi Makiuchi, Yoshitaka Murakami, Ken-ichiro Imai and Paul Horton (16A)

09:30 – 10:00 Dual targeting of aminoacyl-tRNA synthetases to the plastid and mitochondrion in a chlorarachniophyte alga.
Yoshihisa Hirakawa, Fabien Burki and Patrick Keeling (16B)

10:00 – 10:30 Genomics-enabled insight into the periplastidial compartments of cryptophyte and chlorarachniophyte algae.
Goro Tanifuji and John M. Archibald (16C)

10:30 – 10:50 Coffee break

10:50 – 11:20 Apicomplexan parasites and plant hormones.
Kisa Nagamune, Syed Bilal Ahmad Andrabi and Ryuma Matsubara (16D)

11:20 – 11:50 A model for endosymbiotic genome reduction: rhopalodiacean diatoms and their nitrogen-fixing organelles, spheroid body.
Yuji Inagaki (16E)

11:50 – 12:50 POSTER SESSION (A-M) and LUNCH

17

Parallel session 17: Chloroplastid evolution and function
Chair: Patrick J. Keeling and Ales Horak

12:50 – 13:10 A new driving force for the functional gene transfer during endosymbiotic evolution: pol II promoter de novo origination.
Junichi Obokata (17A)

13:10 – 13:30 How many endosymbiotic gene transfers in eukaryotes?
Fabien Burki and Patrick Keeling (17B)

13:30 – 13:50 Comparative analysis of nucleomorph genomes among three chlorarachniophytes.
Shu Shirato Shigekatsu Suzuki, Takuro Nakayama, Yoshihisa Hirakawa, Sayaka Koike and Ken-ichiro Ishida (17C)

13:50 – 14:00 10 min break

14:00- -14:20 Genome evolution of a tertiary dinoflagellate plastid.
Tove M. Gabrielsen, Marianne A. Minge, Mari Espelund, Alexander J. Nederbragt, Kamran Shalchian-Tabrizi, Christian Otis, Monique Turmel, Claude Lemieux and Kjetill S. Jakobsen (17D)

14:20 – 14:40 Tetrapyrrole pathway reflects an evolutionary history of algae with complex plastid.
Miroslav Oborník, Luděk Kořený and Jaromír Cihlář (17E)

14:40 – 15:00 Coffee break

18

Parallel session 18: Mitochondrion-related organelles
Chair: Andrew J. Roger and Jan Tachezy

15:00 – 15:20 Adaptations to anaerobiosis in the hydrogenosome of *Andalucia incarcerate.*
Michelle M. Leger, Laura A. Hug and Andrew J. Roger (18A)

15:20 – 15:40 Broad distribution of TPI-GAPDH fusion proteins among eukaryotes: evidence for glycolysis in the mitochondrion?
Takuro Nakayama, Ken-ichiro Ishida and John M. Archibald (18B)

15:40 – 16:00 Hydrogenosomes in the diplomonad parasite *Spironucleus salmonicida.*
Jon Jerlström-Hultqvist, Elin Einarsson, Karin Hjort, Daniel Steinhauf, FeiFei Xu, Jan O. Andersson and Staffan G. Svärd (18C)

16:00 – 16:20 FeS proteins and FeS cluster assembly machinery in anaerobic protist *Mastigamoeba balamuthi* and *Entamoeba histolytica.*
Eva Nývltová, Miroslava Šedinová, Robert Sutak, Ivan Hrdy, Čestmír Vlček, Jan Paces and Jan Tachezy (18D)

16:20 – 16:40 Coffee break

16:40 – 17:00 Mitochondrion-like organelle of *Trimastix pyriformis*.
Z. Zubacova, L. Novak, J. Hlavackova, J Ridl, V.Vacek, I. Hrdy,J. Tachezy, C. Vlcek, V. Hampl (18E)

17:00 – 17:20 The anaerobic adaptations of Blastocystis mitochondrion-related organelles and the evolution of mitochondria.
Anastasios D. Tsaousis, Eleni Gentekaki, Eva Nývltová, Grant C. Stevens, Ivan Hrdy, Andrew J. Roger and Jan Tachezy (18F)

17:20 – 17:40 Evolutionary analysis of mitochondrial ancestry of diplomonads: Phylogenomics of mitochondrial-related organelles in Carpediemonas-like organisms.
M Kolisko, R. Kamikawa, K. Takishita, I. Čepička, Q. Zhang, T. Hashimoto, Y. Akinori, I. Inagaki, A.J. Roger and A.G.B. Simpson (18G)

Parallel session 19: Ciliates

Chair: Denis Lynn and Virginia P. Edgcomb

12:50 – 13:10 Phylogeny of the peniculistomatid genera Mytilophilus and Peniculistoma and relationships among pleuronematid scuticociliates.
M. C. Strüder-Kypke, D. H. Lynn, G. A. Antipa and L. Obolkina (19A)

13:10 – 13:30 Molecular phylogenetics of peritrich ciliates (Ciliophora, Peritrichia) with emphasis on the genus Epistylis.
Laura R. P. Utz, Taiz L. L. Simão, Lúcia S. L. Safi and Eduardo Eizirik (19B)

13:30- -13:50 Diversity of planktonic ciliates in deep hypersaline anoxic basins in the Eastern Mediterranean Sea.
A. Stock, W. Orsi, V. Edgcomb, H.-W. Breiner, S. Filker and T. Stoeck (19C)

13:50 – 14:00 10 min break

14:00 – 14:20 Novel benthic ciliates (Protozoa) from marine sediments of the UK.
Xiaozhong Hu and Alan Warren (19D)

14:20 – 14:40 Utility of small and large subunit rRNA genes for morphospecies discrimination and phylogenetic inference in the order Tintinnida (Ciliophora, Spirotrichea).
Luciana F. Santoferrara, George B. McManus and Viviana A. Alder (19E)

14:40 – 15:00 Coffee break

Parallel session 20: Phylogeny of obscure species

Chair: Mustafa H. Fakhri and Anna Maria Fiore-Donno

15:00 – 15:20 Fine structure of *Telonema subtile* Griessmann, 1913: A unique cytoskeletal structure among eukaryotes.
Akinori Yabuki, Wenche Eikrem and Kiyotaka Takishita (20A)

15:20 – 15:40 *Blastocystis* sp. Nand II strain: an evolutionary mosaic or a deeply divergent organism?
E. Gentekaki, L. Eme, B. Curtis, A. Tsaousis, J. Archibald and A. J. Roger (20B)

15:40 – 16:00 Will the real picobiliphyte please stand up?
Linda K. Medlin, Ramkumar Seenivasan and Michael Melkonian (20C)

16:00 – 16:20 Coffee break

16:20 – 16:40 The phylogenetic position and mitochondrial genome sequence of the enigmatic discobid *Tsukubamonas globosa*.
Ryoma Kamikawa, Yuki Nishimura, Akinori Yabuki, Martin Kolisko, Alastair G. B. Simpson, Andrew J. Roger, Ken-ichiro Ishida, Tetsuo Hashimoto and Yuji Inagaki (20D)

16:40 – 17:00 RY-coding and non-homogeneous models can ameliorate the maximum-likelihood inferences from nucleotide sequence data with parallel compositional heterogeneity.
Sohta Ishikawa, Yuji Inagaki and Tetsuo Hashimoto (20E)

17:00 – 17:20 EST investigation of an early branching dinoflagellates *Psammosa pacifica* and early evolution of dinoflagellates.
Noriko Okamoto, Fabien Burki, Behzad Imanian and Patrick Keeling (20F)

19:00 – SOCIAL PROGRAM: Banquet

Friday, August 3

21

Symposium 5: Classification and mega-phylogeny (Session 21)

Chair: Alastair Simpson and Fabien Burki

09:00 – 09:30 Revisions to the classification of protists.
Sina Adl and many others (21A)

09:30 – 10:00 The cytoskeleton of Breviata, and the nature of the ancestral eukaryote flagellar apparatus.
Aaron A. Heiss, Giselle Walker and Alastair G. B. Simpson (21B)

10:00 – 10:30 Finding homes for outcast protistan lineages through phylogenomics; the case of the breviates.
Matthew W Brown, Susan C Sharpe, Jeffrey D Silberman, Alastair GB Simpson, Aaron A Heiss and Andrew J Roger (21C)

10:30 – 10:50 Coffee break

10:50 – 11:20 Loukozoa and Sulcozoa: the significance of groovy flagellates for eukaryote deep phylogeny.
Thomas Cavalier-Smith (21D)

11:20 – 11:50 Phylogenomics of eukaryote proteins of Bacterial origin.
Ding He, Johan Viklund, Siv Andersson and Sandra Baldauf (21E)

11:50 – 12:20 Phylogenetic analyses on the evolution of eukaryotes using concatenated ribosomal protein sequences.
Tani Leigh and Wei-Jen Chang (21F)

12:20 – 12:50 Pinpointing the root of extant eukaryotic diversity.
Laura Eme and Andrew J. Roger (21G)

12:50 – 13:20 Final remarks.

13:20 – 14:20 LUNCH

List of posters (in alphabetical order by title)

Poster session Wednesday, August 1, 12:40 – 14:30

- A new freshwater peritrichous ciliate from West Lake, Hangzhou, China, *Epistylis hangzhounensis* n. sp. (Sessilida: Epistylididae).
    - *Chuanqi Jiang, Xinlu Shi, Guijie Liu and Xiaozhong Hu*
- A new species candidate of marine ciliate, *Brooklynella* sp. (Phyllopharyngea: Dysteriida: Hartmannulidae) from Jeju in Korea.
    - *Ji Hye Kim and Mann Kyoon Shin*
- A new strategy of organellar genome sequencing incorporating rolling circle amplification: protist mitochondria genomes.
    - *Yuki Nishimura, Ryoma Kamikawa, Yuji Inagaki and Tetsuo Hashimoto*
- A Novel Alveolate in gill epithelium cells of bivalves with chemosynthetic bacteria Inhabiting deep-sea methane seeps in Sagami Bay, Japan.
    - *Fumiya Noguchi, Kiyotaka Takishita, Masaru Kawato, Takao Yoshida, Yoshihiro Fujiwara and Katsunori Fujikura*
- A Rieske monooxygenase highly conserved in animals is the sterol-C7 desaturase of *Tetrahymena thermophila*.
    - *Sebastián R. Najle, Alejandro D. Nusblat, Claudio H. Slamovits, Clara B. Nudel and Antonio D. Uttaro*
- Adaptations of the cytosolic iron/sulfur cluster assembly machinery in microbial eukaryotes.
    - *Anastasios D. Tsaousis, Eleni Gentekaki, Daniel Gaston and Andrew J. Roger*
- An international research coordination network for biodiversity of ciliates.
    - *John C. Clamp*
- Anaerobic adaptations of the mitochondria in *Naegleria gruberi.*
    - *Anastasios D. Tsaousis, Eva Nývltová, Ivan Hrdy & Jan Tachezy*
- Application of protist communities for monitoring water quality in the Hangzhou section of Jing-Hang Grand Canal, southern China.
    - *Xinlu Shi, Guijie Liu, Henglong Xu, Xiaojiang Liu, and Zhiqiang Sun*
- Assembling and annotating the nuclear genome of the jakobid flagellate *Andalucia godoyi* (Discoba, Excavata).
    - *Vladimir Klimes, Cestmir Vlcek, Marek Elias, Jan Fousek, Jan Paces, Michael W. Gray, Michelle Leger, Andrew J. Roger and B. Franz Lang*
- Association of methanogenic archaea with individual protozoa species and its influence on ruminal methanogenesis.
    - *Johanna O. Zeitz, Michael Kreuzer and Carla R. Soliva*
- *Balantidion pellucidum*, a freshwater haptorid ciliate with resting cysts covered by heteromorphic lepidosomes: light and scanning electron microscopy and cytochemistry.
    - *William A. Bourland*
- Characterization of hydrogenosome in anaerobic protist *Mastigamoeba balamuthi.*
    - *Eva Nývltová, Ivan Hrdy and Jan Tachezy*
- Characterization of long non-coding RNAs from the parasite *Trichomonas vaginalis.*
    - *Gary Kusdian, Christian Wöhle, Claudia Radine, Giddy Landan, William Martin and Sven B. Gould*
- Characterization of the Charged Repeat Motifs of Alveolin Proteins.
    - *H. El-Haddad, J. Pryzborski, W. Martin and S. B. Gould*
- Coiling direction does not depend on water temperature in a planktic foraminifer.
    - *Yurika Ujiie and Takahiro Asami*
- Comparative analysis of nuclear and nucleomorph gene expression in cryptomonad and chlorarachniophyte algae.
    - *Goro Tanifuji, Naoko T. Onodera, Christa E. Moore, Julia Hopkins and John M. Archibald*
- Comparative distribution of the two ichthyotoxic dinoflagellates *Gambierdiscus* spp. and *Ostreopsis* spp. in the coastal waters of Jeju Island, Korea.
    - *Bora Jang, Hyung Seop Kim, Mi Ryeong Oh, Jung Rae Rho and Wonho Yih*
- Differential analysis and comparison on the proteins profile of *Urostyla grandis* under different physiology conditions.
    - *Chen Ji-wu, Zheng Li-na, Wang Bang-zheng and Gu Fu-kang*
- Distribution of novel apicomplexan lineage in the Gulf of Aqaba.

- *Ales Horak and Ondrej Prasil*

- Diversity of the genus Monocercomonoides and its genetic codes.
  - *E. Šrámová, KK Novotná, P Smejkalová, I Čepička and V Hampl*
- Ecto-phosphatase activity in *tritrichomonas foetus*: differential expression in the pseudocyts (endoflagellar form) and its participation in the cytotoxicity.
  - *Antonio Pereira-Neves, José Roberto Meyer-Fernandes and Marlene Benchimol*
- Expanded molecular phylogeny of Armophorea (Ciliaphora: Intramacronucleata).
  - *da Silva Paiva, Bárbara do Nascimento Borges, In·cio Domingos da Silva Neto*
- Finding the role of *Chromera velia* in its environment.
  - *Marjorie Linares, Jan Slapeta and Dee Carter*
- First glance at the genome of the Phytomyxae club root pathogen *Plasmodiophora brassicae.*
  - *Arne Schwelm, Johan Fogelqvist and Christina Dixeliu s*
- Genetic differentiation in populations of desmid species *Micrasterias rotata* (Zygnematophyceae, Streptophyta).
  - *Katarína Nemjová, Pavel Škaloud, Frederik Leliaert, Jana Veselá and Helena Bestová ·*
- Genome sequencing project of *Acrasis kona.*
  - *Chengjie Fu, and Sandra L. Baldauf*
- Ecological significance of chlorophyll metabolism of herbivorous protists in aquatic ecosystems.
  - *Yuichiro Kashiyama, Akiko Yokoyama, Hideaki Miyashita, Kanako Ishikawa, Akira Ishikawa, Isao Inouye and Hitoshi Tamiaki*
- Himatismenida Page, 1987 (Amoebozoa) is a proper taxonomic home for Parvamoeba Rogerson, 1993: morphological and molecular evidence.
  - *Alexander Kudryavtsev and Jan Pawlowski*
- Intragenomic spread of plastid-targeting presequences in the coccolithophore *Emiliania huxleyi.*
  - *Fabien Burki, Yoshihisa Hirakawa and Patrick J. Keeling*
- Is the replacement of a gene encoding plastid-targeted GAPDH on-going in the dinoflagellate genus in Karenia?
  - *Euki Yazaki, Ryoma Kamikawa, Tetsuo Hashimoto and Yuji Inagaki*
- Localization of *Chromera velia* heme pathway enzymes by xenotransfection.
  - *Jitka Kručinská, Lilach Sheiner, Luděk Kořený, Boris Striepen and Miroslav Oborník*
- Microbial eukaryotes in the arctic marine ecosystem: Identity and seasonality.
  - *Miriam Marquardt, Archana Meshram, Anna Vader, Marit Reigstad and Tove M. Gabrielsen*
- Microtubular Organelles in *Euplotes patella* (Ciliophora: Hypotrichida) Revealed by fluorescent Labeling.
  - *Gu Fu-Kang Lin Qin and Fan Xin-Peng*
- Mitochondrial preprotein translocase of kinetoplastids is homologous to Tom40.
  - *Vojtěch Žárský, Jan Tachezy and Pavel Doležal*
- Molecular differentiation within *Paramecium dodecaurelia* from the *P. aurelia* spp. complex reveals recent speciation process.
  - *E. Przybos, S. Tarcz, M. Prajer, M. Surmacz, N. Sawka, and M. Rautian*
- Morphology and molecular analysis of six antarctic tintinnid ciliates.
  - *Sun Young Kim, Joong Ki Choi, John R.Dolan and Eunjin Yang*
- Morphology and phylogeny of a new imbricatean flagellate (phylum Cercozoa).
  - *Takashi Shiratori, Akiko yokoyama and Ken-ichiro Ishida*
- Morphology and ultrastructures of a new Frontonia ciliate (Ciliophora, Peniculida) from Harbin, Northeastern China.
  - *Ying Chen, Wenqiao Ding, Xuming Pan, Zijian Qui and Weibo Song*
- *Neospora caninum* antibodies in cats from the Czech Republic.
  - *K. Sedlak and E. Bartova*
- New anaerobic member of the jakobid genus Andalucia.
  - *T Pánek, P Taborsky and I Čepička*
- Newly isolated, branching and network-forming naked amoebae enhance the morphological, genetic and ecological diversity of class Variosea (Amoebozoa).

- *Cédric Berney, Stefan Geisen and David Bass*

- Parasitic protists (Syndiniales, Dinophyta) and fungi in sea ice and winter-time water in the Baltic Sea.
  - *Markus Majaneva, Janne-Markus Rintala, Maria Piisilä, David P. Fewer and Jaanika Blomster*
- Persistent structures of trypanosomatid cryptogene editing domains.
  - *Alexander A Kolesnikov and Evgeni S Gerasimov*
- Phylogenetic analyses of *Discomorphella* sp. n. show that odontostomatids are polyphyletic.
  - *Bárbara do Nascimento Borges, Wallax Augusto Silva Ferreira, Maria Lúcia Harada, Inácio Domingos da Silva Neto and Thiago da Silva Paiva*
- Phylogenetic composition and distribution of picoeukaryotes in the hypoxic northwestern coast of the Gulf of Mexico.
  - *Emma Rocke, Hongmei Jing, Takafumi Kataoka, Liangliang Kong and Hongbin Liu*
- Phylogenetic relationships in Pyrenomonadaceae (Cryptophyta) inferred from nuclear and nucleomorph SSU rDNA and chloroplast rbcL genes.
  - *Iina Remonen, Markus Majaneva, Janne-Markus Rintala and Jaanika Blomster*
- Phylogenomics reveals deep relationships of Rhizaria.
  - *Roberto Sierra, Mikhail Matz, Galina Aglyamova, LoÔc Pillet, Johan Decelle, Fabrice Not, Colomban de Vargas and Jan Pawlowski*
- Phylogeny and domain configuration of fatty acid synthases and polyketide synthase-like proteins in protists.
  - *Aika Yamaguchi, Maiko Tamura and Holger Jenke-Kodama*
- Properties of the unique insert in the ribosomal stalk protein, phosphoprotein P0, shared by members of the Ciliophora.
  - *G. Pagano, R. King , L.M. Martin, J. Schumacher and L.A. Hufnagel*
- Protein import into mitosomes of *Giardia intestinalis*.
  - *Eva Martincová, Ivana Fixová, Vojtěch Žárský, Jan Tachezy, Trevor Lithgow and Pavel Doležal*
- Protist phylogeography: Effects of climate and geographic distance on the genetic diversity of the testate amoeba *Hyalosphenia papilio* (Amoebozoa: Arcellinida).
  - *Thierry J. Heger, Edward A.D. Mitchell and Brian S. Leander*
- PUF proteins in the biology of *Giardia intestinalis.*
  - *Vladimíra Najdrová and Pavel Doležal*
- Purification and Characterization of LdFrataxin and LdIsd11 proteins involved in Iron Sulfur Clusters (ISC) machinery of L. donovani.
  - *Amir Zaidi, Krishn Pratap Singh, Pradeep Das and Vahab Ali*
- Quantification and influencing factors of the protozoic Si pool in forested mature ecosystems.
  - *Daniel Puppe, Otto Ehrmann, Michael Sommer and Manfred Wanner*
- Quantitative comparison and analysis on the differentially expressed genes of resting cyst and vegetative cell from *Pseudourostyla cristata.*
  - *Chen Ji-wu, Zheng Li-na, Wang Bang-zheng and Gao Qiu-xia*
- Radiolaria: a reservoir for marine alveolates.
  - *Anders K. Krabberød, Jon Bråte, Jane K. Dolven, Randi F. Ose, Tom A. Kristensen, Kjell R. Bjørklund, and Kamran Shalchian-Tabrizi*
- Redescription of a poorly known *Metopus setosus* Kahl, 1927 (Armophorea, Armophorida, Metophidae) from Korea.
  - *Choon Bong Kwon and Mann Kyoon Shin*
- Seasonal dynamics of harmful algae in the outer Oslofjord monitored using microarrays, qPCR, and microscopy.
  - *Vladyslava Hostyeva, Simon M. Dittami, Viljar Alain Skylstad, Wenche Eikrem and Bente Edvardsen*
- Seasonal succession of eukaryotic community detected by environmental sequences in Tokyo Bay, Japan.
  - *Akiko Yokoyama, Jun Kikuchi, Yuri Tsuboi, Shigeharu Moriya, Yuji Inagaki, Tetsuo Hashimoto and Isao Inouye.*
- Seasonal variation in the picoeukaryote *Micromonas pusilla* in an arctic fjord, Svalbard, as revealed by quantitative PCR.

- *Lene Christensen, Wenche Eikrem, Anna Vader and Tove M. Gabrielsen*

- Seasonality of marine parasites of Syndiniales in the Isfjord system, Svalbard.
  - *Stuart Thomson and Tove Gabrielsen*
- Simple repeat polymorphisms are not simply induced by DNA repair.
  - *John A Burns, Moinuddin Chowdhury and David A Scicchitano*
- *Strombidium paracalkinsi* (Ciliophora: Oligotrichea: Oligorichida) Revised: living morphology, infraciliature, and small subunit ribosomal DNA.
  - *Eun Sun Lee, Dapeng Xu, Sun Young Kim and Young-ok Kim*
- Studies on the morphogenesis and phylogeny of various Hypotrichous (s. l.) Ciliates.
  - *Chen Shao, Weibo Song and Alan Warren*
- Study on subpellicular ultrastructures of six ciliate species.
  - *Zijian Qiu, Ying Chen, Jing Gao, Na LI, Wenwei Liang and Wenqiao Ding*
- Study on Trichodinidae phylogeny inferred from 18S ribosomal DNA, ITS and 5.8S rDNA sequences.
  - *Fahui Tang and Yuanjun Zhao*
- Symbiosis between a euglenid and verrucomicrobial bacteria with extrusive structures. Extrusomes in the making?.
  - *Breglia S, Yubuki N, Leander B and Slamovits C.*
- Targeting into *Trichomonas vaginalis* hydrogenosomes is evolving towards a mechanism independent of cleavable N-terminal targeting sequences.
  - *Verena Zimorski, Peter Major, Kathrin Hoffmann, Xavier Pereira Brás, William F. Martin and Sven B. Gould*
- The Acetabularia chloroplast genome.
  - *Jörn Habicht, Christian Wöhle, Gregor Christa, Katharina Händeler, William F. Martin, and Sven B. Gould*
- The community of ciliates forming on different substrates..
  - *Ivan Mukhin*
- The complete mitochondrial genome sequence of the eustigmatophyte alga *Trachydiscus minutus.*
  - *Veronika Zbrankova, Jan Fousek, Cestmir Vlcek and Marek Elias*
- The differentiation of cortical ciliature microtubules of *Oxytricha platystoma* under different physiological conditions.
  - *Ni Bing, Guo Jian, Chen Ji-wu, Gu Fu-kang*
- The genus Tetracystis (Chlamydomonadales, Chlorophyceae): another highly polyphyletic taxon of coccoid green algae.
  - *Tereza Hasíková, Alena Lukešová, Eva Sýkorová and Marek Eliáš*
- The diversity in the Vischeria/Eustimatos complex (Eustigmatophyceae): morphological and molecular perspectives.
  - *Katerina Prochazkova, Lira A. Gaysina, Martina Pichrtova, Alena Lukesova and Marek Elias*
- The plastid genome of eutreptiella provides a window into the process of secondary endosymbiosis of plastid in Euglenids.
  - *Stepanka Hrda, Jan Fousek, Jana Szabova, Vladimir Hampl and Cestmir Vlcek*
- The role of algae in assessing the water quality of Hulan River wetland.
  - *Yawen Fan, Hongkuan Hui and Ninaemeka Emmanuel Okpala*
- Ultrastructural and molecular characterization of cyanobacterial endosymbionts in polycystine radiolarians.
  - *Osamu Takahashi, Tomoko Yuasa and Takeo Horiguchi*
- Ultrastructure and molecular phylogeny of dinoflagellate symbiont from solitary polycystine radiolarian.
  - *Tomoko Yuasa, Takeo Horiguchi and Osamu Takahashi*
- What can phenotypic plasticity tell us about coccolithophore evolution?
  - *Andrea Gerecht, Bente Edvardsen, Ian Probert and Jorijntje Henderiks*

Abstracts – Symposia (in alphabetical order by title)

A model for endosymbiotic genome reduction: rhopalodiacean diatoms and their nitrogen-fixing organelles, spheroid body (16E)

Yuji Inagaki

Members of the diatom family Rhopalodiaceae possess two distinct cyanobacteria-derived organelles, plastids for photosynthesis and 'spheroid bodies (SBs)' for putative nitrogen fixation. The origin of plastids found in a broad spectrum of eukaryotes can be traced back to early eukaryotic cell evolution. On the other hand, the SBs are only found in a subgroup of diatoms, suggesting that a nitrogen-fixing cyanobacterial endosymbiont was transformed into the organelle in the ancestral rhopalodiacean diatom cells after the extant major diatom lineages diverged. We regard that the SBs represent an intermediate intracellular structure between non-obligatory cyanobacterial endosymbionts and organelles that are completely integrated into the host cell systems (i.e. Mitochondria and plastids). Here we present the results from our recent investigations on rhopalodiacean diatoms and their SBs. We examined the evolutionary origin of the SBs in rhopalodiacean diatom species, and the nitrogen fixation activity by tracing 15N. Finally, we introduce our on-going sequencing of the SB genome in a rhopalodiacean diatom *Epithemia turgida*.

Assessing spatial variation in ecophysiological diversity - does selection shape free-living protist populations? (2C)

Chris Lowe (2C)

Apicomplexan parasites and plant hormones (16D)

Kisa Nagamune, Syed Bilal Ahmad Andrabi and Ryuma Matsubara

Recently, we found that the parasitic protist, Toxoplasma gondii, produces a plant hormone, abscisic acid (ABA). ABA stimulated the production of cyclic ADP-ribose, which is a second messenger for the cytosolic calcium release, in T. gondii. ABA also induced the parasite-egress from the parasitophorous vacuoles. Conversely, fluridone, a specific ABA biosynthesis inhibitor, prevented parasite-egress and promoted differentiation into the resting, bradyzoite stage. Further, we could detect other plant hormones cytokinins in T. gondii. A natural cytokinin, trans-zeatin (tZ), accelerated the proliferation of T. gondii. In contrast, a synthetic cytokinin, thidiazuron, inhibited the proliferation. tZ hastened the cell cycle progression from G1 to S phase, similar to plant, while thidiazuron caused the halt there. Quantitative-PCR (qPCR) analysis revealed that tZ upregulated the expression of one cyclin like as higher plant, whereas thidiazuron downregulated its expression. These suggest this cyclin plays a crucial role in controlling the cell cycle in T. gondii and concomitantly its proliferation. Since cytokinins are also known to regulate chloroplast development in plants, we examined whether cytokinins affect apicoplast, which is a remnant organelle derived from an algae endosymbiont, in T. gondii. Immunofluorescence microscopy and qPCR analysis revealed tZ increased the number of apicoplasts but thidiazuron extinguished apicoplast.

Evolutionary morphology of uncultivated euglenozoans living in low-oxygen marine environments (7E)

Naoji Yubuki and Brian S. Leander

The Euglenozoa is a large group of protists with diverse modes of nutrition. The group consists of three main subclades (euglenids, kinetoplastids, diplonemids) that have been confirmed with molecular phylogenetic data and a combination of shared ultrastructural characteristics. Several poorly understood euglenozoans live in low oxygen marine environments, such as *Bihospites bacati, Calkinsia aureus* and *Postgaardi mariagerensis*.. Molecular phylogenies demonstrate that *B. bacati* and *C. aureus* group strongly with environmental sequences from low-oxygen sediments, forming the Symbiontida, a new subclade within the Euglenozoa. *B. bacati* possesses a feeding rod and a distinctive surface organization reminiscent of features present in phagotrophic euglenids. *C. aureus* and *P. mariagerensis* share novel features associated with the feeding apparatus, such as an array of finger-like projections. Members of the Symbiontida are enveloped by rod-shaped epsilon proteobacteria. The surface of *B. bacati* is also adorned with rows of spherical extrusive bacteria, called "epixenosomes". Similar epixenosomes have also been observed on a ciliate, and molecular phylogenetic data demonstrate that these episymbionts are novel members of the Verrucomicrobia. Moreover, molecular phylogenetic analyses of SSU rDNA sequences from the hosts and the epsilon proteobacterial episymbionts demonstrate co-evolutionary patterns that have improved our understanding of prokaryote-eukaryote symbioses in marine environments.

Dual targeting of aminoacyl-tRNA synthetases to the plastid and mitochondrion in a chlorarachniophyte alga (16B)

Yoshihisa Hirakawa, Fabien Burki and Patrick Keeling

Chlorarachniophyte algae have plastids acquired by a secondary endosymbiosis of a green algal endosymbiont. Each plastid is surrounded by four envelope membranes and possesses the relict endosymbiont's nucleus, namely nucleomorph, in the intermembrane space (periplastidal compartment: PPC). Therefore, chlorarachniophytes contain four genomes, and protein synthesis occurs in the cytoplasm, mitochondria, plastids, and PPC. These four compartments require a full set of aminoacyl-tRNAs for protein translation, but almost all aminoacyl-tRNA synthetase (aaRS) genes are absent in three organelle genomes. This implies that organelle aaRSs would be encoded by the nuclear genome, and targeted to each compartment. To confirm this idea, we searched aaRSs in the complete genome sequence of a chlorarachniophyte, *Bigelowiella natans*, and characterized the intercellular localization and

phylogenetic position of several aaRSs. We found the genome encoded three copies of histidyl-RSs and two of glycyl-RSs. Interestingly, one of each aaRS seems to be derived from some bacterium by lateral gene transfer, and was targeted into both plastids and mitochondria. The dual targeted aaRSs have two translation variants, and these products encoded an N-terminal plastid- and mitochondrial-targeting sequence, respectively. We also characterized cytoplasmic and PPC-targeted aaRSs, and we here would like to discuss the evolutionary process of aaRSs in secondary algal groups.

Endosymbiosis, plastid acquisition and the chlorarachniophytes (1A)

Ken-ichiro Ishida

Fundamentals of eukaryotic cellular system were established and have diversified in the evolution of protists. Endosymbiosis is one of major factors that drove this evolutionary process. Studying the endosymbiotic evolution in protist cells, therefore, lead us toward the correct understanding of the eukaryotic cellular system. Plastid is one of cellular organelles that have evolved by the endosymbioses. Acquisition of the plastid by a primary endosymbiosis introduced the photosynthesis into a eukaryote lineage. Subsequent plastid lateral transfers to different eukaryote lineages by multiple secondary endosymbiosis expanded the distribution of photosynthesis in the eukaryotes and created the amazing diversity of photosynthetic organisms. Efforts to elucidate how the photosynthetic cell system was established in each primary and secondary endosymbiosis are currently underway. In this presentation, the recent progress in understanding the endosymbiotic acquisitions of plastids will be summarized with special reference to a secondary algal group, the chlorarachniophytes, which acquired their plastids from an endosymbiotic green alga independently.

Field Protistology: Importance and challenges (7A)

Thierry J Heger, Naoji Yubuki and Brian S. Leander

The discovery and characterization of protist communities from various environments are crucial for understanding the overall evolutionary history of life on earth. The invention of light microscopy in the 17th century, the applications of electron microscopy to biological materials in the mid 1900's, and recent developments in molecular biology have provided important findings in protistology. However, major questions about the diversity, ecology and evolutionary history of protists remain unanswered, notably because data obtained from the field and protist cultures, especially of heterotrophic species, remains limited. In this presentation, we will highlight how field studies in protistology lead to the (1) discovery of new lineages that expand the tree of eukaryotes, (2) the recognition of novel evolutionary patterns and processes, and (3) the untangling of ecological interactions and functions involving protists.

From single cells to communities - deciphering the ecology and evolution of microbial symbionts in termite/cockroach hindguts using multiple sequencing approaches (2D)

Vera Tai and Patrick Keeling

To aid in their digestion of wood, the hindguts of termites and cockroaches from the genus Cryptocercus are known to harbor a diverse community of protist, primarily parabasalid and oxymonad, and prokaryotic symbionts. We examined the ecology and evolution of this microbial community at 3 different scales. At the community scale, we used 454 pyrosequencing of the 18S and 16S rRNA genes to characterize the diversity of the protist and prokaryotic communities from several termite species. These data show that the composition of the symbiont community is host-specific and demonstrates the co-evolution of the symbionts with their insect hosts. To examine speciation within the community, we sequenced the internal transcribed spacer (ITS) region and 18S rRNA genes of Trichonympha and Trichomitopsis species from *Zootermopsis angusticollis* Trichonympha species from *Z. angusticollis* clustered together to the exclusion of other parabasalid sequences, as did the Trichomitopsis species, suggesting that speciation occurred after the divergence of the Zootermopsis lineage. Lastly, at the organismal level, we have investigated the symbiosis between Barbulanympha (a parabasalid symbiont of the cockroach *Cryptocercus punctulatus*) and their bacterial ectosymbionts. The DNA from a single Barbulanympha cell (including its ectosymbionts) was sequenced using Illumina's sequencing-by-synthesis technology. This sequencing effort showed that it is possible to retrieve large genome fragments showing the metabolic capabilities of the bacterial ectosymbionts from a single protist cell. Together, these approaches provide a way forward to understand the role of protists in ecosystems and their evolution.

Has there been an "inordinate fondness for diatoms" and if so why? Insights into diatom speciation and diversification from genetic, mating, and morphological data (2B)

David G. Mann and Pieter Vanormelingen

Before the development of molecular systematics, diatoms were probably already the most taxon-rich of the major protist groups, their elaborate shells providing ample reasons (excuses?) for splitters to create a panoply of species and infraspecific taxa. It might have been expected, perhaps, that the greater objectivity of nucleotide data would instill some discipline into this extravagant world, and that the number of taxa would fall as they were shown to be phenotypic variants or life cycle stages. However, the message from an increasing number of molecular studies is that earlier generations of diatom systematists were in fact rather stingy: there are apparently many more species than they thought, both among the predominantly planktonic centric lineages and in the mostly benthic pennates. Some of these are morphologically distinct, others are not.

Meanwhile, morphology-based taxonomy, perhaps reassured by molecular and mating studies, is in a new phase of rapid expansion, claiming the discovery of numerous endemic taxa mostly in previously understudied areas. Studies of speciation and reproductive biology are sometimes seen as a valuable or essential companion of taxonomy or as a conceptually separate endeavour, but are for sure in their infancy in diatoms. A few studies have now begun to examine mating system evolution and suggest, that as in 'higher' eukaryotes, transition to obligate auto- (or apo-)mixis is usually an evolutionary dead-end; but they also hint that such transitions are not infrequent in some groups and may produce arrays of 'microspecies' like those that have long been familiar to angiosperm systematists (e.g. in *Crepis*, *Taraxacum*, *Rubus*). On the other hand, other groups seem to be predominantly allogamous and speciation seems to involve the development of prezygotic reproductive isolation; a few examples have emerged where this is incomplete and detectable hybridization still occurs in nature. It is still very unclear whether speciation occurs predominantly in allopatry, or whether sympatric speciation is common. The impression from much morphology-based taxonomy is that endemism is common, and hence that allopatric speciation is plausible, but there is no doubt that diatom floras are grossly undersampled and progress is also hampered by lack of knowledge of ecological controls. Certainly, pairs of closely related diatoms sometimes grow side-by-side in the same water-body, but our sampling of diversity is rarely enough to be sure whether we have observed pairs of sister species, still less do we know where such species arose. To show that allopatric speciation is plausible, it must be shown that dispersal constrains distributions (contrary to extreme versions of the ubiquity hypothesis); population genetic and statistical analyses of diversity data suggest that it does, but whether it does so enough to permit widespread allopatric speciation is unclear and is currently an active focus of research.

Genomics-enabled insight into the periplastidial compartments of cryptophyte and chlorarachniophyte algae (16C)

Goro Tanifuji and John M. Archibald

Endosymbiosis has been a driving force in the evolution of eukaryotic cells. The genetic and biochemical re-organization that occurs during the evolution of organisms with plastids of secondary (i.e., eukaryote-eukaryote) endosymbiotic origin is particularly complex. The periplastidial compartment (PPC) of secondary plastid-bearing algae corresponds to the remnant cytosol of the eukaryotic endosymbiont, and in cryptophyte and chlorarachniophyte algae, the PPC harbors a 'nucleomorph', which is the residual endosymbiont nucleus. Because sequenced nucleomorph genomes are extremely limited in terms of their coding capacity (with <1,000 genes), the diversity of biochemical processes taking place in the PPC is unclear. As part of a large International collaborative effort, we have carried out an exhaustive survey of PPC-targeted proteins encoded in the *Guillardia theta* and *Bigelowiella natans* nuclear genomes, which were sequenced by the Joint Genome Institute's Community Sequencing Program. 1,196 and 2,560 PPC-targeted proteins were predicted in *B. natans* and *G. theta*, respectively, of which ~34 % could be assigned a function. A surprisingly complex array of 'housekeeping' proteins and metabolic enzymes were found, particularly in *G. theta,* whose PPC is predicted to play an important role in the organism's carbohydrate metabolism. Phylogenomic analyses of PPC-targeted proteins in *G. theta* and *B. natans* suggest that both host- and endosymbiont-derived gene products currently function in the PPC.

Insights provided by recent field studies of protists in low-oxygen/anoxic marine environments (7D)

Virginia Edgcomb and Joan Bernhard

Field studies have formed the foundation of what we know about protozoa. Ultrastructural analyses continue to provide revised circumscriptions of different groups previously classified based on light microscopic examination of field (and medical) samples and of cultured isolates. Powerful molecular approaches provide new avenues for exploring protistan diversity, taxonomy and phylogenetic relationships, and recently have facilitated studies of gene expression and gene content within single specimens and whole communities. However, field studies remain essential for advancing our knowledge of protozoa and the role they play in the environment. This talk highlights recent field studies of oxygen depleted to anoxic/sulfidic marine sedimentary and water-column environments. Emphasized will be recent studies of Santa Barbara Basin (USA), Eastern Mediterranean deep hypersaline anoxic basins, Saanich Inlet (Canada) and Cariaco Basin (Venezuela). Such studies have expanded knowledge of protist groups with tolerance to low oxygen, sulfide, and hypersaline conditions, improved understanding of potential impacts of protists on biogeochemcial cycling, and yielded novel taxa thereby improving phylogenies. Symbioses between protists and bacteria and/or archaea have long been known, however, field studies provide further insights into the prevalence and role of symbioses in dysoxic/sulfidic marine habitats. Funded by NSF grants OCE-0849578 and OCE-1061391.

Meta-protistomics (7D)

Colomban de Vargas

The current revolution in high-throughput sequencing and imaging technologies is pushing protistology from a models-based science into a systems-based science, where protistan genetic and phenotypic makeups and their variations can be assessed at the community and ecosystems levels. In the EU and international research programs BioMarks and Tara-Oceans, respectively, we developed several new approaches to explore the frontiers of marine planktonic total genomic and organismic biodiversity. Whole communities DNA, RNA, and cellular bodies were collected and preserved from 4 organismal size fractions covering the entire range of protistan diversity. A pipeline integrating metabarcoding, metatranscriptomics, single-amplified genomics, to high-throughput imaging was build up to assess the biocomplexity of environmental eukaryomes. The first analyses show

that total planktonic protistan biodiversity can be explored close to saturation using Illumina-sequencing of >1 million metabarcodes (V9 rDNA amplicons) per sample. Several ten thousands different ribosomal OTUs (97%) are typically retrieved from each water sample, while the ribosomal diversity of the global photic oceans reaches >1 million OTUs. In addition, RNA-based metabarcodes are more appropriate for molecular ecology than DNA-based ones, as shown by their significantly higher diversity and closer matches to environmental and organismal diversities. A first glimpse into planktonic protistan total metagenic diversity using 29 metagenomes and metatranscriptomes from the Mediterranean sea shows that >85% of predicted genes are orphans, illustrating the phenomenal knowledge gap we are facing in terms of protistan metabolic and behavioral functional diversities.

Mitosomal transport system in *Entamoeba histolytica* (16A)

Tomoyoshi Nozaki, Takashi Makiuchi, Yoshitaka Murakami, Ken-ichiro Imai and Paul Horton

Under anaerobic environments, the mitochondria have undergone remarkable reduction and transformation into highly reduced structures in the form of mitosomes, hydrogenosomes, and mitochondrion-related organelles (MRO). In agreement with the concept of reductive evolution, the mitosomes of Entamoeba histolytica lack most of the canonical structures associated with the mitochondria like the TOM (translocase of the outer mitochondrial membrane) complex, a multiunit assembly that mediates targeting and membrane translocation of mitochondrial preproteins into MROs. Investigation of the TOM complex in highly divergent MROs may shed light on evolutionary pressures acting on the transport machinery of endosymbiont-derived organelles. Here we show in E. histolytica the presence of a 600-kDa TOM complex composed of Tom40, a central pore-forming subunit, and Tom60, a newly identified mitosomal protein. Tom60 was localized in the outer membrane of mitosomes and in the cytosol, and served as a receptor/carrier molecule of mitosomal preproteins. Our data indicated that E. histolytica mitosomes uniquely perform cargo recognition and transport of molecules via Tom60. It is likely that lineage-specific components like Tom60 may have evolved as a consequence of anaerobic adaptation in eukaryotes. Interestingly, Entamoeba Tom60 contains multiple tetratricopeptide repeats (TPRs), which are implicated in the protein-protein interaction of Tom20 and Tom70, membrane-anchored mitochondrial receptors in aerobic mitochondria. The conservation of TPRs in mitochondrial receptors between the typical aerobic mitochondria and the anaerobic mitosomes indicates that the domain plays an important role in the mitochondrial import machinery.

Origin of photosynthetic eukaryotes: Inferring traits of pre-green ancestors (7C)

Eunsoo Kim

The rise of photosynthetic eukaryotes by endosymbiosis was a critical evolutionary event that fundamentally changed the trajectory of life on Earth. However, many questions surrounding the origin and diversification of photosynthetic eukaryotes remain. One mystery is the identity of the plastid-less protists that are most closely related to so-called primary plastid-containing groups (i.e., green algae plus land plants, red algae, and glaucophytes). The number of endosymbiotic events that gave rise to the current diversity of protists is another unresolved issue. I will present newly obtained transcriptome and ultrastructural data from select protist flagellates and discuss the traits of pre-green protist ancestors that existed prior to the emergence of the first photosynthetic eukaryotes.

Towards a molecular taxonomy: benefits, risks and applications in plankton ecology (2A)

David A. Caron

The increasing use of genetic information for the development of methods to study the diversity, distributions and activities of protists in nature has spawned a new generation of powerful tools. For ecologists, one lure of these approaches lies in the potential for DNA sequences to provide the only immediately obvious means of normalizing the diverse morphology-based taxonomies that exist for protists. A single, molecular taxonomy would allow studies of diversity across a broad range of species, as well as the detection and quantification of particular species of interest within complex, natural assemblages; goals that are not feasible using traditional methods. These charms are not without their potential pitfalls and problems, however. Conflicts involving the species concept, disagreements over the true (ecological) meaning of genetic diversity, and a perceived threat by some that sequence information will displace knowledge regarding the morphologies, functions and physiologies of protistan taxa, have created debate and doubt regarding the efficacy and appropriateness of some genetic approaches. These concerns need continued discussion and eventual resolution as we move towards the irresistible attraction, and potentially enormous benefits, of genetic applications in protistan ecology.

Abstracts - Parallel sessions (in alphabetical order by title)

A calcified cyst-producing dinoflagellate from freshwater: fine-structure characterization and phylogeny (4A)

Sandra C. Craveiro, Mariana S. Pandeirada, Niels Daugbjerg, Øjvind Moestrup and António J. Calado

Calcified structures in dinoflagellates are presently known only within the peridinioid group, in an assemblage that includes resting stages in the fossil record and of extant species of Scrippsiella and allied genera. In addition, coccoid vegetative stages of the closely related Thoracosphaera-group also display a calcareous outer layer. As far as known, all these forms are marine. A small peridinioid (16-25 μm long, 13-23 μm wide) with roundish cells was isolated in March 2011 from a shallow meso-eutrophic pond near Aveiro (NW Portugal). Despite being situated between the two southern channels of the Aveiro coastal lagoon, the pond contains freshwater throughout the year (conductivity mostly 300-400 μ ms/cm) and the organism was grown in 4x L16 medium supplemented with vitamins. Cysts surrounded by a thick organic wall and an outer layer of crystals developed in the cultures. The crystal layer readily dissolved in acetic acid and contained a large fraction of calcium (detected by EDS-X ray spectrometry). Swimming cells contained pyrenoids and a peduncle supported by a three or four-layered microtubular basket. Phylogenetic analysis based on ribosomal SSU, ITS and LSU domains 1 and 2 placed the organism as a sister group to a clade containing Scrippsiella and Duboscquodinium.

A new driving force for the functional gene transfer during endosymbiotic evolution: pol II promoter de novo origination (17A)

Junichi Obokata

During endosymbiotic evolution, genes of the organelle genome were gradually moved to the host nucleus, and integrated into the nuclear gene network. For this functional gene transfer, it is a prerequisite for the translocated genes to become transcriptionally active in the genomic integration loci. Several cases were reported that organelle-derived coding sequences acquired eukaryotic promoters in the past by trapping preexisting nuclear gene. This promoter acquisition mechanism is easy to understand, but one promoter acquisition event will result in one disruption of preexisting genes. Therefore alternative mechanism might be necessary to explain how thousands of organelle-derived coding sequences have become transcriptionally active in the nuclear chromosomes. To explore this mechanism, we carried out a model experiment utilizing gene trap screening system of plants. As a result, we found that the insertion of the promoter-less reporter ORF in the transcriptionally silent genomic regions sometimes causes chromatin remodeling its 5' vicinity, and de novo origination of pol II promoter activity was observed. Molecular mechanism and biological impact of this phenomenon is discussed.

A strategy for 454-pyrosequencing of ribosomal SSU DNA and RNA to more accurately assess haptophyte diversity and relative abundance (12C)

Elianne Egge, Lucie Bittner, Stéphane Audic, Colomban de Vargas, Hervè Moreau and Bente Edvardsen

Next generation sequencing of ribosomal DNA is increasingly used to assess the biodiversity and structure of microbial communities. Our aim was to develop a method to depict the species-level diversity and community structure of marine planktonic haptophytes. We used a mock community consisting of equal number of cells of 11 species representing all known haptophyte orders to investigate the ability of 454-pyrosequencing to detect and assess the number of species present, and assess the relative abundance in terms of cell numbers and biomass. We compared DNA to RNA/cDNA, 454-pyrosequencing to Sanger sequencing of clone libraries, and two different V4-SSU rDNA haptophyte-biased primer pairs. Further, we tested five different strategies to clean the sequence data from errors. Up to 6500 unique tags (from 16500 reads) were obtained before sequence cleaning and clustering, and higher diversity was obtained from cDNA than DNA. Filtering reduced this number up to 200-fold. The proportion of reads assigned to each species was significantly different from the initial proportions of cells or biomass in the culture-mix, but cell numbers were better reflected by proportion of cDNA than DNA reads. Here we propose a strategy to more accurately depict the haptophyte diversity and assess relative abundance using 454-pyrosequencing.

Adaptations to anaerobiosis in the hydrogenosome of *Andalucia incarcerata* (18A)

Michelle M. Leger, Laura A. Hug and Andrew J. Roger

Mitochondrion-related organelles (MROs) have arisen independently in a wide range of anaerobic microbial eukaryotes. To date, these organelles have been studied mainly in parasitic organisms, which limit our ability to distinguish adaptations to anaerobiosis from adaptations to parasitism. Here, we describe the MRO of *Andalucia incarcerata*, a free-living excavate, and based on bioinformatics analyses of extensive transcriptomic data, we predict a biochemical map of the pathways present in this organelle. 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 tentative classification as a hydrogenosome. While *A. incarcerata's* MRO contains a number of canonical hydrogenosomal functions it has retained more complete mitochondrial functions including ten more enzymes involved in amino acid metabolism pathways that are not found in the hydrogenosome of *Trichomonas vaginalis*. 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.

Benthic foraminifera in plankton samples: the illusion of environmental DNA study (13A)

Jan Pawlowski, Emanuela Reo and Franck Lejzerowicz

Foraminifera are omnipresent in marine environment but only about 50 species are known to live in plankton or are adapted to the tychopelagic mode of life. Therefore, it was very surprising to find that the DNA and RNA extracted from the filtered water samples contain a high number of foraminiferal sequences that have been assigned to benthic species. Within the framework of BIOMARKS project, we analyzed 45 samples from coastal waters of North Sea, Mediterranean Sea and Black Sea. We found foraminiferal sequences in all of them, although we know that the planktonic foraminifera were uncommon in these waters. Indeed our analyses identified only few planktonic species. The majority of foraminiferal cDNA/RNA sequences were assigned either to known shallow-water benthic species or to the undetermined environmental clades. The origin of these sequences could be multiple. They may originate from adult specimens washed out of the coast, the propagules dispersed in the water column or the extracellular DNA present in the water samples. Our study suggests that the genetic studies of environmental samples provide an extraordinary source of information about the richness of protist communities but the ecological interpretation of these results should be done with caution.

BLASTGrabber - visualization and taxonomic analysis of BLAST output files

Ralf Neumann, Surendra Kumar and Kamran Shalchian Tabrizi

Due to new sequencing technologies and increasing database sizes, the popular Basic Local Alignment Search Tool (BLAST) algorithm will often produce massive textual output. The BLASTGrabber program will visualize this output, facilitating the analysis of high-throughput BLAST data on a normal computer. Three modes of analysis are supported: alignment visualization, text-mining and hit categorization based on up till three BLAST statistics at once. Taxonomical analysis can be done using an integrated interactive tree structure. Data of interest can be "grabbed" (selected and copied to the local clipboard) in all three modes of analysis and saved as a BLASTGrabber file. This enables a recursive approach, repeatedly trimming down (and possibly joining) subsets of the BLAST data. BLASTGrabber is a Java program that will run on Windows, Mac or Linux computers. It is intended for an audience of computer laymen, not familiar with scripting languages or databases. BLASTGrabber can process the standard textual BLAST output; alternatively a BLAST pipeline installed on the University of Oslo HPC server (www.bioportal.uio.no) can produce BLASTGrabber files complete with taxonomical identifiers.

Biogeography and diversity of dictyostelid social amoebae in high-latitude environments (8I)

A.L. Perrigo, M. Romeralo and S.L. Baldauf

The dictyostelids, also known as cellular slime molds or social amoebae, are a group of soil microbes within Amoebozoa with a unique aggregative multicellular life phase. Dictyostelids are known from terrestrial habitats worldwide, with approximately 150 described species. A meta-analysis of 27 existing distributional studies, combined with novel distribution data, has shown that high latitude environments tend to have lower dictyostelid diversity than localities closer to the equator. This finding is consistent with the latitudinal gradient of species diversity. The latitudinal gradient is a recognized trend in larger organisms (namely plants, animals and fungi) but its relevance to smaller organisms, such as protists, has been the source of considerable debate in recent years. Despite the lower diversity of dictyostelids, high-latitude environments are by no means devoid of species. Recent surveys have been carried out in Iceland and northern Sweden, where four and nine species, respectively, were recovered, including two previously undescribed ones. These findings highlight the need for continued sampling, as well as the utility of molecular data for species identification.

Blastocystis sp. Nand II strain: an evolutionary mosaic or a deeply divergent organism? (20B)

E. Gentekaki, L. Eme, B. Curtis, A. Tsaousis, J. Archibald and A. J. Roger

Blastocystis sp. Nand II strain is an opportunistic parasite that infects humans preferentially. Phylogenetic analyses place Blastocystis within the stramenopiles in a deep-branching position. Recently, we carried out a transcriptomic survey of *Blastocystis* sp. Nand II strain. We were able to generate a total of 1000 transcripts. The transcripts were translated and those less than 50 amino acids long were discarded. The remaining transcripts were used to explore the diverse evolutionary histories of the various proteins. A blast search against a local database was performed and phylogenetic trees were constructed. In many cases, Blastocystis clustered in a basal position to a variety of groups, making it very difficult to determine and tease apart the true phylogenetic affinities of the proteins. In the cases where phylogenetic affinities were noted Blastocystis formed a long branch, thus it was not possible to determine whether the placement was real or a result of long-branch attraction artifact (LBA). By employing sophisticated methods of analyses involving estimation of branch lengths and rates of evolution per site we were able to tease apart and distinguish between cases of LBA and real phylogenetic affinities thus determining the degree and sources of lateral gene transfer (LGT) in Blastocystis.

Broad distribution of TPI-GAPDH fusion proteins among eukaryotes: evidence for glycolysis in the mitochondrion? (18B)

Takuro Nakayama, Ken-ichiro Ishida and John M. Archibald

Glycolysis is a central metabolic pathway in eukaryotic and prokaryotic cells. In eukaryotes, the textbook view is that glycolysis occurs in the cytosol. However, fusion proteins comprised of two glycolytic enzymes, triosephosphate isomerase (TPI) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), were found in members of the stramenopiles (diatoms and oomycetes) and shown to possess amino-terminal mitochondrial targeting signals. In this study, we found that mitochondrial TPI-GAPDH fusion protein genes are widely spread across the known diversity of stramenopiles, including non-photosynthetic species (*Bicosoeca* sp. and *Blastocystis hominis*). Our study also showed that TPI-GAPDH fusion genes exist in three cercozoan taxa (*Paulinella chromatophora*, *Thaumatomastix* sp. and *Mataza hastifera*) and an apusozoan protist, *Thecamonas trahens*. Unexpectedly, subcellular localization predictions for other glycolytic enzymes in stramenopiles and cercozoan showed that a significant fraction of the glycolytic enzymes in these species have mitochondrial-targeted isoforms. These results suggest that part of the glycolytic pathway occurs inside mitochondria in these organisms, broadening our knowledge of the diversity of mitochondrial metabolism of protists.

Combining molecular data with classical morphology for uncultured phagotrophic euglenids (11B)

Gordon Lax and Alastair Simpson

Heterotrophic euglenids are one of the most diverse and important groups of heterotrophic flagellates in sediment systems, and hold the key to understanding the evolution of photosynthetic euglenids. However, relatively little is known about their biodiversity, functional roles and evolution. Even though a wealth of light microscopy-based information is available, little progress has been made in associating this information to molecular sequence data. In addition, environmental sequencing projects likely underestimate the diversity and abundance of euglenid taxa due to the divergent nature of their ribosomal RNA genes. We established an experimental protocol combining morphological and molecular data from single euglenid cells that were uncultured. We isolated individual cells from freshwater and marine benthic samples by micropipetting and sorted them according to their morphological characteristics. After high-resolution photomicrosopy, single cells were subjected to nested PCR using newly-designed euglenid-specific primers in combination with universal eukaryotic primers, generating full-length 18S rDNA sequences. We characterized seven individual isolates and subjected them to phylogenetic analyses. We combined this information with their respective morphological data and found discrepancies between their current taxonomic assignments and our molecular phylogenetic reconstructions. Our experimental protocol provides a useful starting point for future analyses on euglenid biodiversity and their evolutionary relationships.

Comparative analysis of nucleomorph genomes among three chlorarachniophytes (17C)

Shu Shirato Shigekatsu Suzuki, Takuro Nakayama, Yoshihisa Hirakawa, Sayaka Koike and Ken-ichiro Ishida

The plastid of chlorarachniophytes is accompanied by a nucleomorph, the vestigial nucleus of an ancient green algal endosymbiont. The nucleomorph contains a small compacted genome which represents an intermediate stage of endosymbiont's genome reduction in the process of secondary endosymbiosis. Therefore the chlorarachniophytes can be a good model group to study the reductive evolution of endosymbiont's genome. Previous studies demonstrated that the chlorarachniophyte nucleomorph contains three chromosomes and the genome size varies from ca. 330 Kbp to ca. 1 Mbp among the members of chlorarachniophytes. *Bigelowiella natans* is an only chlorarachniophyte of which complete nucleomorph genome has been sequenced sequenced so far. In order to know the nucleomorph genome evolution, we sequenced nucleomorph genomes of two chlorarachniophytes, *Amorphochlora amoebiformis* and *Lotharella vacuolata*, of which nucleomorph genome sizes have been estimated to be 330 Kbp and 450 Kbp, respectively, and performed a comparative analysis. Our analysis suggested that the common ancestor of chlorarachniophytes already had experienced large genome compaction, and the existence of syntenic blocks among three spices indicates that the chromosome rearrangements were frequently occurred separately independently in each clade of chlorarachniophytes. Those findings may indicate that the trend of nucleomorph genome evolution in chlorarachniophytes is similar to that in cryptophytes.

Comparison of eukaryotic microbial communities from a broad range of free-living and host-associated environments (3A)

Laura Wegener Parfrey and Rob Knight

The eukaryotes that reside in the human gut remain relatively unexplored with high-throughput methods. However, many eukaryotic parasites are major sources of morbidity and mortality, especially in developing countries. Initial pyrosequencing results from geographically and socioeconomically diverse populations demonstrate that the eukaryotic component of the human gut microbiome is highly variable across individuals. Family members tend to share a large proportion of taxa, but geography does not drive overall patterns. These results suggest that the presence or absence of specific taxa may be the most important factor in structuring eukaryotic communities in the gut. We also combine these data with additional samples from a wide range of environments to compare the diversity patterns between free-living and host-associated microbial eukaryotic communities. The patchy taxonomic distribution across people obscures the distinction between free-living and host-associated

communities in analyses of beta-diversity. However, using the open-source tool Topiary Explorer to visualize these communities within the eukaryotic tree of life reveals the fundamental differences between host-associated and free-living communities. These data are also combined with community sequence data for bacteria to assess patterns of co-occurrence among taxa and gain an initial window into multilevel trophic dynamics in microbial communities.

Cox I and 18S rDNA genes as barcodes for species differentiation and study of population structure in two lineages of lobose amoebae (8A)

A. Glotova, V. Zlatogursky, A. Kudryavtsev A. Smirnov and J. Pawlowski

The genetic structure of amoebae morphospecies remains virtually not studied; range of current hypotheses varies from clonal population structure to discontinuous genetic diversity. Data on vannellid amoebae show that amoeba morphospecies consists of a limited number of genetic units; the best DNA barcode to distinguish them seems to be the Cox I gene. In the present study we test the genetic structure of amoeba morphospecies in two more phylogenetic lineages. We have studied variability of Cox I and SSU genes for 11 isolates of lobose amoebae of the genus Korotnevella (Discosea, Dactylopodida) and 5 isolates of Flamella (Variosea, Varipodida). Phylogenetic analysis for both genes reveals several clusters of genotypes containing one or several isolates. Cox I gene identifies more clades within a morphospecies than SSU and definitely shows higher resolution of genetic structure of the studied morphospecies. These data confirm that an amoeba morphospecies consists of a limited number of genetic units, however taxonomic meaning of these units is not yet clear. Supported with IZLR Z3_1 28338 and RBRF 12-4-1825 research grants.

Different types of locomotive activity in heliozoans (5E)

V.V. Zlatogursky

Heliozoa is a non-taxonomic name applied for polyphyletic assemblage of radially symmetrical predatory protists. Among them, order Centrohelida includes ca. 80% of heliozoan species. We have found that beside of morphologically different scales, centrohelid heliozoans have very distinctive patterns of locomotive activity. As it seen on videotapes, taken from pure cultures there are many different types of behavior (attachment to the substratum, “lying” on the substratum, rolling movement, or floating) shared by all members of the group, but in different species some of the behaviours may considerably predominate. During the development of culture, different types of behavior subsequently change into each other. On the early stages cells are mostly attached in some species (most representatives of Choanocystis) or are mostly rolling (in all studied species of Acanthocystis). The mechanism of rolling movement in Acanthocystis is clearly different from that in actinophryid heliozoans and includes not only retraction but also active bending of axopodia. Floating often can be found in older cultures when the density of cells is comparatively high. Members of another order of heliozoa - Desmothoracida at some stages of the life cycle show amoeboid activity and possess flagellar stages. Thus, heliozoan morphotype of the cell organization provides many different possibilities for locomotion.

Distribution of conventional and non-conventional introns in tubA and tubB genes of euglenoids (10A)

Rafał Milanowski, Anna Karnkowska-Ishikawa, Abiba Boulahdjel, Takao Ishikawa and Bożena Zakryś

Euglenoids together with heterotrophic flagellates, diplonemids and kinetoplastids, form the group Euglenozoa within the kingdom Excavata, one of the most ancient groups among Eukarya. Three types of introns were found in nuclear genes of euglenoids: (1) conventional spliceosomal introns with canonical GT/C-AG borders, (2) non-conventional introns for which a splicing mechanism is unknown (non-canonical and variable borders, stable secondary structure of intron ends) and (3) so-called intermediate introns. Because of scarce data availability it was difficult to answer some basic questions as: whether the conventional, unconventional or intermediate introns are maintained in conserved positions in different evolutionary lineages, and whether introns of a given type can be replaced by introns of another type. We obtained genomic sequences of tubA and tubB genes from nineteen species representing eleven genera of phototrophic and secondary heterotrophic euglenoids. Analysis of intron distribution showed that conventional introns with canonical borders are present in the same positions in green euglenoids; non-conventional introns emerged in new sites suggesting that they are relatively new structures in genomes. Additionally it seems that intermediate introns have evolved from non-conventional type, which indeed suggests the evolutionary association between non-conventional and conventional introns.

Diverse Trichomonas lineages associated with columbids (11C)

A. Peters and S. R. Raidal

Trichomonas gallinae is a parabasalid long associated with the domestic pigeon (*Columba livia*) and birds of prey. Recent genotypic investigations supported a cryptic diversity of Trichomonas in pigeons and doves, and after extensive field sampling of wild Australian and New Guinean columbids we have discovered several phylogenetic ally divergent Trichomonas lineages. These appear to show host-specificity although a complex correspondence between host and parasite phylogenies exists. The relationship of these organisms with other members of their genus and the conservation of habitat amongst the diverse lineages has implications for the ancestral identity of Trichomonas, potentially including the human sexually transmitted parasite *T. vaginalis* and suggests a deep historical association of Trichomonas with pigeons and doves.

Diversification of cytoskeletal genes following multiple whole genome duplications in Paramecium species (10B)

Yana Eglit, Casey L. McGrath, Lydia J. Bright, Thomas G. Doak and Michael Lynch

Gene duplication appears to be a key mechanism of cellular evolution, leading to subdivided and novel paralogue functions (sub- and neofunctionalisation, respectively), as well as paralogue loss. The *Paramecium aurelia* species complex underwent a round of whole genome duplication prior to its relatively recent radiation – on top of two preceding rounds in the class Oligohymenophora (Aury et al. 2006, Nature) – rendering it an excellent model for investigating the ramifications of gene duplications. The Paramecium cytoskeleton features massively expanded gene families, and earlier work on actins shows a high degree of specialisation in some paralogues (Sehring et al. 2006, J Cell Biol). By employing *P. caudatum* and *P. multimicronucleatum* to reconstruct the ancestral functions of actins, we can follow the fates of recently duplicated paralogues in *P. aurelia* species by examining their intracellular localisation and co-localisation patterns, as a proxy for function. We are currently creating actin:GFP/RFP constructs to transform into multiple *P. aurelia* species, as well as *P. caudatum* and *P. multimicronucleatum*. Furthermore, a paired set of constructs will be made with myosins, whose functional diversification should correlate to the greatly expanded vesicular trafficking system in Paramecium spp. This work should shed light on an important mechanism in the expansion of cellular complexity and novelty exhibited by numerous protist lineages.
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Diversity and seasonal dynamics of haptophytes in outer Oslofjorden revealed by 454 pyrosequencing (12B)

Elianne Egge, Wenche Eikrem, Vladyslava Hostyeva, Tom Andersen, Lucie Bittner, Torill Johannesen, Ruth-Anne Sandaa and Bente Edvardsen

Haptophytes constitute an important part of the marine plankton community and play a major role as primary producers. Bloom formers may have large biogeochemical and economic impact through production of toxins harmful to marine biota and carbon sequestration through calcite scale formation in coccolithophores. Knowledge on the biodiversity and seasonal dynamics of haptophytes at species level is limited because species identification often requires electron microscopy or molecular methods. Here we present results from a study using 454-pyrosequencing to investigate the seasonal dynamics of the haptophyte community in outer Oslofjorden. Samples of the subsurface nano-and picoplankton were collected monthly for two years, and the haptophytes were targeted by amplification with group-specific SSU rDNA V4 primers. The pyrosequencing data were supplemented with clone libraries and microscopy. Considerable diversity is revealed, particularly within the order Prymnesiales. Members of more than 20 genera were detected, distributed among all 8 known haptophyte orders. The pyrosequencing data retrieved species that have not previously been observed by microscopy in the area, and also clades consisting only of hitherto uncultured species (clade D and E). Analysis of the diversity in relation to physicochemical factors is in progress to shed light on mechanisms driving changes in haptophyte community structure.

Diversity of insect trypanosomatids: all that is hidden shall be revealed (11D)

Julius Lukeš, Jan Votýpka, Vyacheslav Yurchenko and Dmitri A. Maslov

The history of research on insect trypanosomatids has quietly passed its sesquicentennial milestone. The period of descriptions of new species from insects culminated with creation of the morphology-based taxonomy of Trypanosomatidae that largely remains in use today. One of its caveats is that it forces all strains to be ascribed into just a few genera, potentially ignoring the actual diversity that had evolved within the group under the cover of a monotonous morphology. This has changed in the era of molecular biology, which provides better means of following the real diversity of these flagellates. Current view on the diversity of non-Trypanosoma trypanosomatids, based on the results obtained from our extensive collections of new strains/species from hemipterid and dipterid hosts captured on different continents will be presented. Moreover, we will introduce a newly isolated strain that represents the earliest diverging trypanosomatid and can thus shed light on the emergence of parasitism in these omnipresent protists.

Diversity of marine Prasinophyceae and associated viruses (14C)

Evelyne Derelle, Camille Clérissi, Yves Desdevises, Gwenael Piganeau, Nigel Grimsley, Bente Edvardsen and Hervè Moreau

The order Mamiellales (Prasinophyceae) comprises freshwater and marine “green” unicellular photosynthetic eukaryotes. Many of these have very small cell sizes and are named “picoeukaryotes” (cell diameter <2µm). The ecological importance of marine Mamiellales in coastal waters, their easy manipulation and their small genome sizes have fostered genome projects in several different species. In this presentation, recent analyses of the 6 available genomes from the Bathycoccus, Micromonas and Ostreococcus genera are presented and the insights gained into the species diversity, their ecology and the evolution of these green algae are reviewed. Furthermore, these picoeukaryotes, as all phytoplankton populations are largely controlled by hugely diverse populations of viruses. Double-stranded (ds-DNA) viruses specific to these three genera have been sequenced. Genome comparison of these giant viruses revealed a high degree of conservation, both for orthologous genes and for synteny, except few differences. These viruses encode a gene repertoire of certain amino acid biosynthesis pathways never previously observed in viruses that are likely to have been acquired from lateral gene transfer from their host or from bacteria. Pairwise comparisons of whole genomes using all coding sequences with homologous counterparts, either between viruses or between their corresponding hosts, revealed that the evolutionary divergences between viruses are lower

than those between their hosts, suggesting either multiple recent host transfers or lower viral evolution rates.

Diversity of planktonic ciliates in deep hypersaline anoxic basins in the Eastern Mediterranean Sea (19C)

A. Stock, W. Orsi, V. Edgcomb, H.-W. Breiner, S. Filker and T. Stoeck

Salt concentrations up to saturation, high pressure, anoxia, high methane and sulfide concentrations challenge basic processes of life in some Eastern Mediterranean deep-sea basins (DHABs – Deep Hypersaline Anoxic Basins). Yet, in the last few years molecular diversity surveys in these DHABs revealed initial proof for the existence of life from all three domains of life. In the domain Eukarya, ciliates, dinoflagellates and kinetoplastids belong to the dominant taxon groups of the protistan plankton. Using a next generation high throughput sequencing strategy we here compare the ciliate communities in different DHABs. Because each DHAB is characterized by a unique set of hydrochemical parameters and isolated from each other, these habitats are ideal model systems to study the influence of environmental selection on planktonic ciliate communities. We show that each basin is characterized by a unique ciliate community and identify environmental parameters explaining a large proportion of the observed spatial variability.

Diversity, evolution and systematics of the Diphyllatea: understanding by combining conventional culture and environmental survey (12D)

Sen Zhao, Kamran Shalchian-Tabrizi, Akinori Yabuki, Makoto M. Watanabe and Dag Klaveness

The heterotrophic Diphylletea is an 'orphan lineage' that seems not to belong to any of well-established major eukaryote supergroups. Recent phylogenomic evidence reveals that it occupies a deep and basal position on the eukaryote tree of life. In spite of the well-illuminated phylogeny of this group, its diversity and the geographic distribution remain incompletely understood. Here, by both classical culturing and environmental PCR techniques, we identified a small unknown diversity of phylotypes and uncovered internal phylogeny in Diphylletea. All of the sequences were recovered into four supported clades (sub-clades) and most of these clades were composed of phylotypes from different geographic regions. The global distribution of Diphylletea suggests this group goes through several expansions since it early diverged in eukaryote tree. Yet, its limited diversity in environmental samples may indicate a low abundance in nature. In addition, we reported Diphylletea culture strains consume a variety of picoplanktons, and a light microscopy survey show a possible symbiotic relationship between *Chlorella pyreuoidosa* and *Collodictyon* under cultural condition. Overall, the group Diphylletea should hold an ecological importance in the food web system. Its true diversity would be better understood, as the group-specific primers are used in further sampling strategies.

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Ecological and biological researches on *Mesodinium rubrum*, one of the major red tide protists in Korea (6c)

Wonho Yih, Hyung Seop Kim, Jong Woo Park, Myung Gil Park and Hae Jin Jeong

Mesodinium rubrum is a cosmopolitan red tide ciliate and also an obligate mixotroph requiring cryptophycean prey for sustainable photosynthesis. Bloom formation by *M. rubrum* has been recorded in Korea since early 1980s, and distributional ecology of Korean *M. rubrum* populations were firstly reported in an article about the red tide occurrence and species succession in Jinhae Bay (Park et al. 1988). The first temperate strain of *M. rubrum* was established from Gomso Bay, Korea in 2002 (Yih et al. 2004), which was followed by a series of research on the biological and ecophysiological characteristics of the ciliate strain MR-MAL01 and then by the first successful cultivation of a DSP dinoflagellate, *Dinophysis acuminata* strain DA-MAL01 in 2006 (Park et al.). The occurrence of *M. rubrum* in Korean coastal waters encompassing from the national wide long-term monitoring program to the spatiotemporally fine-scaled regional studies will be characterized. In parallel, on the basis of the current ecophysiological and cell biological research using Korean *M. rubrum* cultures we will project our perspectives on the future direction of *M. rubrum* research.

Effects of oil pollution on attached microbial communities in short term indoor microcosms (15D)

Young-Ok Kim, Seung-Won Jeong and Eun-Sun Lee

An indoor microcosm experiment was carried out in order to investigate the effect of oil pollution on attached marine microbial communities. Microbial assemblages including ciliates on acrylic plates dipped in 10 L-liter cubic container filled with WAF (Water Accommodated Fractions of crude oil) were monitored during 10 days and compared with the communities in the control. Total ciliate abundances were largely decreased in the WAF. Especially, dominant species at the initial time before the oil exposure, *Eufolliculina* sp. and *Aspidisca* spp., were greatly crashed. However, peritrich ciliates were increased and sustained during the experiment period. In the case of microalgal community, *Thalassionema frauenfeldii*, which was a dominant diatom at the initial time, was considerably damaged while *Nitzschia directa* showed durable in WAF under available silicates. A rapid growth of heterotrophic bacteria was observed and followed by increase of heterotrophic nanoflagellates, which can provide good prey conditions for the peritrich sustainable survival.

Entamoeba diversity and prevalence in cockroaches (8C)

Mustafa H. Fakhri, Courtney A. Cagle and Jeffrey D. Silberman

While the parasitic Amoebozoan *Entamoeba histolytica* has been well-studied for its role in human pathogenesis, the biodiversity of invertebrate-inhabiting Entamoeba has been scarcely investigated. Using molecular methods, we searched

for Entamoeba in the guts of cockroaches from 4 of the 6 Blattodean families. Entamoeba small-subunit rRNA genes were recovered from all 7 cockroach species tested, 4 of which represent newly discovered hosts. Phylogenetic analysis of over 190 sequences revealed a novel and highly diverse clade of invertebrate-inhabiting Entamoeba, separate from the clade predominated by vertebrate-inhabitants. In addition to doubling the known genetic diversity of Entamoeba, these results strengthen the basis for mapping character-state transitions and understanding the evolution of host-commensal relationships.

Environmental diversity and lineage discovery in Haplosporidia (13B)

Hanna Hartikainen, Cedric Berney, Beth Okamura, Stephen Feist, Grant Stentiford, Craig Austin-Baker and David Bass

Haplosporidia (comprising the genera Haplosporidium, Urosporidium, Minchinia, and Bonamia) are endoparasites of many, mostly marine invertebrates, including oysters, mussels, chitons, shrimps, polychaetes, limpets, and trematodes, among many others, and are responsible for some commercially important diseases such as MSX disease of oysters. Haplosporidia are closely related to other parasites of marine invertebrates, e.g. copepods (Paradinium) and Pandalus, several uncharacterized clades of environmental sequences, and the paramyxean genera Marteilia and Paramarteilia. As part of a collaboration with the UK's Centre for Environment, Fisheries, and Aquaculture Science (cefas) we used several haplosporidian lineage-specific primers to investigate the "hidden" diversity of the group in two key study sites on the UK's south coast - a rocky shore with tidepools and a silty saline lagoon partially separated from the sea by a shingle spit. We revealed a large diversity of novel lineages, some closely related to known taxa, some highly distinct. We present a phylogenetic analysis of the novel lineages in the context of all available haplosporidian SSU rDNA sequences. Our results are being integrated with histological and life-cycle information to develop a more realistic understanding of haplosporidia in coastal marine habitats.

EST investigation of an early branching dinoflagellates *Psammosa pacifica* and early evolution of dinoflagellates (20F)

Noriko Okamoto, Fabien Burki, Behzad Imanian and Patrick Keeling

Dinoflagellates exhibit unique features compared other protists, such as RNAs with trans-spliced leader sequence and unconventional organellar genomes. Interestingly, its sister lineage, perkinsids shares some oddities with dinoflagellates, including trans-splicing of nuclear mRNA, though distinct leader sequence from dinoflagellates, as well as RNA editing of mitochondrial RNA. It is of great interest to understand when and how those molecular characteristics have been developed during the early evolution of the dinoflagellates. Recently described *Psammosa pacifica* is one of the early branching dinoflagellates. *Psammosa pacifica* is a free-living, heterotrophic flagellate that lacks typical dinoflagellate morphology but still retains apical complex, a structure found in other myzozoans i.e., perkinsids, colpodellids, apicomplexans, but secondarily lost in the other dinoflagellates. Molecular phylogeny based on SSU rRNA and Hsp90 suggests Psammosa is the earliest evolving dinoflagellate branched right after the divergence of dinoflagellates and perkinsids. Here we investigated ESTs of actively growing *P. pacifica* to gain an insight of the early evolution of dinoflagellates. Our EST library includes transcripts with canonical dinoflagellate trans-spliced leaders. In addition, we're reporting some evolutionary insights gained through search of transcripts that related to the apical complex proteins or those involved in the plastid-related metabolic pathways.

Evolutionary analysis of mitochondrial ancestry of diplomonads: Phylogenomics of mitochondrial-related organelles in Carpediemonas-like organisms (18G)

M. Kolisko, R. Kamikawa, K. Takishita, I. Čepička, Q. Zhang, T. Hashimoto, Y. Akinori, I. Inagaki, A.J. Roger and A.G.B. Simpson

Metamonads are a large group of anaerobic protists consisting of parabasalids and Fornicata (diplomonads, retortamonads, and their free-living relatives the Carpediemonas-like organisms), plus Trimastix and oxymonads. Recent advances in understanding their diversity and phylogenetic relationships make metamonads an excellent system for studying the evolution of anaerobic mitochondria-related organelles. Mitochondria-related organelles in Metamonads range from the relatively large hydrogenosomes of parabasalids, which possess a wide variety of functions and are involved in energy generation, to the extremely reduced and tiny 'mitosomes' of the diplomonad Giardia, whose only known function is iron-sulfur cluster assembly. Other metamonads, including the free-living Trimastix and Carpediemonas-like organisms usually possess relatively large double-membrane bound organelles, but their functions and metabolism remain almost entirely unknown. In the presented study we performed extensive transcriptome sequencing of several of the least studied metamonads, including Trimastix, retortamonads, and several Carpediemonas-like organisms. We have used thorough in-silico analyses of the transcriptomic data to infer putative proteomes of mitochondrion-related organelles of each of these organisms. We compare these in the context of the well-resolved phylogenomic tree of metamonads and suggest the evolutionary steps leading from very complex ancestral mitochondrial organelles to the highly reduced mitosomes of Giardia.

Evolutionary questions raised by RNAi studies of Paramecium parafusin (10C)

Birgit H. Satir, Peter Satir, Søren T. Christensen, Johan Kolstrup and Elzbieta Wyroba

Parafusin (PFUS), a member of the PGM superfamily without enzymatic activity, is a well-characterized Paramecium protein that primarily is part of the scaffold surrounding the trichocysts (dense core secretory vesicles) prior to exocytosis, but may also

be found in the nucleus. Post-translational dephosphoglucosylation of PFUS and liberation into the cytoplasm is essential for DCSV exocytosis. Evolutionarily, PFUS is closely related to eubacterial PGM but not archeal PGM. Liu et al. (2011) described RNAi experiments knocking down PFUS that caused a block in exocytosis, resulting from a shutdown of total DCSV scaffold synthesis, rather than just a PFUS deficiency. Preliminary evidence indicates that in mammals PFUS might be localized to the ciliary pocket and possibly to nucleoli. These observations suggest that PFUS originated as a cytoplasmic scaffold protein, but perhaps at the time of evolution of the eukaryotic nucleus and cilium assumed some function in nuclear control of protein synthesis.

Evolutionary significance of chlorophyll metabolisms of herbivorous protists in aquatic ecosystems (5C)

Yuichiro Kashiyama, Akiko Yokoyama, Takashi Shiratori, Isao Inouye, Ken-ichiro Ishida and Hitoshi Tamiaki

Chlorophylls are crucial strategic biomolecules for phototrophic protists since they are essential for photosynthesis. However, chlorophylls can be highly phototoxic, if their excitation energy after absorbing light is not managed carefully, generating singlet oxygen that causes severe damages on cells. Therefore, not only phototrophs but also protists hosting symbiotic algae should have evolved well-organized metabolic processes on chlorophylls. Moreover, it is also a significant concern for the protists indirectly depending on phototrophs including herbivorous and kleptochloroplast-harboring protists because they must manage phototoxicity of chlorophyll liberated from their lysed plastids. We report detoxifying metabolism on chlorophylls that is widely distributed among herbivorous protists belonging to the Stramenopile-Alveolate-Rhizaria (SAR) and Cryptophyte-Centrohelid-Telonemid-Haptophyte (CCTH) clades. Heterotrophic protists belonging to these two clades are known to be dominant in marine surface environments. The SAR and CCTH clades are also known to include secondary algae and many of protists with photosymbiotic/kleptochloroplasts. We will discuss their evolutionary successes from viewpoints of chlorophyll metabolisms.

Exploring slime moulds biodiversity with environmental RNA analysis in high-altitude forests and grasslands (8B)

Anna Maria Fiore-Donno, Akiko Kamono, Marianne Meyer, Manabu Fukui and Thomas Cavalier-Smith

Myxomycetes, the plasmodial slime-moulds, a monophyletic taxon (ca. 900 species) in the phylum Amoebozoa, are distinctive amoebae with a complex life cycle culminating in the formation of mostly macroscopic fruiting bodies. They are found in nearly every terrestrial biome and as amoeboflagellates in aquatic environments where they cannot form fruiting bodies. Recently it has been shown that they are one of the major components of the soil protistan community. Despite this, they are absent from nearly all environmental sampling studies, probably because of their highly diverging SSU rDNA gene sequences. For the first time, Myxomycetes partial SSU rDNA sequences have been obtained from soil-extracted RNA by using specific primers. Soil samples were collected in three ranges of mountains (French Alps, Scotland and Hokkaido), next to remaining snow patches in spring, a habitat particularly rich in Myxomycetes that have very narrow ecological requirements, the nivicolous species. Seventy-three genotypes were retrieved, the majority (74%) had less than 98% percent similarity with known Myxomycetes sequences; only few genotypes were common to all sites. Our study provides insights into the community composition of an important group of protists and marks a direction for generalized studies about their distribution and abundance.

FeS proteins and FeS cluster assembly machinery in anaerobic protist *Mastigamoeba balamuthi* and *Entamoeba histolytica* (18D)

Eva Nývltová, Miroslava Šedinová, Robert Sutak, Ivan Hrdy, Čestmír Vlček, Jan Paces and Jan Tachezy

Mitochondrion is a central organelle for formation of cellular FeS clusters. This function is mediated by iron-sulfur cluster (ISC) assembly machinery that was inherited from α-proteobacterial ancestor of mitochondria. Uniquely among eukaryotes, in *Entamoeba histolytica* and its free- living relative Mastigamoeba balamuthi, the ISC machinery was replaced by ε-proteobacterial NIF-like system. In M. balamuthi genome, we identified two paralogues of NifS (cysteine desulfurase) and NifU (scaffold protein). One paralogue of each protein was equipped with amino-terminal extension that targeted the protein to mitochondria (MbNifS-M, MbNifU-M), while the second paralogue was found in the cytosol (MbNifS-C, MbNifU-C). Accordingly, cysteine desulfurase activity was detected in both cytosolic and organellar fractions of *M. balamuthi.* Dual localization of NIF system corresponded to the dual localization of FeS proteins (hydrogenase, pyruvate:ferredoxin oxidoreductase). Entamoeba possessed only single EhNifS and EhNifU that we identified in cytosol, but not in mitosomes, reduced forms of mitochondria. Our results suggest that *M. balamuthi* acquired the genes for NifS and NifU from the bacterial donor. Both genes were duplicated and destined to function in the cytosol and in mitochondria, where they replaced the original eukaryotic ISC machinery. The mitochondrial form of NIF machinery was most likely lost in Entamoeba.

Finding homes for outcast protistan lineages through phylogenomics; the case of the breviates (21C)

Matthew W Brown, Susan C Sharpe, Jeffrey D Silberman, Alastair GB Simpson, Aaron A Heiss and Andrew J Roger

There are several orphan lineages that elude clear eukaryotic supergroup affiliations. These include flagellates such as the breviates, apusomonads, and ancyromonads that have been variously proposed to be somehow related to the Amoebozoa and Opisthokonta (i.e. the “unikonts”). Here we describe a novel anaerobic amoeboflagellate (PCB) isolated

from estuarine sediment from Cape Cod, Massachusetts. PCB has a unique multiphase lifecycle with two different flagellate cell types. The amoeboid uniflagellate stage closely resembles the breviates such as Breviata and Subulatomonas, whereas the swimming biflagellate cell type resembles members of the apusomonads. Ultrastructurally, PCB is very similar to *Breviata anathema*, except that its posterior basal body has an emergent flagellum. Using RNA-seq transcriptomic data, we constructed a robust 159 protein super-matrix and report phylogenomic analyses of this dataset. These analyses strongly show that PCB + *B. anathema* clade are the sister group of the apusomonad *Thecamonas trahens*. Collectively, the breviates + Thecamonas are the strongly supported sister group Opisthokonta and are not related to the Amoebozoa as previously suggested for *B. anathema*. Interestingly, we also recovered a nearly complete integrin adhesome from PCB, which is like that of the recently described components of Thecamonas. These data supports the existence of an opisthokont + breviate + apusomonad eukaryotic 'mega-group' and show that multicellular signaling systems like integrins evolved well before the origin of Metazoa and have been secondarily lost by Fungi and choanoflagellates.

Fine structural observation of shell formation in a rhizarian testate amoeba *Paulinella chromatophora* (5B)

Mami Nomura, Takuro Nakayama, Taizo Motomura, Chikako Nagasato and Ken-ichiro Ishida

Paulinella chromatophora possesses a shell that is made of approximately 50 siliceous scales. The scales are formed inside of mother cell and secreted out from the aperture of its shell. The new shell is assembled out side of the cell before cell division. Our previous study revealed that these scales are assembled into the shell by a thick pseudopodium. However, we still don't know how and where the silica deposited to form the scales in the mother cell. The purpose of this study is to reveal the process of scale formation in mother cell with transmission electron microscopy (TEM) and an elemental analysis by scanning transmission electron microscopy (STEM). The TEM observation revealed the scale was made one by one in a silica deposition vesicle (SDV) that was lined with an array of microtubules and located at the posterior end of the cell, which was close position to the Golgi body and endoplasmic reticulum. The STEM elemental analysis showed silicon is accumulated in the SDV. The small amount of silicon was also detected in the SDV that has low electron density. It indicates the scale is made in the SDV and the silica deposition starts after SDV membrane formation.

Fine structure of *Telonema subtile* Griessmann, 1913: A unique cytoskeletal structure among eukaryotes (20A)

Akinori Yabuki, Wenche Eikrem and Kiyotaka Takishita

Telonema is a heterotrophic biflagellate genus occurring in marine environments around the world. Based on recent phylogenetic analyses it has been suggested that *Telonema* is related to cryptomonads, haptophytes and heliozoans (collectively known as "CCTH group" or "Hacrobia"). However, more recently, such a relationship has not been consistently recovered in the analyses of larger datasets. Therefore, the phylogenetic position of *Telonema* in the tree of life still remains unclear and other approaches to address this issue would be required. Previous observations of the ultrastructure of *Telonema* in the electron microscope have revealed the existence of an intricate cytoskeletal structure. Hitherto, this three-dimensional cytoskeleton has not been reconstructed nor discussed, although traditionally the cytoskeletal structure is regarded as one of the most important characteristics when considering the taxonomic/phylogenetic position of a protist. In the present study we observed serial ultra-thin sections and reconstructed the ultrastructure of *T. subtile* including the cytoskeleton. We also discuss the morphological similarity and uniqueness of *Telonema* in comparison with other eukaryotes.

Genome evolution of a tertiary dinoflagellate plastid (17D)

Tove M. Gabrielsen, Marianne A. Minge, Mari Espelund, Alexander J. Nederbragt, Kamran Shalchian-Tabrizi, Christian Otis, Monique Turmel, Claude Lemieux and Kjetill S. Jakobsen

The dinoflagellates have repeatedly replaced their ancestral peridinin-plastid by plastids derived from a variety of algal lineages. We characterized the genome of the haptophyte-derived tertiary plastid in *Karlodinium veneficum* in order to understand the evolutionary processes that have shaped the organelle since it was acquired as a symbiont cell. The plastid genome was analyzed using the 454-pyrosequencing technology, PCR and clone library analyses. The sequences were assembled into a single contig of 143 kb, encoding 70 proteins, 3 rRNAs and a nearly full set of tRNAs. Several genes of probable extrachromosomal origin were also identified, including genes encoding the chaperone DnaK, the rubisco large subunit, two tRNAs and several photosystem genes. Comparative genomics revealed massive rearrangements and gene losses in the *K. veneficum* plastid genome compared to the haptophyte plastid. Despite the reduced number of genes identified, the *K. veneficum* plastid genome has retained a large size due to expanded intergenic regions. Some of the plastid genes are highly diverged and may be pseudogenes or subject to RNA editing. Gene losses, fragmentation and rearrangements are also features of the genomes of the peridinin-containing plastids, suggesting a host-driven process shaping the plastid genomes of dinoflagellates.

Global analysis of plastid diversity reveals new lineages of apicomplexan related protists associated with coral reef environments (3B)

Patrick J. Keeling

The presence of relict non-photosynthetic plastids in obligate intracellular apicomplexan parasites (e.g. Plasmodium) proved puzzling in many ways, but the recent discovery of their photosynthetic relative, *Chromera velia*, has begun to shed much needed light on their origin and evolution. The intense

interest that this single species generated demonstrates how surprisingly little we know about photosynthetic relatives of apicomplexans as a whole. We have investigated global plastid diversity and distribution by comprehensively searching existing prokaryotic sequence surveys for eukaryotic plastids. From more than 1.6 million bacterial sequences, we identified 9,799 plastid-derived sequences, most of which were mis-identified as 'novel bacteria'. Almost all of these sequences could be assigned to well-defined algal lineages, most often green algae, diatoms, and haptophytes. The exceptions were 121 sequences, all of which were related to apicomplexan parasites, and nearly all of which were derived from coral reef environments. Including shorter sequences raised this to nearly 500 sequences in eight Apicomplexan-Related Lineages (ARLs). Close relatives of *C. velia* were rare, but two other clusters were more common and globally distributed, one of which, ARL-V, was strictly associated with corals. We addressed the possible nature of this association using fine-scale bacterial sequence surveys. In a transect between corals and epiphytic algae, ARL-V is specifically associated with the coral, in contrast to other algal types (including diatoms, haptophytes, prasinophytes, and photosynthetic apicomplexan relatives, Chromera and Vitrella), which are associated with macroalgae. ARL-V is associated with at least 20 species of symbiotic corals through extended time periods and large geographic distances. It is predominantly found in healthy coral tissue at shallow reef depths. Altogether, the evidence points to a specific relationship between ARL-V and corals and is suggestive of symbiosis, perhaps based on photosynthesis

Good families gone bad: The origin and evolution of pathogenic genes In the oomycete secretome (9D)

Ian Misner, Guy Leonard, Eric Bapteste, Philippe Lopez, Thomas Richards and Christopher Lane

Saprobic organisms, such as fungi, bacteria, and a variety of protists, rely on secreted proteins to obtain nutrients and breakdown complex macromolecules; these proteins are collectively termed the secretome. While free-living saprobes play a vital biological role in decomposition, it is the parasitic relatives of these organisms that are of greater scientific and economic interest. Parasites rely on the secretome to not only obtain nutrients, but for host attachment, cellular breakdown, and host defense avoidance. Little is known about how parasites have obtained these novel functions or how the evolved from free-living relatives. To understand the evolutionary processes at work we have sequenced the genomes, and identified the secretomes, of two Saprolegnian oomycetes, the free-living *Thraustotheca clavata* and the facultative parasite *Achlya hypogyna*. Combining our data with the available secretomes from parasitic Peronosporalen taxa we have assembled a core oomycete secretome. Using these methods we have identified unique gene family expansions, contractions, and horizontal gene transfer events that are key to the evolution of parasitism within this diverse group of organisms.

GTPases of the ROCO family: a component of an ancient eukaryotic immune system? (9A)

Marek Elias

Most, if not all, cellular organisms are apparently attacked by specific pathogens (or parasites) and hence need to employ appropriate defensive strategies. However, the putative immune systems of most organismal groups, including most protist lineages, remain uncharacterized. Recently, we hypothesized (Zambounis et al. 2012, Mol. Biol. Evol. 29:1263-76) that the brown alga *Ectocarpus siliculosus* uses as immune receptors proteins of the ROCO family. This family is defined by a conserved core composed of a GTPase and a dimerisation domain, and has been found in some prokaryotes and many eukaryotic groups. Cellular roles of very few eukaryotic ROCO proteins have been characterized in detail, and the immunity-related function proposed in Ectocarpus remains to be corroborated experimentally. It was thus interesting to note that ROCO families in genomes of phylogenetically diverse lineages (choanoflagellates, filastereans, rhizarians, cryptophytes, lycophytes and others) also exhibit features pointing towards their possible involvement in pathogen defence: apparent evolution by a rapid birth-and-death process and domain architectures including evolutionary versatile domains for highly specific protein-protein interactions and/or domains found in known immunity-related proteins (leucine-rich repeats, ankyrin repeats, TIR). I therefore argue that ROCO proteins are a common and ancestral component of the eukaryotic immunity toolkit.

Host genotype by parasite genotype interactions underlying the resistance of toxic microalgae species to the protist parasite *parvilucifera sinerae* (9B)

R.I. Figueroa, L. Råberg, E. Alacid, M. Turon and E. Garcès

Parasites are thought to have an important effect on host population genetic diversity, and may even be the selective force maintaining sexual reproduction. However, models focusing on the evolution of virulence commonly assume that parasite populations are genetically homogeneous; disregarding that different trade-offs between parasite virulence in different host types may exist. The protist parasite genus Parvilucifera infects a wide range of phytoplanktonic species within the group of microalgae known as dinoflagellates. Therefore, it is considered a generalist parasite. "Generalist" parasites have however a high potential to become specialized on different host species. If so, it may then be expected that parasite-microalgae relationships be based on host genotype by parasite genotype interactions. In such systems, certain hosts are resistant to one subset of the parasite's genotypes, while other hosts are resistant to a different subset. To investigate this hypothesis we have studied patterns of genotype interactions between 10 different parasite genotypes and 9 different host genotypes by assessing the proportion of infected hosts, the number of parasite spore stages and maximum host cell density achieved during infection. A genetic analysis of the 10 parasite

genotypes employed is for first time shown in the species *Parvilucifera sinerae* (18S, LSU and ITS1 sequences). Preliminary results indicate that host resistance varies widely, being one strain resistant to all parasites. However, resistant genotype interactions involving other host strains were also found.

How many endosymbiotic gene transfers in eukaryotes? (18B)

Fabien Burki and Patrick Keeling

The transition from endosymbiont to organelle in eukaryotic cells involves the transfer of significant numbers of genes to the host genomes, a process known as Endosymbiotic Gene Transfer (EGT). The detection of EGTs largely relies on automated phylogenomic pipelines, but, so far, the outputs of such analyses have shown great inconsistencies. Most importantly, the interpretation of the number of inferred EGTs remains subjective for the reason that no unambiguous rule exists to distinguish between HGTs from related sources and EGTs. Here, we propose to discuss the issues associated with the detection of EGTs and evaluate their occurrence in 5 different photosynthetic eukaryotes, each allowing us to address a specific aspect: a) the chromerid *Chromera velia*, which provides an independent reanalysis of a published dataset; b) the dinoflagellates *Karenia brevis* and *Karlodinium micrum*, which possess a tertiary plastid of haptophyte origin acquired relatively recently compared to most cases of endosymbiosis; and c) the rhizarian *Bigelowiella natans* and the cryptophyte *Guillardia theta*, whose nuclear genomes have recently been sequences, thus providing a complete set of proteins.

Hydrogenosomes in the diplomonad parasite *Spironucleus salmonicida* (18C)

Jon Jerlström-Hultqvist, Elin Einarsson, Karin Hjort, Daniel Steinhauf, FeiFei Xu, Jan O. Andersson and Staffan G. Svärd

Mitosomes and hydrogenosomes are mitochondrial remnant organelles (MROs) found in diverse eukaryotic microbes inhabiting anaerobic or microaerophilic environments. Essentially nothing is known about the MRO in diplomonads other than *G. intestinalis*, which harbors a mitosome. Recently, the diplomonad parasite *Spironucleus vortens* was reported to evolve hydrogen in comparable amounts to the hydrogenosome-bearing parasite *Trichomonas vaginalis*. We confirm that hydrogen production is prevalent in Spironucleus and identify MROs by immunoEM in the fish parasite Spironucleus salmonicida. Comparative genomics of mitosomal and hydrogenosomal proteins in *S. salmonicida* was used to identify candidate MRO proteins. Co-localization with MRO markers was used to assign candidate proteins to the MRO allowing the construction of a minimal MRO proteome of 21 proteins that includes potentially novel enzymatic activities and the hydrogenase maturation machinery (HMM), previously not found in diplomonad MROs. Two of the seven [FeFe]-hydrogenases in the *S. salmonicida* genome localized to the MRO, indicating that the organelle might be classified as a hydrogenosome. The presence and phylogenetic history of the HMM in *S. salmonicida* hydrogenosomes might indicate that the ancestors of extant diplomonads contained a hydrogenosome-like organelle. Hydrogenosomes appear to be an ancient metabolic adaptation perhaps predating the split of the diplomonads and trichomonads.

Intermediate fragmentation per se provides stable predator-prey metapopulation dynamics (6A)

Jen Cooper, Jiqiu Li and David Montagnes

The extent to which a landscape is fragmented will affect the persistence of predator-prey dynamics. Increasing fragmentation concomitantly imposes conditions that stabilise and destabilise metapopulations, and we assess the hypothesis that intermediate levels provide optimal conditions. We examine four structural changes that arise from increased fragmented: increased fragment number; decreased fragment size; increased connectedness (corridors scaled to fragment); increased fragment heterogeneity (based on connectedness). Using the model predator-prey system (Didinium-Paramecium) we support our hypothesis, by examining replicated metapopulations at five fragmentation levels over time. While both species became extinct without fragmentation, prey survived at low and high levels, and both species survived at intermediate levels. By examining time to extinction, maximum abundances, and population asynchrony we conclude that fragmentation produces structural heterogeneity (independent of environmental heterogeneity) that influences stability. Our analysis suggests why some theoretical, field, and microcosm studies present conflicting views of fragmentation effects on the persistence of populations.

Investigating the effect of light on the flagellated form of *Chromera velia* (15E)

Kate Weatherby, Jan Slapeta and Dee Carter

Chromera velia was recently discovered associated with Australian stony corals. This unicellular alveolate possesses housekeeping genes and ultrastructural features typical of apicomplexans, and has active photosynthetic pathways related to dinoflagellates. This unique connection suggests that *C. velia* can be used to understand the evolution of apicomplexan parasites from their algal ancestors. *C. velia* exists in either an immotile coccoid state or an active motile flagellated form. The life cycle is not well understood and little is known about what triggers the transformation between states, and how this translates to ecological conditions. The aim of this study was to explore the effect of light on *C. ve*lia and its ability to flagellate. Cultures grown under different wavelengths of light in light-dark cycles were found to exhibit differing flagellation patterns. Blue wavelengths induced the highest levels of flagellating cells. *C. velia* also exhibited a temporal cycle of motility over 24 hours similar to that of the coral symbiont Symbiodinium. This pattern continued when the cells were grown in constant dark, suggesting that the cycle is not completely light dependent. Phototactic responses to light were also observed for the flagellated form providing insight into how *C. velia* moves through its natural environment.

Large scale DNA-based characterization of eukaryotic communities from bromeliad tank waters (3C)

Laura R. P. Utz, Adriana Giongo, Eric Triplett, Raquel Dias, Cèsar A. F. De Rose, Claudio A. Mondin, Renata Medina da Silva, Leandro V. Astarita and Eduardo Eizirik

Next-generation sequencing technologies are beginning to shed light onto the composition and dynamics of environmental communities. However, few studies so far have focused on eukaryotes or on poorly known habitats such as bromeliad tank waters. Using Illumina high-throughput sequencing of 18S rDNA, we surveyed the eukaryotic communities inhabiting tanks of the bromeliads *Vriesea platynema* and *Aechmea gamosepala* in southern Brazil. We analyzed a total of 76,406 sequences, representing a per-sample average of 7,778 reads for V. platymena and 4,674 reads for *A. gamosepala*. Sequences were classified with BLAST using a TaxCollector-modified NCBI database. Both species harbored a very diverse eukaryotic community, whose similarity varied depending on the taxon assignment criterion. For example, using 95% identity as a cutoff for assigning sequences to the same taxon, a total of 616 different units were observed, of which 195 were found only in *V. platynema* and 124 only in *A. gamosepala*. Many sequences could be identified at genus level, often matching protists that were also visually detected in the same sample. Still, the most frequent category (>20% in each bromeliad species) was “unidentified eukaryote environmental sample”, highlighting the task ahead of describing and understanding the biodiversity of these environments.

Lectin binding patterns in marine raphidophytes (5A)

Anette Engesmo, Richard Dillaman, Carmelo Tomas and Wenche Eikrem

Lectins are a diverse group of molecules that bind with varying specifically to carbohydrates present on cell surfaces. Since these carbohydrates can play a crucial role in transmitting biochemical signals between and among cells, the carbohydrate composition of cell surfaces is likely to be closely linked to their biology. The marine raphidophytes *Heterosigma akashiwo, Chattonella subsalsa, C. antiqua, Fibrocapsa japonica* and *Haramonas dimorpha* were, along with the haptophyte *Prymnesium parvum* and the dinoflagellate *Gymnodinium instriatum*, preserved in 3 % paraformaldahyde, stained with 14 different fluorescent-labeled lectins and examined under a confocal microscope. *Fibrocapsa japonica* was the only species that tested positive for UEA I (which binds α-linked fucose residues) and ECL (galactose residues), while only *Chattonella antiqua* tested positive for GSL II (α - and β -linked N-acetylglucosamine). All species examined, except Haramonas dimorpha tested positive for LCA (which binds a-linked mannose residues). We observed substantial fluctuations in binding intensity, even within the same species and a number of clearly distinct binding patterns were evident. We believe the variability in lectin binding patterns reflects both temporal and species differences in the biology of toxic algae and may therefore be a valuable tool for species identification

Loukozoa and Sulcozoa: the significance of groovy flagellates for eukaryote deep phylogeny (21D)

Thomas Cavalier-Smith

I shall compare the cytoskeletons of two key paraphyletic phyla of groovy phagotrophic flagellates: the more ancient Loukozoa and more derived Sulcozoa. I shall discuss protist deep phylogeny with special reference to (1) flagellate ultrastructure and body plan diversity; (2) results of recent multi-gene sequence trees, presenting a new one with ~175 genes and many sulcozoan taxa; and (3) my recent ideas on location of the root of the eukaryote tree. I now see eukaryote evolution in terms of three supergroups: the basal protozoan subkingdom Eozoa (comprising Euglenozoa and Excavata, between which the root probably lies); and two huge derived clades: corticates (kingdoms Chromista and Plantae), ancestrally characterised by cortical alveoli (membranous sacs), and podiates (protozoan subkingdom Sarcomastigota, plus kingdoms Fungi and Animalia) which ancestrally were characterised by pseudopodia used for feeding (lost when walls evolved independently in Fungi, Ichthyosporea, Corallochytrium). Sarcomastigota comprise phyla Amoebozoa, Choanozoa, and new phylum Sulcozoa comprising subphyla Apusozoa (Apusomonadida only) and Varisulca (Planomonadida, Discocelida, Mantamonadida, probably all related; Rigifilida, and Diphyllatea). I shall focus especially on evolution within Sulcozoa, the basal podiate phylum, but will touch on other topics, including arguing that Loukozoa should be expanded by including the secondarily anaerobic Metamonada as a subphylum.

Mitochondrion-like organelle of *Trimastix pyriformis* (18E)

Z Zubacova, L Novak, J Hlavackova, J Ridl, V Vacek, I Hrdy, J Tachezy, Vlcek C and V Hampl

Trimastix (Metamonada, Excavata) is a heterotrophic tetraflagellate inhabiting oxygen poor environments and its mitochondria are reduced accordingly to double membrane bounded electron dense vesicles without cristae. In the transcriptome of *Trimastix pyriformis* were detected 19 proteins that, in other eukaryotes, typically function in mitochondrial organelles. N-terminal extensions, putative targeting sequences, were detected in four of them – Cpn60 and H, T and P1 proteins of glycine cleavage system (GCS). We have used tree methods to investigate the localisation of some of these candidates – immunoblots with cell fractions, immunofluorescence slides and expression of GFP tagged proteins in yeast. The evidence from these methods suggests that the proteins of GCS and HydG (hydrogenase maturase) are localised within the organelle. On the other hand, pyruvate:ferredoxin oxidoreductase (PFO) and [FeFe]hydrogenase are probably localised in the cytoplasm. The presence of HydG in the organelles, however, indicates that other form of [FeFe]hydrogenase (Trimastix has three) may localise to the organelles. Presence of GCS in the organelle suggests that it is involved in the amino acid metabolism and production of NADH and

methylation cofactors. Annotation of 454 sequences of Trimastix transcriptome, which is in progress, promises to complement the picture of putative functions of this compartment.

Molecular characterization of *Ichthyophthirius multifiliis* (4B)

Matthew Therkelsen and Wei-Jen Chang

Ichthyophthirius multifiliis (Ich) is a ciliate parasite that causes white spot disease in freshwater fish. While Ich's life cycle is well known, whether it undergoes sexual reproduction remains unclear. Due to the lack of well-characterized genetic markers in Ich, serotyping has been the most commonly used method in describing different isolates of Ich. Serotyping, however, is not useful in addressing Ich's method of reproduction, or in providing information regarding the evolution and geographic distribution of Ich. Here we report the use of single nucleotide polymorphisms (SNPs) to help characterize three geographically different isolates of Ich. The SNPs were identified from the Ich genome projects, and a group of those that could be distinguished by restriction enzyme digestions were selected for further testing. Based on the SNP positions, we found that the isolate NY4 is the most distantly related to the other two isolates, G5 and NY7. As both NY4 and NY7 have an unknown serotype, our genetic markers proved to be more successful at classifying strains of Ich than serotyping.

Molecular phylogenetics of peritrich ciliates (Ciliophora, Peritrichia) with emphasis on the genus Epistylis (19B)

Laura R. P. Utz, Taiz L. L. Simão, Lúcia S. L. Safi and Eduardo Eizirik

Epistylis is one of the most speciose genera among peritrich ciliates, being recognized as a taxonomic entity. However, previous molecular phylogenetic studies have indicated that it contains taxa that are placed in the orders Vorticellida and Operculariida. So far a single species has been placed in Operculariida, whereas all other sampled Epistylis were positioned within Vorticellida. Different analyses have supported the monophyly of species morphologically identified as Epistylis, and often placed them as basal lineages within Vorticellida. In the present study, we have obtained 18S rDNA sequences from six different Epistylis species collected in freshwater environments in Rio Grande do Sul state, Brazil. Five of them consistently grouped with other Epistylis species within the order Vorticellida, while the sixth was robustly placed in order Opercullariida. This result corroborates the previous findings that morphologically-identified Epistylis may represent lineages from the two peritrich orders, supporting the proposition that their diagnostic features may be shared plesiomorphies. Within Vorticellida, 'Epistylis' species formed at least two different clades, whose joint monophyly was not supported. Within this main Epistylis group, a strongly supported sub-clade was composed by three new species that present a thick stalk, indicating that this feature may be a synapomorphy for this group..

Neofunctionalization of rab7 duplicated genes studied by molecular biology tools and high resolution imaging techniques (10D)

Elzbieta Wyroba, Rafał Bartosiewicz and Magdalena Osińska

Rab proteins mediate membrane trafficking by interaction with their effectors. RILP is an effector of Rab7. There are several evidences for presence of RILP-like proteins in *Paramecium octaurelia* obtained by cloning, RT-PCR and immunoblotting. The cross-immunoprecipitation confirmed interaction of Rab7a with RILP1, whereas confocal and ultrastructural studies revealed their colocalization at the late stage of phagocytosis. Post-transcriptional silencing of rab7a gene abolished this interaction and suppressed latex ingestion by 70% due to impairment of V-ATPase recruitment. To visualize interaction of these proteins we have developed a novel technique of 3D reconstruction in electron microscopy (JEM 1400) covered by our EP1146157.3 patent application (pending) that is based on 141 images acquired with tomographic holder. This high-resolution tool proved trafficking of Rab7a, RILP1 and V-ATPase within lysosomal compartment indispensable for phagosome maturation. Rab7b does not interact with RILP1 and its knocking down did not evoke any significant phenotypic effect. STED confocal laser microscopy revealed the distinct targeting of Rab7b to the oral apparatus: in double immunolabeling with α-tubulin it was mapped partially to the region of basal bodies arranged into the rows called peniculus and quadrulus. These results point out to a new function acquired by rab7b gene in *P. octaurelia*.

Neospora spp. And *Toxoplasma gondii* antibodies in donkeys from Southern Italy (4C)

E. Bartova, T. Machacova, K. Sedlak, G. Fusco and U. Mariani and V. Veneziano

The sera of 238 donkeys from 19 donkey farms in southern Italy were tested for *T. gondii* antibodies by Latex Agglutination Test (LAT) and by the Indirect Fluorescent Antibody Test (IFAT); a titre ≥ 50 was considered positive. The same sera were tested for *N. caninum* antibodies by a Competitive-Inhibition Enzyme-linked Immunosorbent Assay (cELISA); ≥30% inhibition was considered positive. Antibodies against *T. gondii* were found in 12 (5%) and 19 (8%) donkeys by LAT and IFAT, respectively. Antibodies against *Neospora* spp. were found in 28 (11.8%) donkeys with inhibition ranging from 30.07% to 44.34%. In case of both *T. gondii* and *N. caninum*, no statistical difference (P>0.05) was found between genders, age, use and their seropositivity. We found statistical difference (P > 0.05) in breeds (18%) compared to crossbreeds (5%) for *N. caninum* with different seroprevalence in individual breeds; however no statistical difference was found for *T. gondii*. The presence of cats or dogs in farms, the size of farms, grazing or keeping of donkeys inside were not confirmed as a risk factor. The present study describes for the first time the presence of *Neospora* spp. and *T. gondii* in donkeys from Italy. This study was funded by the grant no. MSM6215712402 from the Ministry of Education,

Youth and Sports of the Czech and by the grant 6/2012/FVHE from IGA VFU Brno, Czech Republic.

New species of gregarine parasites from invertebrates in Japan and the UK (4D)

Sonja Rueckert

Gregarines are apicomplexan parasites that can be found in terrestrial, freshwater and marine habitats. They infect the intestine, coelome and reproductive organs of their invertebrate hosts. Gregarines have been lumped conveniently into three major groups (archigregarines, eugregarines and neogregarines), but only the archi- and eugregarines occur in marine environments. The diversity of marine gregarines is enormous and is due to ultrastructural and behavioural adaptations. Especially the feeding stages, the trophozoites show a huge variability in their morphology. Still, the number of gregarine apicomplexans described on ultrastructural and also molecular level is quite limited. The impact of gregarine apicomplexan parasites on their hosts varies from harmless to severe: commensalism, reduction of a host's ability to assimilate food, delayed development, decrease of body weight and longevity. I will present a case study on a gregarine as probable disease causing agent in an inland culture of the ascidian *Ciona intestinalis* in Japan. My talk will also address some new data on the morphology and phylogeny of novel gregarines from polychaetes in the UK..

Novel benthic ciliates (Protozoa) from marine sediments of the UK (19D)

Xiaozhong Hu and Alan Warren

Benthic ciliates are important components of the food chain of the marine sediments, however their morphological and ecological diversity is comparatively little known worldwide; moreover for the majority of described species, molecular data are lacking. As part of an ongoing Marie Curie project about the diversity and systematics of this group of microorganisms, a faunistic survey has been carried out along the British coasts for two years. A variety of habitats have been sampled including sandy beaches, mud flats and salt marshes. About fifty species from 42 genera have been investigated in detail and identified to species based on modern taxonomic criteria of ciliate protozoan. Of these, twelve species are new records for the UK and five are new to science representing two new genera. This study also demonstrates that some species represented in the literature were misidentified, and thus the true diversity of benthic ciliates is still far from understood. Here we present taxonomy and phylogeny of these five new species. This work is funded by the National Natural Science Foundation of China (project number: 41176119) and the EU Marie Curie International Incoming Fellowship.

Nutritional history matters to predator-prey population dynamics: the effect of past food experience on Didinium eating Paramecium, a model system (15A)

David J.S. Montagnes and Jiqiu Li

Virtually all simple predator-prey models and most complex ecosystem models that incorporate predator-prey dynamics are structured, directly or indirectly, on the Lotka-Volterra couple of differential equations for predator-prey dynamics. One underlying assumption of these is that predator ingestion (and for some models growth rate) is dependent on the present abundance of prey, without considering the past nutritional experience of the predator. However, there is good evidence that "nutritional history" will affect the ingestion rate, growth rate, and even size of predators. Exploring the consequences of incorporating such nutritional history into models is, therefore, of importance. We use a model predator-prey system (Didinium-Paramecium) to first reveal that nutritional history does influence the relationship between ingestion, growth rate, and predator cell size vs. prey concentration (i.e., the functional, numerical, and volume responses). We then indicate how incorporating these responses will alter model outputs of predator-prey dynamics. In doing so, we use this model-protozoan system to illustrate the need for further work in this direction, on general ecological scales.

Organellar interactions in red algal host/parasite heterokaryon cells (9C)

Nicolas A. Blouin and Christopher E. Lane

Many virulent eukaryotic pathogens and parasites have either directly evolved from a photosynthetic ancestor or are hypothesized to have plastid containing ancestry. The primary barrier to understanding the early stages of evolution of these parasites, however, has been the difficulty in finding parasites with closely related free-living lineages with which to make comparisons. Parasites found throughout the florideophyte red algal lineage provide a unique and powerful model to investigate the genetic origins of a parasitic lifestyle. This is because they share a recent common ancestor with an extant free-living red algal (adelphoparasitism). Cytological studies have shown that the adelphoparasite *Gracilariophila oryzoides* contains a non-photosynthetic plastid (proplastid) that is derived from its host, *Gracilariopsis andersonii* while still maintaining it own mitochondria. We have identified candidate nuclear-encoded genes in the host-parasite pair through homology searches against known plastid proteomes and target signal prediction of transcriptome data. We present ongoing bioinformatics research to determine which nuclear encoded, plastid-targeted genes remain in the parasite's genome and are transcriptionally active. Further, we investigate whether or not key ATP synthase genes (sdhC and atp8) not found in the parasite's mitochondrion genome have been transferred to its nucleus or lost completely.

Phylogenetic analyses on the evolution of eukaryotes using concatenated ribosomal protein sequences (21F)

Tani Leigh and Wei-Jen Chang

Constructing a robust eukaryotic phylogenetic tree has proven to be one of the most challenging tasks faced by evolutionary biologists to this day. As more molecular and morphological data have become

available, various studies now claim that all eukaryotes can be classified into six “super groups”, as well as two over-arching clades, the unikonts and bikonts. Much discrepancy remains, though, as to whether these categorizations are entirely valid. Given that ribosomal proteins are universally present and are typically well-conserved, we selected them as a basis for reconstructing eukaryotic phylogenetic trees representing 34 eukaryotic species spanning five of the six super groups. Our results showed that the topology of the trees generated using ribosomal proteins as markers did not match the topology of trees advocating the unikont-bikont grouping. However, this was largely due to the fact that amitochondriate species consistently grouped together rather than with their expected super groups. Without amitochondriate species, the five eukaryotic super groups were well supported by using ribosomal protein sequences as phylogenetic markers. This occurrence suggests that the lack of mitochondria may have driven eukaryotic ribosomal proteins to evolve differently, an event which was most likely the result of convergent evolution as implied by our data.

Phylogenomics of eukaryote proteins of Bacterial origin (21E)

Ding He, Johan Viklund and Siv Andersson and Sandra Baldauf

The eukaryote proteins of Bacterial origin (euBacs) contribute significantly in shaping eukaryote genome evolution. The nuclear-encoded euBacs were results from endosymbiotic gene transfer that transformed symbionts into mitochondria and ultimately gave birth of modern eukaryotes. We have conducted a systematic survey focusing on ubiquitous euBacs that potentially possess ancestral information to unveil the early eukaryotic evolution. Specifically we are using euBacs with various phylogenetic analyses to test some of the most important outstanding questions, such as the root of eukaryote tree of life and the origin of mitochondria.

Phylogeny and Biodiversity of Phytomyxea (“Plasmodiophorids”) (3E)

Sigrid Neuhauser, David Bass and Martin Kirchmair

Phytomyxea (Rhizaria, Endomyxa; common name plasmodiophorids) are 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. Currently the group is subdivided into two orders: Phagomyxida - which are parasites of diatoms and brown algae - and Plasmodiophorida, parasites of green plants. Extensive environmental sampling and sequencing revealed, however, that these two orders are not concordant with higher level host taxonomy: The oomycete parasite *Woronina phytii* is a member of the Plasmodiophoridia, whereas the seagrass parasite *Plasmodiophora diplantherae* is affiliated with the Phagomyxids. These results suggest that phytomyxid parasitism of stramenopiles and higher plants developed at two different, independent evolutionary events. We also found a diverse set of well supported 18S rDNA clades in environmental samples, which very likely represent undescribed species. The results from these DNA-samplings suggest that phytomyxids are ubiquitous in suitable environments worldwide, but also that the sampling point rather than sample size is the major factor influencing the biodiversity detected within one sample. The number of species within each sample was small and strongly influenced by the plants growing at the point of sampling. These results will be used to inform more targeted samplings and to identify the so far cryptic lineages and the host they are associated with.

Phylogeny and DNA barcoding of the Himatismenida based on three genes (8E)

Alexander Kudryavtsev, Alexey Smirnov and Jan Pawlowski

The order Himatismenida Page, 1987 (Amoebozoa) comprises discoid lobose amoebae in which part of the plasma membrane surface is covered with a flexible layer of organic material secreted by the cell. Currently the order includes families Parvamoebidae, Cochliopodiidae and Goceviidae, with still largely unclear phylogenetic relationships. We present an overview of the morphological and genetic diversity of the Himatismenida comparing the performance of nuclear SSU rRNA, actin and mitochondrial Cox1 genes for reconstructing the phylogeny and DNA barcoding of species in Himatismenida. We conclude that SSUrRNA and Cox1 genes perform similarly as DNA barcodes for the genus Cochliopodium revealing an extensive cryptic speciation in at least one species. Both genes are also suitable as phylogenetic markers for himatismenids yielding the best results when analysed together in concatenated alignment. Phylogenetic relationships reconstructed using actin gene are obscured by the presence of multiple copies and paralogs, and different strains of the same morphospecies may often demonstrate higher level of actin polymorphism than observed between different morphospecies. Yet, actin polymorphism within the morphospecies also corresponds to multiple genotypes revealed by SSU rRNA and Cox1. Supported by the grant IZLR Z3_128338 from the Swiss-Russian Science and Technology Cooperation Programme and a RFBR grant 12-04-01835.

Phylogeny and ecology of Endomyxa, a bewilderingly diverse assemblage of lineages within Cercozoa (Rhizaria) (1C)

Cédric Berney, Patricia Dyal and David Bass

Endomyxa is a possibly paraphyletic assemblage of various lineages within phylum Cercozoa (part of the eukaryotic supergroup Rhizaria). The morphological, ecological and genetic diversity of Endomyxa is huge: they include at least three major lineages of amoeboid organisms (the vampyrellids, Filoreta, and Gromia), two major lineages of parasitic organisms (the phytomyxean plant and algal pathogens and the ascetosporean invertebrate parasites), plus a collection of lineages known so far only from environmental surveys. As part of a project about the diversity, ecology and evolution of large, naked, ramose or reticulose amoebae, we present several

newly isolated amoebae belonging to Endomyxa, mostly among vampyrellids. An intensive screening of environmental clones in the NCBI and BioMarKs databases by Blast combined with preliminary results from new Endomyxa-enriched 18S rDNA environmental libraries further highlight the astounding diversity of this major cercozoan assemblage in both marine and freshwater habitats. We also present an updated phylogeny of Endomyxa based on 18S rDNA and concatenated 18S+28S rDNA, and ongoing efforts to identify endomyxan lineages so far only known from environmental 18S rDNA surveys. In light of our results, we discuss possible higher-level relationships and major evolutionary and ecological trends within Rhizaria as a whole.

Phylogeny and origin of parasitism in Archamoebae (8F)

Eliska Ptackova, Lukas Falteisek, Alexei Y. Kostygov, Lyudmila V. Chistyakova, Alexander O. Frolov, Giselle Walker and Ivan Čepička

The human pathogen *Entamoeba histolytica* is the most studied member of Archamoebae. Recent phylogenetic analyses showed that the genus Entamoeba is unrelated to the other parasitic Archamoebae (genera Endolimax and Iodamoeba). We have established five free-living and two endobiotic strains of the genus Rhizomastix whose phylogenetic position has been unclear. Phylogenetic analyses of SSU rDNA surprisingly showed that the genus Rhizomastix is a member of Archamoebae and is a close relative of the parasitic genus Entamoeba. Moreover, our TEM study of a Rhizomastix strain showed it possesses a peculiar cell structure. The genus Rhizomastix comprises both free-living and parasitic members. Therefore it is an excellent model organism for studying the evolution of parasitism within Archamoebae. In addition, we have successfully established several strains of genera Mastigamoeba and Mastigella, and, for the first time, four strains of Pelomyxa. We determined actin gene sequences of most strains. The analyses showed that most of our Mastigella strains are closely related to Pelomyxa. Overall, it seems that Rhizomastix, Entamoeba, Pelomyxa, and Mastigella form a major clade of Archamoebae the second one comprising genera Mastigamoeba, Endolimax and Iodamoeba. Our results suggest that the parasitism has arisen at least three times independently within the Archamoebae.

Phylogeny of the peniculistomatid genera Mytilophilus and Peniculistoma and relationships among pleuronematid scuticociliates (19A)

M. C. Strüder-Kypke, D. H. Lynn, G. A. Antipa and L. Obolkina,

The Family Peniculistomatidae Fenchel, 1965 was established for the distinctive *Peniculistoma mytili* (Morgan, 1925) Jankowski, 1964, which is commensal in the mantle cavity of the Atlantic blue mussel *Mytilus edulis*. Antipa and Dolan (1985) described *Mytilophilus pacificae* as a commensal of the mantle cavity of the Pacific mussel *Mytilus californianus*. We collected samples of these ciliates from along the west coast of the United States and from the coast of Helsingor, Denmark. To examine more broadly relationships among pleuronematid scuticociliates, we have collected *Sulcigera comosa* from Lake Baikal. We have used standard techniques to sequence their nuclear small subunit rRNA (SSUrRNA) genes and their mitochondrial cytochrome c oxidase subunit 1 (cox-1) genes and to examine phylogenetic relationships of these pleuronematid scuticociliates. Our preliminary analyses suggest that: 1) Mytilophilus and Peniculistoma are closely related based on both genes and clearly form a separate clade, supporting the Family Peniculistomatidae; 2) the Family Histiobalantidiidae, including the genera Histiobalantium and Sulcigera, is a separate clade; and 3) the Family Pleuronematidae may not be monophyletic. Using paleontological estimates of the maximum age of *M. californianus*, we calibrate the SSUrRNA and cox-1 molecular clocks and compare these estimates to those of other pairs of ciliate species.

Pinpointing the Root of Extant Eukaryotic Diversity (21G)

Laura Eme and Andrew J. Roger

Determining the root of the eukaryotic tree is of crucial importance to determine the sequence of events at the earliest stages of eukaryotic evolution. Recent studies of mitochondrion-derived genes have suggested that the root may fall between unikonts and bikonts, whereas a gene family evolutionary analysis indicated that the root may fall between opisthokonts and other super-groups. However, deep level analyses like these are plagued with artefacts and the historical signal versus systematic error in rooted phylogenomic analyses of eukaryotes have not been comprehensively examined. We have addressed this question by carefully selecting and analyzing three classes of eukaryotic genes that can be outgroup rooted: (1) genes whose closest orthologs are from the Archaea (~200 genes) ; (2) genes of potential mitochondrial origin, where alpha-proteobacterial homologues can be used as the outgroup (~100 genes) ; (3) genes that duplicated before the last eukaryotic common ancestor (~400 genes), as phylogenetic reconstruction from paralogous gene families can be used to generate reciprocally rooted trees. Orthologs from a large sample (~80) of eukaryotes from all supergroups were assembled for each gene, and sophisticated phylogenetic methods, exploratory data analysis and 'robustness' procedures were used to determine support for alternative eukaryotic root positions.

Polyphyly of the genus Pelomyxa and general issues in the systematics of Archamoebae (8G)

Alexei Y. Kostygov, Lyudmila V. Chistyakova and Alexander O. Frolov

Biodiversity, taxonomy and phylogeny of genus Pelomyxa Greeff, 1874 became a matter of controversy soon after taxon description. Recently we rejected a hypothesis of genus monotypy by Whatley & Chapman-Andresen having reisolated and described de novo nine species of these archamoebas. Formerly multinuclearity and multiple flagella were distinguishing features of the genus Pelomyxa. However phylogenetic analysis of pelobiontid morphological characters led us to

conclude that it is polyphyletic. Here we provide a molecular phylogenetic proof of our hypothesis. On 18S rRNA phylogenetic trees the genus Pelomyxa breaks up into two groups. The first one comprises *P. stagnalis*, P. *belevski* and the type species *P. palustris*. It obviously represents the proper Pelomyxa genus. The following morphological traits support this clade: lack of lateral and basal kinetosomal rootlets, reduction of radial microtubules cone, multiple nucleoli and compound nuclear envelope. The genus Entamoeba appears to be its sister clade. *P. gruberi* and *P. prima* form the second pelomyxoid group joining uniflagellate archamoebas cluster. This group is characterized by presence of both lateral and basal kinetosomal rootlets, well-developed radial microtubules cone, simple nuclear envelope and single non-fragmented nucleolus. The described situation isn't unique within Archamoebae. Other genera are also morphologically heterogeneous and may represent artificial taxa.

Responses of an Intertidal Microbial Community to Petroleum Hydrocarbons at a Bioremediation Site on Prudence Island in Narragansett Bay (15G)

Gaytha A. Langlois

The release of petroleum byproducts into shallow coastal bays and estuaries can lead to significant changes in ecosystem dynamics, including reduced biodiversity and selective damage to some species of organisms, thus leading to alterations in food chains in the biotic community. The damaging effects of these organic compounds may be especially evident in soft mud sediments. This study of an intertidal microbial community at a contamination site in Narragansett Bay, located at the south end of Prudence Island, characterizes some of the ecosystem changes resulting from chronic release of gasoline and diesel fuel residues linked to underground fuel storage tanks placed there by the military and then later removed as part of cleanup and mitigation efforts. Changes in microbial population dynamics, trophic relationships, species composition, and predation patterns were observed in field samples collected over a 5-year period, and results were compared to a control site, other intertidal locations, and to microcosm studies at the Marine Ecosystems Laboratory (MERL), Graduate School of Oceanography at the University of Rhode Island. These comparisons confirm that the response of a marine microbial community to low-level exposure to petroleum hydrocarbons represents a shift to an altered, stable ecological community during low-level, chronic oil exposure.

Revisions to the classification of protists (21A)

Sina Adl and many others

The new revisions to the classification of protists update the previous revision dated 2005. Several nodes at the base of the eukaryotic phylogeny remain unresolved and problematic. Several nodes further in the tree have been ameliorated by improved sequence diversity and analysis. New issues have come to fore regarding the naming of several clusters which are discussed in the paper. Several issues noted in the previous revision remain to be addressed. Advances in our treatment of environmental samples, from a technical and analytical perspective, are promising and begin to address previously unknown diversity from several environments. These will impact our perception of protist diversity and its role in the environment.

RY-coding and non-homogeneous models can ameliorate the maximum-likelihood inferences from nucleotide sequence data with parallel compositional heterogeneity (20E)

Sohta Ishikawa, Yuji Inagaki and Tetsuo Hashimoto

In phylogenetic analyses of nucleotide sequences, 'homogeneous' substitution models, which assume the stationarity of base composition across a tree, are widely used. However, a homogeneous model-based analysis can yield an artifactual tree when two distantly related sequences achieved similar base frequencies in parallel. Such potential difficulty can be countered by two approaches, 'RY-coding' and 'non-homogeneous' models. The former approach converts four bases into purine and pyrimidine to normalize base frequencies across a tree, while compositional heterogeneity is explicitly incorporated in the latter approach. Although these approaches have been applied to real-world sequence data, their basic properties have not been fully examined by pioneering simulation studies. Here, we assessed the performances of the maximum-likelihood analyses incorporating RY-coding and a non-homogeneous model (RY-coding and non-homogeneous analyses) on simulated data with parallel convergence to similar base composition. Both RY-coding and non-homogeneous analyses showed superior performances compared with homogeneous model-based analyses. Curiously, the performance of RY-coding analysis appeared to be significantly affected by a setting of the substitution process for sequence simulation relative to that of non-homogeneous analysis. The performance of a non-homogeneous analysis was also validated by analyzing a real-world sequence data set with significant heterogeneity in AT content.

SEM, TEM, and nanosims imaging of microbes from the hindgut of a lower termite: Evidence for carbon transfer between protists and their bacterial symbionts (15B)

Kevin J. Carpenter, Peter K. Weber, M. Lee Davisson, Jennifer Pett-Ridge, Michael I. Haverty and Patrick J. Keeling

The hindguts of lower termites harbor highly diverse and endemic microbial biotas that are essential to the termite's ability to digest wood. However, the ecological roles of many of these microbes are unknown, partly because almost none of the protists can be cultivated. Many of the protists associate with bacterial symbionts, but hypotheses for their respective roles in nutrient exchange are based on genomes of only two such bacteria. To elucidate ecological roles of protists and nutrient transfer with symbiotic bacteria, we took an in-situ, culture-independent approach that combines stable isotope

(13C-cellulose) labeling of the lower termite Paraneotermes simplicicornis with analysis of fixed hindgut microbes using SEM, TEM, and high resolution imaging mass spectrometry (NanoSIMS). Results suggest that the oxymonad protist Oxymonas dimorpha plays a role in phagocytosis and enzymatic degradation of wood fragments and transfers carbon from this to its bacterial surface symbionts. Such a role is consistent with certain large parabasalids, as inferred from other studies. In contrast, results suggest the parabasalid protist *Hoplonympha natator* receives carbon from its bacterial surface symbionts, and is not a cellulose degrader itself. Our approach provides evidence independent of molecular data for use in testing and forming ecological hypotheses of these organisms.

Sex or no sex: The probe for sexual reproduction in fish parasite *Ichthyophthirius multifiliis* (5F)

Wei-Jen Chang, Ke Xu, Yonghyun Song, Matthew Therkelsen, Donna Cassidy-Hanley and Theodore Clark

The parasitic ciliate *Ichthyophthirius multifiliis* (Ich) is responsible for “white spot” disease in freshwater fish, a major problem in commercial aquaculture worldwide. While it is generally assumed that Ich reproduces sexually to produce genetic variants that are crucial in environmental and/or host adaptation, there is no conclusive data to support this one way or the other. To test whether Ich undergoes sexual reproduction, we examined the expression profiles of a number of meiosis and conjugation specific genes in different stages of the life cycle by real-time PCR. Consistent with the idea of sexual reproduction, these genes showed stage-specific differences in mRNA expression with highest levels being seen at the beginning of the tomont stage when parasites are just coming off the fish. Despite this, microscopic observations showed no evidence of micronuclear meiosis, macronuclear degradation, or anlagen formation at this or later time points (up to the theront stage) in any cells examined. We also examined the genome of the G5 strain of Ich, and found that ~ 30% of genes in Ich were homozygous, but substantial amount of heterozygosity was also detected, thus ruling out continuous selfing. We will discuss potential models of reproduction in Ich based on our findings.

Soil C & N nutrient transfers through food web functional groups using stable isotope methods (15C)

Sina Adl and Felicity Crotty

Our stable isotope tracer studies improved on the resolution of results from radioactive tracer studies, and improved on older data. Our approach combined natural abundances of the 13C and 15N isotopes in soil organisms, with enriched intact soil columns, and laboratory microcosm experiments. We identified previously undescribed interactions in the food web. In particular we were able to identify the consumers of protozoa in the soil, which include many micro-invertebrates besides nematodes. The patterns in soil N and C utilization were quantified separately for labelled bacteria added to soil columns, followed by labelled protozoa cultures to distinguish parts of the pathways; then the fungivory pathway using labelled fungi in microcosm food webs. This was followed by microcosm studies of protozoa nutrient uptake and respiration rates. The results provide a more complete description of soil food web pathways; we have improved the resolution of quantifiable nutrient transfer rates and respiration between soil compartments; we have identified new interaction between soil functional groups. These results also indicated errors in the older data since the 1970’s used for modeling soil food webs which can be corrected. These improvements impact estimates of soil nutrient utilization and budgeting gas emissions from soil.

Species discovery and evolutionary history of marine gregarine apicomplexans (4E)

Kevin C Wakeman and Brian S Leander

Marine gregarines are poorly understood endoparasites of marine invertebrates that are critical for understanding the earliest stages in the evolution of the Apicomplexa. Both morphological and molecular data suggests that some lineages of marine gregarines (e.g., so-called “archigregarines”) form a stem group from which all other gregarines, and possibly apicomplexans as a whole, evolved. Other lineages of gregarines (e.g., so-called “eugregarines”) represent a vast collection of parasites that have diversified into many lineages of invertebrate hosts, especially species living in marine environments. I have explored the west coast of British Columbia, Canada in search of marine gregarines in a variety of invertebrate hosts. High-resolution light and scanning electron microscopy, as well as DNA extracted from single-cell isolations has been vital for elucidating the sometimes-cryptic diversity and evolutionary history of these marine parasites. These methods have also led to the identities of ambiguous environmental sequences belonging to novel clades of marine eugregarines. Moreover, I will present comparative data using fluorescence confocal microscopy of immuno-labeled tubulin to fully characterize the cytoskeletal diversity of marine gregarines within a molecular phylogenetic context.

Taxonomical enigma - genus Kelleromyxa (Myxogastria): evidence from the SSU rDNA gene (8D)

Daria A. Erastova, Mikchail V. Okun, Anna Maria Fiore-Donno, Yuri K. Novozhilov and Martin Schnittler

Kelleromyxa fimicola (Dearn. & Bisby) Eliasson is a coprophilous myxomycete that was first described in 1929 as *Licea fimicola* because of its superficial resemblance to *Licea biforis*. However the detailed studies on morphology of sporocarps and life cycle revealed some characters of order Physariida, not Liceida, therefore a new genus name for this species was given. Recent advances in phylogeny of Myxogastria have revealed the existence of two major clades: the dark-spored (Stemonitida, Physariida) and the bright-spored clades (Liceida, Trichiida). To shed light on the systematic affiliation of *K. fimicola* 4 samples from herbarium of Komarov Botanical Institute (LE) were taken for molecular study and 3 partial and 1 whole sequence of SSU

rDNA gene were obtained. The dendrogram including representatives of Myxogastria clearly shows the relationship of *K. fimicola* to the dark-spored clade and the position of it between two families - Didymiidae and Physariidae (Physariida) - with a high bootstrap. Thereby on the basis of molecular and morphological analysis *K. fimicola* may be placed among dark-spored myxomycetes of Physariida.

Temperature-driven changes in growth and bacterivory in a common mixotrophic chrysophyte (15F)

Robert W. Sanders, Sarah B. DeVaul and Adam W. Heinze

Mixotrophic phytoplankton use both photosynthesis and ingestion of other organisms to obtain energy and organic matter. Dinobryon, a common mixotrophic genus that can seasonally dominate the planktonic assemblages of lakes, is reported in the literature at temperatures ranging from 3 to 23∞C, but maximum population sizes were found at intermediate temperatures. In our laboratory experiments, physiological processes showed differential responses to increasing temperature with growth and feeding rates increasing with rising temperature up to 8 and 12˚C, respectively. Growth and feeding declined at higher temperatures, with gradual acclimation, and encystment increased. Climate change is predicted to result in increased temperature fluctuations and higher absolute temperatures in many temperate zone lakes, suggesting that cold-water adapted species like Dinobryon will be further restricted spatially and seasonally.

Tetrapyrrole pathway reflects an evolutionary history of algae with complex plastid (17E)

Miroslav Oborník, Luděk Kořený and Jaromír Cihlář

Tetrapyrroles are components essential for life. Phototrophic and primarily heterotrophic eukaryotes differ in the synthesis of the first precursor δ - aminolevulinic acid (ALA). In primary heterotrophs, ALA is formed by C4 pathway in mitochondrion and cytosol respectively. In contrast, eukaryotic phototrophs synthesize ALA from glutamate in C5 pathway in the plastid. However, several exceptions to this arrangement exist. Although apicomplexan parasites and chromerids synthesize ALA in the mitochondrion using C4 pathway, the rest of the route is localized to the plastid (chromerids) or the last two steps are relocated to the mitochondrion (apicomplexans). Thus, chromerids are unique phototrophs synthesizing chlorophyll from glycine. In *Euglena gracilis*, two tetrapyrrole pathways are functioning in the cell; one heterotrophic-like in the cytosol and mitochondrion, while the second one is plastid localized. Similar arrangement was found in chlorarachniophyte Bigelowiella natans. Contrary, cryptophyte *Guillardia theta* synthesizes tetrapyrroles in the plastid, with the last step (ferrochelatase) localized in both, plastid and mitochondrion. We propose that presence of two tetrapyrrole pathways in one cell indicates recent acquisition of the plastid. Since cryptophytes are the only algae with red complex plastid having remnant of the mitochondrial pathway, we propose later acquisition of its plastid when compared to stramenopiles and haptophytes.

The anaerobic adaptations of Blastocystis mitochondrion-related organelles and the evolution of mitochondria (18F)

Anastasios D. Tsaousis, Eleni Gentekaki, Eva Nývltová, Grant C. Stevens, Ivan Hrdy, Andrew J. Roger and Jan Tachezy

In contrast to anaerobic organisms that possess canonical mitochondria, microbial eukaryotes from strictly anoxic and low oxygen environments harbor mitochondrion-related organelles (MROs). Some of these organelles, known as hydrogenosomes, generate ATP anaerobically, producing molecular hydrogen in the process. Blastocystis, a unicellular (protistan) anaerobic intestinal human parasite, is a particularly interesting organism in which to study the function and evolutionary origin of MROs. The nature of Blastocystis' MROs is puzzling, as they contain cristae and DNA, but appear to lack classical aerobic mitochondrial pathways and function in the complete absence of oxygen. To clarify their functions, we performed a new broad genome-level transcriptomic study of Blastocystis and more than 200 clusters were identified encoding putative mitochondrial and hydrogenosomal proteins. By immunofluorescence microscopy, proteomics and in vivo assays, we show that the hydrogenosome-specific proteins including pyruvate:ferredoxin oxidoreductase (PFO) pyruvate:NADP oxidoreductase (PNO) and [FeFe]-hydrogenase function within Blastocystis MROs, indicating that these organelle may have hydrogenosome-like functions. In addition, bioinformatics, cell biological and biochemical analyses demonstrate that the MROs and the parasite itself have acquired several other proteins as adaptations of parasitism in anaerobic lifestyle. Results of these investigations shed light on the unknown function of the mitochondrion in this anaerobic organism, thereby elucidating the evolutionary history of both anaerobic metabolism and mitochondria or related organelles within eukaryotes.

The coupling and succession of protists in the seaice and water column in an Arctic fjord (3D)

A. R. Meshram, L. Kuckero, A. Vader, Kjetill S. Jacobsen and T. M. Gabrielsen

Temporal variation in the community composition of sea ice and pelagic eukaryotes of size fraction 0.22- - 38 μm was studied during the winter to spring transition in the arctic Billefjorden (Svalbard). Eight 18S rDNA clone libraries were prepared from 4 water samples (5m, 15m, 35m) and 4 icecore samples obtained at different time points (February, April, May and June). A total of 881 sequences were obtained, forming 191 clusters at 98% sequence identity. Several interesting results were obtained. Firstly, the taxonomic affiliation of the sequences differed markedly between sea ice and water samples. While cercozoans were the most abundant groups in the sea ice libraries (April--45%, May - 88%, June- - 69% of total clones), Dinophyceae dominated the water samples (February--49%, April--60%, June- - 53%). Secondly, cercozoans were also

abundant in the June water sample (38%), and 2 OTUs (11 clones) were shared between the May sea ice and June water samples, indicating coupling between the sea ice and pelagos. Finally, although the Dinophyceae and Cercozoa were abundant in water and sea ice respectively, very few clusters overlapped between the different samples. Only 31 clusters were represented in more than one library. Taken together, our results suggest arctic marine protist communities to be highly diverse and to display a large degree of seasonality.

The cytoskeleton of Breviata, and the nature of the ancestral eukaryote flagellar apparatus (21B)

Aaron A. Heiss, Giselle Walker and Alastair G. B. Simpson

Breviata is an amoeboid flagellate that does not branch within any established "supergroup" of eukaryotes. Recent molecular phylogenies suggest affinities to Amoebozoa, Opisthokonta, or apusomonads. Breviata has two basal bodies. The flagellated anterior basal body associates with a fan of ~18 microtubules and a short singlet microtubular root. Three microtubular roots associate with the posterior basal body. One, the right root, is initially a triplet that splits into two parts. The other two are singlets: the left root, and the middle root (the latter arises on the posterior side of the basal body). The middle root, left root and the smaller part of the right root support the left ventral side of the cell, while the larger part of the right root runs down the right ventral. The posterior flagellar apparatus resembles several other groups, particularly ancyromonads and thecamonas (our previous work), and apusomonas, as well as typical excavates, and even myxogastrid amoebozoans. This comparison suggests that the complex flagellar apparatus of myxogastrids is actually plesiomorphic within Amoebozoa. In fact, it is increasingly plausible that the very widely distributed splitting right root and posterior singlet (middle root in Breviata) could be features of the last common ancestor of eukaryotes.

The genetic structure of amoeba morphospecies (6B)

A. Smirnov, E. Nassonova, A. Glotova, A. Kudryavtsev, V. Zlatogurski and J. Pawlowski

The "species problem" in lobose amoebae remains the basic and yet unresolved question due to luck of data on genetic structure of amoebae populations. To approach this problem, we have sequenced Cox 1 and SSU genes from multiple independent strains of lobose amoebae - Vannella simplex and V. miroides (Amoebozoa, Lobosa, Discosea), representing five local populations split with the distance from 200 to 6000 km. Results show that an "amoeba species" consists of a limited number of genotypes best differentiated by Cox I gene barcode. The same genotype may occur in distant locations, while the single location usually contains co-existing population of several genetic lineages. SSU gene distinguishes lower number of genotypes than the Cox 1 gene, but Cox 1 and SSU data are congruent. Both studied genes contain molecular signatures identifying studied morphospecies, groups of genotypes and individual genotypes. The observed pattern show that despite presumably asexual life cycle, amoebae species do not represent discontinuous genetic diversity and are able to maintain their genetic individuality. Occurrence of identical genotypes at distant locations supports the idea of ubiquitous dispersal of microbial species, while presence of locally distributed ones – the model of moderate endemicity in the biogeographic distribution of protists. Supported with IZLR Z3_1 28338 and RBRF 12-04-01825 grants.

The molecular machinery behind Trichomonas morphogenesis during host cell infection (9E)

Gary Kusdian, William F. Martin and Sven B. Gould

The core components of eukaryotic locomotion are the tubulin machinery for flagella-based swimming and the actin machinery for adherent-amoeboid gliding. Both are hardwired into eukaryotic genomes, but the ability to shift between the two types of locomotion appears to be used by only a few protozoa such as *Trichomonas vaginalis*. The phenotypic plasticity of the human pathogen is an essential mechanism for the parasite to successfully infect human tissue of the urogenital tract within only a few minutes. To identify potential key players we used comparative transcriptomics on a mixture of parasite and host mRNA and downstream bioinformatic screening. Among the candidates we identified a protein of the ancient eukaryotic fimbrin family, which is characterized by a tandem of two actin-binding calponin homology domains. TvFIM1 increases the speed of actin filament assembly and bundles F-actin in a parallel and anti-parallel manner. During infection the protein experiences a dramatic re-localization and associates with structures resembling actin cables and at protruding sites of lamellipodia, suggesting the parasite to not only adhere, but actively migrate across host tissue. Our transcriptomic data furthermore provides insight into the action-response pattern of parasite and host during the infection process.
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The phylogenetic position and mitochondrial genome sequence of the enigmatic discobid *Tsukubamonas globosa* (20D)

Ryoma Kamikawa, Yuki Nishimura, Akinori Yabuki, Martin Kolisko, Alastair G. B. Simpson, Andrew J. Roger, Ken-ichiro Ishida and Tetsuo Hashimoto, Yuji Inagaki

Discoba (Excavata) is a unicellular eukaryotic assemblage that comprises four subgroups: Heterolobosea, Euglenozoa, Jakobida and Tsukubamonadida. This clade is especially important for understanding the early evolution of mitochondrial (mt) genomes, as the jakobid *Reclinomonas americana* retains the most ancestral (i.e. gene-rich) genome of any mt genomes determined to date. Amongst discobids, the Tsukubamonadida is the most recently established group and contains a single representative species (*Tsukubamonas globosa*) whose precise phylogenetic position within the Discoba has not yet been resolved. We conducted a pyrosequencing-based transcriptomic survey of *T. globosa* and, based on these data, we assembled a phylogenomic alignment of 157 proteins. Our phylogenomic

analyses show that *T. globosa* branches at the base of the union of Euglenozoa and Heterolobosea. In addition, we completely sequenced the mt genome of *T. globosa* (48,463 bp in length and A+T content of 66.2%). The mt genome of *T. globosa* can be mapped as circular, and encodes 56 putative protein-coding and 27 RNA genes, indicating that *T. globosa* is second only to R. americana in terms of its coding capacity. In light of the relationships amongst the major members of the Discoba clade and their mt genome data, we discuss the evolution of mt gene repertoires in this protist clade.

The *Spironucleus salmonicida* genome (11A)

Feifei Xu, Jon Jerlström-Hultqvist, Elin Einarsson, Staffan G. Svärd and Jan O. Andersson

The diplomonad *Spironucleus salmonicida* is a parasite of Atlantic Salmon. We have sequenced the genome at a high coverage with next-generation sequencing technologies and assembled it. In addition, optical mapping was used to achieve information of genome architecture independent of the sequence data. There was a good correlation of estimated haploid genome sizes around 12.9 Mb between the optical maps, previous flow cytometry studies and the assembly. After manual revision of various sources of evidence 8,346 protein-coding genes were identified. The putative products of 4,104 of these show significant sequence similarity to previously known proteins or domains. We have recently identified an organelle in *S. salmonicida* with a proteome suggesting capability of hydrogen production. This suggests that hydrogen-producing organelles are ancient within Metamonada, and that the mitosome of the diplomonad *Giardia intestinalis* lost the hydrogen-production capacity relatively recently. Here we compare the coding capacity of *S. salmonicida* to *G. intestinalis*. Overall, *S. salmonicida* seems to have a less reduced genome with almost twice as many genes. For example, *S. salmonicida* has a more elaborate system for oxidative stress response than the other diplomonad, which likely is a reflection of the differences of the energy metabolism.

Three-dimensional ultra-structural study of encystment and astaxanthin accumulation in the green alga, *Haematococcus pluvialis* (5D)

Shuhei Ota, Marina Wayama, Nobuhito Nango, Aiko Hirata and Shigeyuki Kawano

Transmission electron microscopy is to provide a two-dimensional representation of high resolution information. More recently, electron tomography (ET) has emerged as a novel technique to provide ultra-structural three-dimensional (3D) reconstruction of cells. However, the application of ET is limited to sections of thickness less than about 400 nm due to image blurring of multiply scattered electrons. In contrast, 3D reconstruction based on serial sections allows entirety visualization of a whole cell. This method was widely used for not only 3D construction of the flagellar apparatus of protists but also the dynamic analysis of mitochondria and other organelles. *Haematococcus pluvialis* is a freshwater species of Chlorophyta and well known for its high content of the strong antioxidant astaxanthin, which is important in aquaculture, various pharmaceuticals, and cosmetics. The high amount of astaxanthin is present in the cysts, which are produced and rapidly accumulated when the environmental conditions become unfavorable for normal cell growth. It is not understood, however, how such high amount of astaxanthin which is soluble in oil becomes possible during the encystment. Here we apply ultra-structural 3D reconstruction based on approximately 350 serial sections to analyze the dynamics of astaxanthin-accumulation and chloroplasts-degradation during the encystment of *H. Pluvialis*.

Trans-splicing and expression of nucleus-encoded genes for chloroplast proteins in *Euglena gracilis* (14B)

Juraj Krajčovič, Bianka Mateášiková-Kováčová, Kristína Záhonová and Matej Vesteg

Euglena gracilis is a flagellate possessing secondary chloroplasts of green algal origin. In contrast to Archaeplastida, the mRNA levels of nuclear genes for chloroplast proteins in *E. gracilis* are not influenced by light and plastid function. However, it was unknown which portion of these mRNAs is trans-spliced and possesses spliced leader (SL) at the 5`-end and if their trans-splicing is plastid- or light-dependent. mRNAs were isolated from *E.* gracilis strains Z and bacillaris, and white mutants WgmZOflL, W3BUL and W10BSmL grown in the light and dark, and cDNAs were synthesized. The PCRs were performed using these cDNAs as templates and SL-leader sequences as forward primer, and forward and reverse primers derived from internal sequences of mRNAs encoding six chloroplast proteins. The PCR products were obtained in all reactions. The levels of trans-spliced and non-trans-spliced mRNAs encoding chloroplast proteins glyceraldehyde-3-phosphate dehydrogenase, cytochrome f, and subunit O of photosystem II were compared more precisely via real-time PCR. These experiments revealed that all mRNAs encoding these proteins contain SL-leaders in white mutants and in green strains whether grown in the light or dark. This contribution is the result of the project implementation (IMTS 26240120027) supported by the OPRaD funded by the ERDF.

Ultrastructural and phylogenetic evidence for the polyphyly of retortamonads (11F)

P. Smejkalová, J. Kulda and I. Čepička

Retortamonads (genera *Retortamonas* and *Chilomastix*) are a small group of heterotrophic flagellates belonging to the Fornicata. The retortamonads were proposed to be monophyletic on the basis of cell structure. Surprisingly, the recent multigene phylogenetic analysis showed that retortamonads are polyphyletic – the genus *Retortamonas* appeared as the close relative of diplomonads, whereas the genus *Chilomastix* branched more basally within free-living "*Carpediemonas*-like organisms". The discrepancy between results of morphological and molecular analyses could be explained by the fact that the previous TEM studies were performed on *Retortamonas* spp. from insects while molecular phylogenetic studies were based on *Retortamonas*

spp. from vertebrates. Here we present new ultrastructural and molecular data confirming the polyphyly of retortamonads. The first TEM study of *Retortamonas* strains from vertebrates showed that their ultrastructure considerably differ from those of other retortamonads. Additionally, we extended the sampling of retortamonads in phylogenetic analyses. We sequenced and analysed SSU rDNA of ten *Retortamonas* strains from vertebrates, five *Retortamonas* strains from insect and nine *Chilomastix* spp. strains. *Retortamonas* isolates formed an internal branch of *Chilomastix* whereas isolates from vertebrates remained closely related to diplomonads. In addition, the genus *Chilomastix* displayed an enormous SSU rDNA sequence length variability.

Unexpected diversity of free-living trichomonads (11E)

Ivan Čepička, Vít Céza, Jeffrey D. Silberman and František Šťáhlavský

Vast majority of ca. 450 known species of Parabasalia are intestinal symbionts of termites and other animals. Although only 6 species of free-living trichomonads have been described so far, they form four independent lineages. Moreover, all free-living trichomonads are considered secondarily free-living. We have isolates 30 strains of trichomonads from freshwater, brackish and marine anoxic sediments worldwide as well as from wastewater treatment plants in Czech Republic. Phylogenetic analysis of SSU rDNA and light-microscopic morphology showed that our strains represent at least 7 undescribed species of Trichomonadidae, Honigbergiellidae and a novel deep parabasalid lineage. Members of the new lineage display unusual morphological features, e.g. motile neck or flagellar vanes. Interestingly, strains from Czech Republic, USA and South-East Asia belonged to different species suggesting that either the real diversity of free-living trichomonads is even much higher or that at least some species are geographically isolated. *Honigbergiella ruminantium* and *Tetratrichomonas prowazeki* contain both endobiotic and free-living strains.

Unicellular opisthokonts diversity and distribution in the European coast (12A)

Javier del Campo, Ramon Massana, Colomban de Vargas and Iñaki Ruiz-Trillo

Unicellular opisthokonts include relevant eukaryotic lineages both from an evolutionary and an ecological point of view. They include interesting organisms such as the bacterivorous choanoflagellates, the poorly known filastereans, the nucleariids or the parasites ichthyosporeans. We analyzed all the published environmental clone libraries in order to have an approximation to the relative abundance of unicellular opisthokonts (except fungi) in the environment. We focus on filastereans, ichthyosporeans and nucleariids, and we update the previous analysis done with choanoflagellates. Based on the information retrieved from these 18S rDNA clone libraries we constructed an opisthokont phylogenetic tree. It has been used as guide-tree to analyze the taxonomy of the 18SrDNA and rRNA data obtained by high-throughput methods from the European coast in the context of the BioMarks project. Using this data we have described the abundance and the distributions of the studied groups in different sampling points and size fractions. Furthermore, the comparison between 18SrDNA and rRNA helped us to determine the most abundant and active groups. The analysis of environmental clone libraries and the use of high-throughput techniques are useful approaches to increase the knowledge on the diversity of unicellular opisthokonts and to have a better sketch of its putative ecological role.

Unravelling the evolutionary forces that shape the B12 requirements of algae (14A)

K.E. Helliwell, G.L. Wheeler, K.C. Leptos, R.E. Goldstein and A.G. Smith

The distribution of vitamin B12 dependence in algae is sporadic and there are several examples of closely-related species which differ in their requirements for this vitamin. Such variable cofactor dependence raises interesting evolutionary questions. Vitamin B12 is an essential cofactor for the vitamin B12 dependent isoform (METH). However, the second isoform (METE) functions independently of vitamin B12. With the completion of several algal genome projects, a wealth of genomic information is now available for representatives of key phylogenetic groups. Using this resource, we have accumulated evidence which points to multiple losses METE as being a key factor in the evolution of B12 dependence in algae (Helliwell et al. (2011) Molecular Biology and Evolution 28: 2921-2933). Moreover, the identification of a METE unitary pseudogene in the B12-dependent green alga *Volvox carteri* suggests B12 auxotrophy evolved relatively recently in this group. Given that eukaryotes must obtain vitamin B12 from prokaryotes, the selective loss of METE in many different algae may have had important physiological and ecological consequences for these lineages. The role of vitamin B12 as a driver of algal-bacterial symbiosis will therefore be discussed.

Using behavioral and chemical cues to resolve crypticity in free-living, commensal, and parasitic Entamoeba lineages (8H)

Avelina Espinosa and Guillermo Paz-y-Miño-C

We demonstrate that by color tagging and pair-mix-culturing six Entamoeba varieties, the difficulty of discerning among apparently cryptic taxa can be resolved. If grown together with different amoeba strains, free-living/opportunistic (*E. moshkovskii* Laredo), commensal (*E. moshkovskii* snake) or parasitic (*E. invadens* IP-1, *E. invadens* VK-1:NS, *E. terrapinae*, *E. histolytica*) trophozoites aggregate only with members of their own lineage. Clusters of trophozoites from each amoeba show distinctive rate of aggregation, density of cells per cluster, and distance between clusters. By using these behavioral cues, and identifying possible cell-signaling for cluster formation, distinctive amoeba taxa can be characterized quantitatively; we postulate that not only Entamoeba varieties, but apparent taxa crypticity in other protists, can be resolved by examining the natural ability of unicellular eukaryotes to discriminate between members and non-members

of a lineage. Thus, phylogenetic relations among protists, which are usually determined by morphology and molecular techniques (the latter often confounded by horizontal gene transfer), could be further understood by incorporating behavior into the evolutionary analysis of this complex group of organisms.

Utility of small and large subunit rRNA genes for morphospecies discrimination and phylogenetic inference in the order Tintinnida (Ciliophora, Spirotrichea) (19E)

Luciana F. Santoferrara, George B. Mcmanus and Viviana A. Alder

Knowing the degree of correlation between molecular and morphological characters in protists is crucial to understand their diversity, biogeography, and ecological roles. We evaluated the small and large subunit rDNA (SSU and LSU, respectively) for their ability to discriminate morphospecies of tintinnid ciliates. Multiple individuals from 29 morphospecies were classified by lorica morphology and sequenced (21 new species for SSU and all of them new for LSU). Two hypervariable SSU regions (V4 and V9) were examined separately. LSU showed a gap in distances within and between species, and discriminated the maximum number of phylotypes (86% at 1% cut-off), thus being a promising barcoding tool. V9 provided the poorer morphospecies delimitation, and even SSU and V4 lumped together several distinctive morphospecies. Although we also found similar morphospecies with divergent SSU and LSU (pseudocryptic species), a general concordance between morphology and sequences was observed at the species level. The agreement between both criteria was lower above the species level, and some phylogenetic relationships remained poorly resolved according to inferences based on either SSU or SSU+LSU. Both analyses indicated the monophyly of the genera Tintinnidium and Eutintinnus, and the clustering of Helicostomella, Stenosemella, the paraphyletic Tintinnopsis, and some species of the polyphyletic Favella.

Will the real picobiliphyte please stand up? (20C)

Linda K. Medlin, Ramkumar Seenivasan and Michael Melkonian

Using vital mitochondrial staining and cell sorting by flow cytometry, a culture of a “picobiliphyte” has finally been established. This clonal culture, which has a “picobiliphyte” 18S rDNA signature, is neither pico or pigmented. Its morphological and ultra-structural characters clearly show that it is slightly elongate and 2-5 um in length with two unequal flagella not covered by hairs or scales. It exhibits unique cell movements (jump, drag, and skedaddle mode of locomotion) but often remains suspended in the water column for extended periods. Light and electron microscopic studies reveal that the cells are naked and that in this isolate, a plastid is lacking. A structure, presumably a feeding apparatus, suggests a rather unique mode of feeding on perhaps DOM is possible. The cells in this culture, thus, are heterotrophic, although their food source could not be determined, and food vacuoles containing bacteria were never observed. The cells harbor several other unique compartments that do not match those in any other known eukaryotes. This uniqueness is corroborated by phylogenetic analyses of the complete nuclear ribosomal operon, although the clade containing “picbiliphyte” sequences has some affinity with the Hacrobia. With their cell morphology now firmly established, we can now formally describe an important and abundant member of the eukaryotic pico/nanoplankton. This group/division is placed in a new phylum “Picozoa” with *Picomonas judraskeda* gen. et sp. nov. as its type species.

Abstracts – Posters (In alphabetical order by title)

A new freshwater peritrichous ciliate from West lake, Hangzhou, China, *Epistylis hangzhounensis* n. Sp. (Sessilida: Epistylididae)

Chuanqi Jiang, Xinlu Shi, Guijie Liu and Xiaozhong Hu

A freshwater peritrich ciliate, *Epistylis hangzhounensis* n. sp., was collected from West Lake, Hangzhou, Zhejiang province, China, and its morphology, infraciliature, silverline system and SSUrRNA gene sequence were investigated based on living or silver-impregnated specimens. The new species measured 162-259 × 52-93 μm in vivo and is characterized by the following features: cytoplasm containing conspicuous black granular substance and diatoms, result in black-colored, single ventrally located contractile vacuole, the C-shaped macronucleus is transversely located, longitudinal striation and hollow stalk. Number of silverlines between peristome and aboral trochal band, 127-176; between aboral trochal band and scopula, 49-84. The SSUrRNA places *Epistylis hangzhounensis* n. sp. as a distinct lineage near *Epistylis wenrichi* and *Epistylis urceola*. Combine the morphology and molecular data, E. *hangzhounensis* n. sp. could be a new species.

A new species candidate of marine ciliate, *Brooklynella* sp. (Phyllopharyngea: Dysteriida: Hartmannulidae) from Jeju in Korea

Ji Hye Kim and Mann Kyoon Shin

A new species candidate of genus Brooklynella Lom and Nigrelli, 1970 was collected from the coastal water off in Jeju, Korea. The morphology and infraciliature of a new Brooklynella species were investigated using live observation and protargol impregnation method. It was characterized by following characteristics: body size 40-60 × 15-20 ㎛ in vivo, elongated ellipsoidal, laterally highly flattened, 14-16 somatic kineties consisted of 5-6 left kineties, 6-7 right kineties posteriorly strong and 3 postoral kineties; 2 longitudinal rows and 1 irregularly fragmented like zigzag pattern, 2 contractile vacuole located diagonally, cytopharynx small and tube-like shape, inconspicuous podite. *Brooklynella* n. sp. is different from the most similar congener *B. sinensis* in body shape (slender ellipsoid vs. oval to reniform), posterior part of right kineties (densely vs. commonly) pattern of postoral kineties (one regularly scattered vs. all longitudinal arranged), cytopharynx (short vs. long), location of terminal fragment (subapical vs. apical)

A new strategy of organellar genome sequencing incorporating rolling circle amplification: protist mitochondria genomes.

Yuki Nishimura, Ryoma Kamikawa, Yuji Inagaki and Tetsuo Hashimoto

Mitochondrial (mt) genomes have enormous diversity with respect to gene content and genome structure. Comparisons of mt genomes, therefore, can be informative for elucidating mt genome evolution, as well as inferring the phylogenetic relationship amongst eukaryotes. In the 'traditional' strategy for mt genome sequencing, a large quantity of mitochondria or mt DNA is firstly purified from a large amount of cells. However, this strategy is not always suitable for all protists of our interests -- many of scientifically interesting species are slowly growing and are difficult to yield a sufficient amount of cells to initiate mt genome sequencing. Thus, we are developing a new strategy combining rolling circle amplification (RCA) using phi29 DNA polymerase and 454 pyrosequencing technique, which requires only several picograms of total DNA. With this strategy, we successfully completed the mt genome of *Tsukubamonas globosa*, a member of the Discoba clade. Now we are trying to determine the mt genomes of the katablepahrid *Leucocryptos marina*, and its prey organism, the haptophyte Chrysochromulina sp. In this presentation, we report the detailed conditions for RCA, and discuss the pros and cons of our new strategy.

A Novel Alveolate in Gill Epithelium Cells of Bivalves with Chemosynthetic Bacteria Inhabiting Deep-Sea Methane Seeps in Sagami Bay, Japan

Fumiya Noguchi, Kiyotaka Takishita, Masaru Kawato, Takao Yoshida, Yoshihiro Fujiwara and Katsunori Fujikura

It has recently been unveiled that a wide variety of microbial eukaryotes (protists) occur in chemosynthetic ecosystems, such as hydrothermal vents and seeps. However, there is little knowledge regarding protists associated with endemic animals inhabiting these environments. In the present study, utilizing PCR techniques, we detected fragments of the small subunit ribosomal RNA gene (SSU rDNA) from a particular protist from gill tissues of the vesicomyid clams *Calyptogena soyoae* and *C. okutanii* complex and of the mussel *Bathymodiolus platifrons* and *B. japonicus*, all of which harbor chemosynthetic endosymbiont bacteria and dominate methane seeps in Sagami Bay, Japan. Based on the phylogeny of SSU rDNA the organism in question was shown to belong to Alveolata. It is noteworthy that this protist did not affiliate with any known alveolate group, although being deeply branched within the lineage of Syndiniales, for which the monophyly was constantly recovered but not

robustly supported. In addition, the protist detected with PCR was localized within gill epithelium cells of *B. platifrons* with whole-mount fluorescent in situ hybridization. This protist may be an endoparasite or an endocommensal of *Calyptogena* spp. and *Bathymodiolus* spp., and possibly have physiological and ecological impacts on these bivalves.

A Rieske monooxygenase highly conserved in animals is the sterol-C7 desaturase of *Tetrahymena thermophila*.

Sebastián R. Najle, Alejandro D. Nusblat, Claudio H. Slamovits, Clara B. Nudel and Antonio D. Uttaro

Tetrahymena thermophila does not require sterols for living, but when sterols are present in the growth media they are incorporated by the cells, inhibiting the synthesis of the sterol surrogate tetrahymanol, and modified by desaturation at positions C5(6), C7(8) and C22(23). This work reports the identification of a Rieske-type monooxygenase, highly conserved in protostome animals, as the sterol C7-desaturase (Des7p). We used RNA interference and knock-out mutagenesis to demonstrate the activity of Des7p in vivo in *T. thermophila* cells. GC-MS analysis of lipid extracts from interfered or knocked-out cultures supplemented with different sterols exhibited, respectively, a significant decrease or the complete abolition of C7-desaturase activity, as compared with controls. Confocal microscopy analysis of a strain expressing a Des7p:GFP fusion shows that the protein is localized in microsomal and phagosomal compartments and its expression seems to be induced in the presence of sterols. A bioinformatic analysis revealed the existence of orthologous proteins in the species *T. malaccensis, T. elliotti* and *T. borealis*, as well as the presence of 6 paralogs in the genome of *Paramecium tetraurelia*. This data raise the hypothesis that the Rieske sterol C7-desaturase is an ancient character, present in ciliates before the divergence of the clade Oligohymenophorea.

Adaptations of the Cytosolic Iron/Sulfur cluster Assembly machinery in microbial eukaryotes

Anastasios D. Tsaousis, Eleni Gentekaki, Daniel Gaston and Andrew J. Roger

The Cytosolic Iron/Sulfur cluster Assembly (CIA) machinery is a fundamental system in all eukaryotic cells that is responsible for the assembly of cytosolic and nuclear iron/sulfur (Fe/S) clusters in Fe/S proteins. The CIA machinery is present only in eukaryotes and comprises at least seven candidate proteins whose origins and distributions across organismal diversity are still controversial. We performed a phylogenomic analysis to clarify the distribution of the components of the CIA machinery across eukaryotic diversity and determine their potential origin. Preliminarily results demonstrated that at least three components are present in all eukaryotes suggesting that these may be the core proteins required for the function of this machinery. In protists the distribution of these components is even more diverse. For example, several components of this machinery are missing from a number of parasitic lineages, suggestive of reductive evolution due to parasitism. We will demonstrate the localization of these components in the unicellular obligate anaerobic parasite Blastocystis, and discuss their role in the parasite´s lifestyle. Results from all of these investigations suggest that the CIA machinery is a vital biosynthetic pathway in eukaryotes, but its function and relation among other iron/sulfur cluster machineries remains unclear. Investigations of these Fe/S systems employing protists as model organisms will clarify the purpose and function of these biosynthetic pathways within the eukaryotic cell.

An international research coordination network for biodiversity of ciliates

John C. Clamp

The International Research Coordination Network for Biodiversity of Ciliates (IRCN-BC) is a joint project between U.S. and Chinese researchers, funded by the National Science Foundation and the Natural Science Foundation of China, that promotes multidisciplinary, integrative research on biodiversity of ciliated protists and fosters international cooperation in studies of biodiversity. It welcomes participation by researchers investigating any facet of biodiversity of ciliates or other protists as well as prokaryotes or multicellular eukaryotes that interact with ciliates in some way. Our goal is to attract a broad input of expertise, outlooks, and technical skills into collaborative research projects. The IRCN-BC sponsors one major workshop or symposium each year and funds travel by researchers to accomplish research collaborations, to visit participating laboratories for specialized training, or to attend workshops and professional meetings. Major objectives of the IRCN-BC are the following: 1) defining the "Grand Challenges" of ciliate/protistan biodiversity and finding strategies for addressing them; 2) fostering new, international research collaborations; 3) generating new grant proposals for integrative biodiversity studies; 4) forming and using working groups to accomplish specific, broadly based, collaborative projects; 5) developing new data-sharing structures; 6) recommendation of new, enhanced standards for deposition of archival material.

Anaerobic adaptations of the mitochondria in *Naegleria gruberi*

Anastasios D. Tsaousis, Eva Nývltová, Ivan Hrdy & Jan Tachezy

Naegleria gruberi is a free-living heterotrophic amoeba well known for its ability to transform from an amoeba to a flagellate to even a cyst form. The genome of *N. gruberi* has been recently published, and in silico predictions demonstrated that Naegleria has the capacity for both aerobic respiration and anaerobic biochemistry to produce molecular hydrogen in its mitochondria. This finding has fundamental implications for the evolution of mitochondrial metabolism, but there are no actual experimental data to support this hypothesis. For this reason, we have decided to investigate the metabolism of the mitochondrion of *N. gruberi*. Using in vivo biochemical assays we have demonstrated that Naegleria has indeed a functional [fefe]-hydrogenase, an enzyme that is attributed to anaerobic organisms. We will demonstrate

preliminary results on the biochemical characterization and localization of this protein along with its maturases and suggest potential implications for the lifestyle of this evolutionarily important organism. Our data is the beginning of a series of experiments focused on understanding the transition from aerobic mitochondrial-based metabolism to anaerobic lifestyle in this biochemically under-studied protist.

Application of protist communities for monitoring water quality in the Hangzhou section of Jing-Hang Grand Canal, southern China

Xinlu Shi, Guijie Liu, Henglong Xu, Xiaojiang Liu and Zhiqiang Sun

Spatial patterns of protist communities and relationships with water quality status were studied in the Hangzhou section of JingHang Cannel, northern China during a 1-year cycle (February 2008–January 2009). Protist communities were sampled monthly at six sampling stations with a spatial gradient of environmental stress, and the type of protist distribution, community structure, species composition, dominant species and species diversity were analyzed. These taxa showed different spatial patterns in species distribution in all six stations. The protist communities showed a clear spatial pattern in response to the changes in environmental parameters, especially COD, TN and TP. Phytoplankton-based saprobien indices may reflect the spatial variation in water quality status (from β- to α-mesosaprobic zone) in the river system. However, the species diversity, richness and evenness failed to discriminate between different levels of water quality status although these parameters are usually used to summarize the relationships with environmental conditions. It is suggested that spatial pattern of protist community and taxonomic biodiversity can be used in assessing water quality of flowing river systems.

Association of methanogenic archaea with individual protozoa species and its influence on ruminal methanogenesis

Johanna O. Zeitz, Michael Kreuzer and Carla R. Soliva

The rumen microbial ecosystem, comprising bacteria, fungi, protozoa and methanogenic archaea, is the key for efficient feed degradation and utilization in ruminants. Individual species of ruminal ciliate protozoa (phylum Ciliophora) are associated with methanogens extra- and intra-cellularly to a different extent thus probably influencing methane formation differently. *Entodinium caudatum* (EC), *Eudiplodinium maggii* (EM) and *Epidinium ecaudatum* (EE) were anaerobically cultivated in vitro together with ruminal bacteria and methanogens for 24h at 39°C in 40-ml cultures (n=4). Substrates for the cultures with or without protozoa and their associated methanogens (protozoa-free control cultures obtained by centrifugation before incubation) were cellulose (0.6g) and wheat gluten (0.18g). The short-chain fatty acid (SCFA) profile consisted of 80% acetate, 15% propionate, and 4% butyrate, typical for ruminal fluid. Bacterial 16S rDNA copies and the amounts of SCFA were similar ($P>0.1$) in all cultures. However, amount and rate of methane formed was higher in EC- and EM-containing cultures compared to their protozoa-free cultures. It was calculated that 64, 34 and 10% of methane formed within 24h was due to protozoa-associated methanogens in EC-, EM- and EE-containing cultures. Protozoa-associated methanogens made up 36, 48 and 9% of total methanogens (16S rDNA copies) in EC-, EM- and EE-containing cultures.

Characterization of hydrogenosome in anaerobic protist *Mastigamoeba balamuthi*

Eva Nývltová, Ivan Hrdy and Jan Tachezy

Mastigamoeba balamuthi is a free-living protist closely related to the human parasite *Entamoeba histolytica*. Although both organisms are adapted to oxygen-poor environment, mastigamoeba is able to inhabit anaerobic freshwater, which is likely an ancestral trait, while entamoeba underwent adaptation to parasitic lifestyle. In the latter organism, the adaptation resulted in reduction of mitochondria to mitosome, the organelle which lost majority of mitochondrial functions including ATP synthesis. In Mastigamoeba we identified 3 different pyruvate decarboxylation enzymes to form acetylcoa: pyruvate:ferredoxin oxidoreductase (PFO), pyruvate:NADP oxidoreductase and pyruvate formate lyase. Although we localized all of them in cytosolic fraction, we detected PFO activity in organelar fraction. Interestingly, we found cytosolic and organelar localization also for another hydrogenosomal enzyme hydrogenase. Importantly, the organelar fraction exhibited an ADP-phosphorylating activity, supporting the presence of energy metabolism in the organelle. Mitochondrial enzymes NAD-specific malate dehydrogenase (MDH), succinate dehydrogenase and D-lactate dehydrogenase were also present in organelar fractions. In contrast, the citric acid cycle enzymes (isocitrate dehydrogenase, fumarase) were found in the cytosol. Similar to *E. Histolytica* we identified sulfate activation system also in mastigamoeba organelles. Our results indicate that organelles of *M. Balamuthi* are metabolically active, ATP-producing organelles similar to hydrogenosomes of anaerobic protists.

Assembling and annotating the nuclear genome of the jakobid flagellate *Andalucia godoyi* (Discoba, Excavata)

Vladimir Klimes, Cestmir Vlcek, Marek Elias, Jan Fousek, Jan Paces, Michael W. Gray, Michelle Leger, Andrew J. Roger and B. Franz Lang

Jakobids (one of the three principal phyla of the Discoba clade in the supergroup Excavata) are free-living heterotrophic flagellates, remarkable for their most primitive mitochondrial genome relative to other eukaryotes (e.g. encoding subunits of the eubacterial-type RNA polymerase). However, a reference genome sequence is still missing for jakobids. Using pyrosequencing, we have assembled a close-to-complete draft of the nuclear genome sequence of the jakobid *Andalucia godoyi* comprising nearly 20 Mbp, distributed over up to 67 chromosomes. Telomeric repeat sequences are of the human type (TTAGGG). The assembly indicates

that *A. godoyi* cells are diploid with the allelic polymorphism ranging from SNPs to more extensive structural differences particularly in subtelomeric regions. Aided by RNA-Seq data and employing Augustus we predicted around 8,500 protein-coding genes. The genome is intron-poor, with >80% of genes devoid of introns and with the U12-dependent introns apparently absent altogether. Manual curation of the predicted gene models and additional analyses are underway, yielding for example identification of a decent complement of selenoprotein-coding genes. Notable features of the *A. godoyi* gene repertoire revealed by our initial analyses include the presence of transcriptionally active genes encoding both the translation factor paralogs EF-1alpha and EFL.

Balantidion pellucidum, a freshwater haptorid ciliate with resting cysts covered by heteromorphic lepidosomes: light and scanning electron microscopy and cytochemistry

William A. Bourland

We studied previously undescribed resting cysts of the haptorid, *Balantidion pellucidum*, by microscopic and cytochemical methods. Trophonts and resting cysts were obtained during a pre-spring bloom. Spherical cysts are ~ 40-50 μm (excluding pericyst) with four layers. The pericyst comprises heteromorphic lepidosomes of three types and an irregular mucous coat containing acid mucopolysccharides. Lepidosomes take three forms: Types I and II (distally fimbriate, ~ 3 μm and 6 μm long respectively) and Type III (~ 7.5 μm long, slender, distally tapered). Tests for chitin yielded conflicting results [i.e. mesocyst positive with Van Wisselingh's test, Calcafluor and chitinase but negative with wheat germ agglutinin (WGA) and Coomassie blue; lepidosomes positive with Calcafluor, Coomassie blue and chitinase but negative with Van Wisselingh's test and WGA]. Lepidosomes are resistant to proteolytic enzymes. Cytoplasmic "precursor stocking" occurs in preencystment trophonts. Cysts of other haptorids and rhynchostomes have isomorphic lepidosomes. *Balantidion pellucidum* is unique among haptorids in having resting cysts with heteromorphic lepidosomes. Although protective and dispersal-promoting roles for cyst lepidosomes have been suggested, the function of these elaborate structures remains unknown. The phylogenetic importance of heteromorphic lepidosomes has yet to be determined. Future work will include more extensive cytochemical and ultrastructural studies.

Characterization of long non-coding rRNAs from the parasite *Trichomonas vaginalis*

Gary Kusdian, Christian Wöhle, Claudia Radine, Giddy Landan and William Martin

The human pathogen *Trichomonas vaginalis* is a parabasalian flagellate that infects millions of people annually and its genome is the largest among those of sequenced protozoa. On six chromosomes, together ~160 Mb large, 59.672 potential proteins are found encoded. Among gene expression data we noticed an unusual high amount of expressed sequence tags (ESTs) that map onto intergenic, non-coding regions - no less than 16 % of all analyzed loci. These long non-coding RNAs (lncRNAs) reach up to 3 kbp, are partially processed and harbor polyA tails. We have verified the expression of some lncRNAs in our T1 lab strain and from experimental case studies can exclude the use of alternative start codons or stop-codon read through. Approximately 50 % of lncRNAs can be traced back to pseudogenes and reflect the reductive/destructive evolution of parts of the Trichomonas genome, after it was duplicated for an unknown amount of times. Our results demonstrate that *T. vaginalis* can energetically afford the expression of thousands of intergenic loci. We hypothesize they offer a large pool for potential innovation, from which for example regulatory RNA units or even orphan genes can be born

Characterization of the charged repeat motifs of alveolin proteins

H. El-Haddad, J. Pryzborski, W. Martin and S. B. Gould

Proteomic profiling of the *Tetrahymena thermophila* pellicle has revealed that the membrane- and cytoskeleton is enriched with proteins containing charged repeat motifs (CRMPs). The largest proportion of CRMPs are of unknown function and about a dozen have been localized to the cytoskeleton of *Tetrahymena thermophila* and the apicomplexan parasite *Toxoplasma gondii*. We have commenced to characterize the function of the repeats themselves and analyzed their involvement in cytoskeletal association, focusing on alveolin proteins. Our results indicate that the repeats themselves are in several cases sufficient for cytoskeletal association, although not necessarily correct localization, which is underpinned by the analyses of a synthetic CRMP. Correct localization of alveolins was furthermore disrupted by a C-terminal GFP-tag and in comparison to the endogenous protein, which associates with the alveoli, the tagged version associates with basal bodies. Both GFP-tagged CRMPs and isolated repeat-containing fragments thereof localize to *Tetrahymena* basal bodies, raising the possibility that cytoskeletal proteins in *Tetrahymena* might, to a certain extent, be “destined by default” To basal body targeting.

Coiling direction does not depend on water temperature in a planktic foraminifer

Yurika Ujiie and Takahiro Asami

Planktic foraminifera form the calcareous shell coiled clockwise (right-coil) or counterclockwise (left-coil). This coiling direction has long been used as a proxy to assess past environmental changes. However, few studies have explicitly tested the classical assumption that the direction depends on water temperature. This test requires examination of morph frequency variation within biological species dimorphic for the direction. Recent molecular studies confirmed that both morphs occur within single genetic types of *Globorotalia truncatulinoides*, and that geographic and vertical distributions of those genetic types are associated with different water masses. Molecular phylogeny with small subunit and internal transcribed spacer rDNA showed that five distinct genetic types occur in *G. truncatulinoides*.

By regression analyses, we found no significant relationship between morph frequency and habitat (water mass) temperature either at sea-surface or a 100m water depth across the world oceans. Morph frequency largely varies within genetic types, but there is no consistent pattern in frequency variation among genetic types across global temperature ranges. These results suggest that water temperature is not directly responsible for coiling direction of the planktic foraminiferal shell and opens a new question; How is dimorphism for coiling direction maintained within water masses?

Comparative analysis of nuclear and nucleomorph gene expression in cryptomonad and chlorarachniophyte algae

Goro Tanifuji, Naoko T. Onodera, Christa E. Moore, Julia Hopkins and John M. Archibald

Cryptomonads and chlorarachniophytes are unicellular algae with plastids derived from secondary endosymbiosis. They are unusual in that they possess nucleomorphs, which are the residual nuclei derived from their algal endosymbionts. At less than one megabase-pairs in size, nucleomorph genomes are the smallest and most gene dense nuclear genomes known. Little is known about nucleomorph gene expression and regulation. Here we used RNA-Seq technologies to investigate nuclear and nucleomorph gene expression in four nucleomorph bearing organisms including three cryptomonads and one chlorarachniophyte. Comparative analysis of nuclear and nucleomorph genes in *Guillardia theta* and *Bigelowiella natans* showed that overall, the mean expression levels of nucleomorph genes are higher than that of nuclear genes. We also found that essentially all predicted nucleomorph genes, including orphan genes of unknown function and with no similar to known genes in other organisms, are expressed. The most highly expressed nucleomorph genes were those encoding plastid-targeted proteins. Interestingly, more than 95% of the nucleomorph genomes, including intergenic spacers, were found in our transcriptome data, suggesting fundamental differences in the hyper-compacted nucleomorph genomes relative to those of free-living organisms.

Comparative distribution of the two ichthyotoxic dinoflagellates *Gambierdiscus* spp. and *Ostreopsis* spp. In the coastal waters of Jeju Island, Korea

Bora Jang, Hyung Seop Kim, Mi Ryeong Oh, Jung Rae Rho and Wonho Yih

We estimated the abundances of the *Gambierdiscus* spp. (GAMBI) and *Ostreopsis* spp. (OSTRE) from macroalgal samples collected at 6 stations in Jeju during the cold-water months of 2011. GAMBI and OSTRE were quite contrasting in spatial distribution and substrate preference. At Seong-san, mean abundance of GAMBI in February was 996 cells g-1 wwt (CGT) on 10 macroalgal species comprising 66.1% of all epiphytic dinoflagellates while 561 CGT in April on 8 substrate algae. The maximum abundance of GAMBI at Seong-san was 4734 CGT on *Hydroclathrus clathratus* in April. OSTRE recorded mean abundance of 138 in February on 5 substrate algae, and 114 CGT in April on 5 macroalgal species with the maximum of 668 in February on *Galaxaura falcate* at Seong-san. At Aeh-wol, however, OSTRE (with maximum of 13366 CGT on *Polysiphonia sp.*) Was far more abundant than GAMBI (with maximum of 30 CGT on Undaria pinnatifida). Whilst GAMBI was not observed at Shin-do in February or at Aeh-wol and Joe-chun in April, it recorded the highest CGT at Seong-san where water salinity and temperature were in the middle among the 6 stations. OSTRE showed rather “General” distribution throughout the whole T-S-station hyperspace when compared with GAMBI.

Differential analysis and comparison on the proteins profile of *Urostyla grandis* under different physiology conditions

Ji-wu Chen, Li-na Zheng, Bang-zheng Wang and Fu-kang Gu

Ciliates forming resting cyst under adversity is a common phenomenon. The molecular mechanisms underlying the resting phenomenon are not quite clear. Therefore, this study used the resting cyst and the vegetative cell of *Urostyla grandis* as experimental materials and investigated differentially expressed proteins profile in the encystment processes by two- dimensional electrophoresis, mass spectrum and bioinformatics. Six up-regulating expressed proteins were sequenced using mass spectrum and the sequencing results were retrieved and compared using protein banks. The results showed at least dozens of differentially expressed proteins found between the resting cyst and the vegetative cell of Urostyla Grandis. Among six sequencing proteins two proteins are unknown and two proteins may be keratin type II, the other proteins may be amino acid ATP-binding cassette transporter and thermostable serine protease, respectively. Amino acid ATP-binding cassette transporter relates with transported substance. Thermostable serine protease relates with resisting adversity. Keratin type II is components of the cyst wall. So relationship between these four proteins up-regulation and resting encystment of *Urostyla grandis* was discussed and analysed. Further analysis on these differentially expressed proteins helps revealing molecular mechanism of encystment of ciliates under adversity. (This project is supported by the National Natural Science Funds of China (No. 31071875)

Distribution of novel apicomplexan lineage in the Gulf of Aqaba

Ales Horak and Ondrej Prasil

Detailed analysis of more than 2.5 million of ssu rRNA sequences deposited in public databases revealed that a rather large number (almost ten thousand) of sequences annotated as bacterial are actually of plastid (and thus eukaryotic) origin. Some formed new lineage related to free-living photosynthetic chromerid algae (Chromera and Vitrella) and also to apicomplexan parasites. Analysis of origin of these sequences showed pan-tropical distribution and strong link to coral reefs and to sunlight (100 % of data come from depths of 20

meters and less), suggesting the new clade may me be the missing step in transition from free-living photosynthetic alga (Chromera) to intracellular parasites (Toxoplasma and Plasmodium). We have searched for the presence of the recently described deep-branching apicomplexan lineage on the coral reefs of the Gulf of Aqaba, Red Sea. Using cotton swabs, we sampled mucus from the surface of 45 specimens belonging to more than 15 species of stony corals. We have also sampled non-coral parts of the reef as well as surrounding area. PCR and consequent sequencing revealed presence of the novel apicomplexans in 17 samples belonging to the 12 coral species, however all the non-coral samples were negative, suggesting strong link between protozoans and polyps.

Diversity of the genus Monocercomonoides and its genetic codes

E. Šrámová, K.K. Novotná, P. Smejkalová, I. Čepička and V. Hampl

Oxymonads (Excavata) are anaerobic protists that occur mainly in the gut of insects with the exception of Monocercomonoides, which also inhabits the intestine of vertebrates. We focused on clarifying the phylogenetic relationships of the morphologically simplest genus Monocercomonoides. We obtained sequences of SSU rDNA from 25 strains. The analysis showed that the representatives formed a monophyletic group however with low bootstrap support. The relationship within Monocercomonoides clade partially reflected the host origin of strains. We also investigated the presence of alternative genetic code in some of these strains. Non-canonical genetic codes evolved in several eukaryotic groups. Oxymonad genus Streblomastix and potentially Monocercomonoides from the gut of wood roach Cryptocercus punctulatus use a code, in which TAA and TAG codes for amino acid glutamine. cDNA sequencing of the strain PA203 from Chinchilla clearly showed that it uses the canonical genetic code. In the partial sequence of α-tubulin gene from 8 different strains POTFIB (from *Potosia fieberi*), B1-10 (from *Bos taurus*), BAT1 (from *Blaberus atropos*), CEAE (from *Cetonischema aeruginosa*), ESMI2, EUDIA3, CHAM1 (from *Chameleo cristatus*), MALA (from millipede) are all glutamine codons coded canonically, which suggests that all these strain use the canonical code. The morphological analysis of selected strains will follow

Ecto-phosphatase activity in *Tritrichomonas foetus*: differential expression in the pseudocyts (endoflagellar form) and its participation in the cytotoxicity

Antonio Pereira-Neves, Josè Roberto Meyer-Fernandes and Marlene Benchimol

The protist parasite *Tritrichomonas foetus* displays a pear-shaped and a pseudocyst form (endoflagellar form). We assessed whether during the pseudocyst formation occurs a modulation of the ecto-phosphatase activity present on the surface of *T. foetus*. A positive correlation between enzyme activity and pseudocyst formation was observed. The ecto-phosphatase activity increased during the time-course of pseudocyst induction. During pseudocyst reversibility, the ecto-phosphatase activity decreased and it was restored to the same level as found in the parasites before pseudocyst induction. The surface localization of the enzyme was confirmed using ultrastructural cytochemistry. The ecto-phosphatase activities of both parasites forms were decreased, with different patterns of inhibition, at high pH and by several well-known inhibitors of acid phosphatases. The involvement of this enzyme in the cytotoxicity of the parasites was also investigated. A direct relationship between ecto-phosphatase activity and cytotoxicity of both parasites forms to epithelial cells was established indicating that this enzyme could represent a virulence marker for *T. foetus*. Irreversible inhibition of the ecto-phosphatase activity partially blocked the pseudocyst induction and resulted in decrease of the cytotoxic effects exerted by both parasites forms suggesting that this enzyme could be involved in the mechanisms of pseudocyst transformation and cytotoxicity of *T. foetus.*

Expanded molecular phylogeny of Armophorea (Ciliophora: Intramacronucleata)

Thiago da Silva Paiva, Bárbara do Nascimento Borges and Inácio Domingos da Silva Neto

The 18S-rDNA phylogeny of class Armophorea, a group of anaerobic ciliates, is hypothesized on the base of 44 sequences (among a total of 195) sampled from the NCBI/Genbank. Emphasis is given on the exploration of different nucleotide alignment criteria, considering both variation of gap-opening and gap-extension parameters and the use of RNA secondary structure to orientate multiple-alignment. Within such context, a sensitivity analysis of 76 data sets was thus performed to assess the effects of indel parameters variation on tree topologies. Bayesian, maximum likelihood and maximum parsimony phylogenetic analyses were performed in order to further explore how different analytic frameworks influence the resulting hypotheses. The sensitivity analysis revealed that relationships among higher-taxa of Intramacronucleata are dependent upon how indels are determined during multiple alignment, and monophyly of Armophorea was rejected most of the times. Phylogenetic analyses indicate the position of Metopidae and Nyctotheridae as related to the Litostomatea, but did not converge on a single solution for the placement of Caenomorphidae, which could be either a sister group of Metopidae + Nyctotheridae, have diverged at the base of the Spirotrichea branch, or even at the base of the Intramacronucleata tree, according to nucleotide alignment criteria and phylogenetic analysis frameworks, thus remaining unresolved.

Finding the role of *Chromera velia* in its environment

Marjorie Linares, Jan Slapeta and Dee Carter

Identifying and classifying a new organism is difficult, however, deciphering and understanding its ecology, lifecycle, and biodiversity is where things become truly complex. *Chromera velia* is a marine photosynthetic protist that was only recently characterized. To date two strains have been

isolated, one from the Great Barrier Reef, and the other from the busy commercial waters of Sydney Harbour. *Chromera* is phylogenetically related to apicomplexan parasites and marine dinoflagellates, but its ecological relationship with the corals from which it was isolated remains elusive. In a project designed to help understand the biodiversity of this new organism, live algal isolations from coral tissue and coral reef sediment to have been optimized to obtain new *Chromera* species or chromerid- like organisms. In parallel, a molecular detection method using PCR has been devised to screen various coral species for presence of *Chromera*. Additionally, a study using pyrotag sequence data to assess eukaryotic biodiversity-including *C. velia*- within coral samples will also be presented. The corals being screened are not only within *C. velia*'s potential host species but also range across different host taxa. Our aim is to catalogue which corals *C. velia* associates with and to understand the extent of the association in order to shed more light on its ecology.

First glance at the genome of the Phytomyxae club root pathogen *Plasmodiophora brassicae*

Arne Schwelm, Johan Fogelqvist and Christina Dixelius

Plasmodiophora brassicae, an obligate biotrophic protist, belongs to the Phytomyxea class of protists within the eukaryotic supergroup Rhizaria. *P. brassicae* is the casual agent of the clubroot disease of the Brassicaceae, one of the most damaging diseases within this plant family. Despite its agricultural importance, the biology of *P. brassicae* remains poorly understood, especially at the molecular level with only approximately 100 genes known to date. Due to its obligate biotrophic nature, *P. brassicae* remains impossible to grow in axenic culture and the typical experimental systems for working with *P. brassicae* are comparatively unsophisticated. We recently succeeded in obtaining the genome sequence from a *P. brassicae* single spore isolate. The total length of the genome sequence we obtained is 22.8 Mb, which broadly corresponded with the previously estimation of 18-20.3 Mb based on PFGE studies. The exploitation of the genome will greatly advance the knowledge of this important but poorly understood pathogen, as well as the Rhizaria in general. The genome will shed light into the evolutionary origin of *P. brassicae* and the biology, which will in the long run help to understand and control clubroot root disease. Here we present our first results of the genome sequence analysis.

Genetic differentiation in populations of desmid species *Micrasterias rotata* (Zygnematophyceae, Streptophyta)

Katarína Nemjová, Pavel Škaloud, Frederik Leliaert, Jana Veselá and Helena Bestová ·

The idea of missing population structure among protists is now overcome. The most of the case studies confirmed a geographical differentiation of particular populations within a protist species. An adaptation to local environmental conditions could drive the genetic differentiation as well as allopatric speciation. Nevertheless, an ecological differentiation was tested just in a few heterotrophic protists. We supposed that desmids could comprise ecologically differentiated populations as they are closely related to the higher plants. Moreover, most of them are highly speciated to their environments, so the exception (*Micrasterias rotata*) with broad ecological range could be differentiated on that scale. An investigation of 30 strains from different natural habitats uncovered an unexpected genetic differentiation in actin marker as well as in glyceraldehyde-3-phophate dehydrogenase introns. The both markers were congruent. The variability among populations corresponded to a geographic origin of the strains. Nevertheless, the variability within actin marker was more complex. To find out whether this variability corresponds to ecological factors, we have analyzed 100 strains isolated from different habitats and subsequently tested the genetic variability in the actin marker against environmental factors such as microhabitat and ph. The preliminary results indicate both ecological and geographical differentiation of 11 detected alleles.

Genome sequencing project of *Acrasis kona*

Chengjie Fu and Sandra L. Baldauf

The *Acrasis* spp. constitutes a clade of aggregative amoeboid Heterolobosea species nested within the major taxon discobids which is considered one of the earliest split of eukaryotic tree of life. Acrasis species are common soil microbes frequently encountered in the soil surveys for the "sorocarpic amoebae". The genera of Acrasis was recently revised, suggesting that some distinct isolates may be more genetically diverse than previously characterized (Brown et al., 2012). As one member at a deep branch in the eukaryote tree, Acrasis is a potential new model system to study the relevant molecular evolution, cell-cell interaction, lateral gene transfer and cellular metabolism pathways. Experimental studies have been carried out to investigate some general genome features in A. kona ATCC strain MYA-3509 (formerly *A. rosea*), which are necessary to prepare Acrasis for genome sequencing. Using a combination of Solexa and 454 sequencing strategies, we hope to obtain a high-quality draft sequence of A. kona genome based on de novo assembly which can be used for comparative studies and help to understand the early steps of evolution of eukaryote.

Ecological significance of chlorophyll metabolism of herbivorous protists in aquatic ecosystems

Yuichiro Kashiyama, Akiko Yokoyama, Hideaki Miyashita, Kanako Ishikawa, Akira Ishikawa, Isao Inouye and Hitoshi Tamiaki

Phototoxicity of chlorophylls is a potential challenge for heterotrophic microbes to ingest into their translucent bodies. In separate presentation, we report a detoxifying metabolism regarding chlorophylls by the herbivorous protists belonging to the Stramenopile-Alveolate-Rhizaria (SAR) and Cryptophyte-Centrohelid-Telonemid-Haptophyte (CCTH) clades. Although SAR and CCTH heterotrophs are known to be a significant

component of marine and perhaps lacustrine microbial ecosystems, they have generally been regarded as bacteriovores. Our geochemical evidences regarding the chlorophyll catabolites produced by the SAR and CCTH herbivores strongly suggest that protistan herbivory is a substantially large, major microbial process in these environments. We infer that some protists prey on picophytoplanktons, which then incorporates large biomass of picophytoplanktons produced in pelagic settings into carbon flows at higher trophic levels, hence eventually contributing to sequestration of carbon out of atmosphere.

Himatismenida Page, 1987 (Amoebozoa) is a proper taxonomic home for Parvamoeba Rogerson, 1993: morphological and molecular evidence

Alexander Kudryavtsev and Jan Pawlowski

The genus Parvamoeba with a single species *P. rugata* Rogerson, 1993 was established within Thecamoebidae to accommodate an extremely small marine naked amoeba with flat ventral hyaline projection and wrinkled dorsal surface. The first SSU rRNA-based molecular phylogenetic study made on another species, *P. monoura* Cole et al., 2010, excluded Parvamoeba from Thecamoebidae leaving it incertae sedis in Amoebozoa. Later, Parvamoeba was included into the order Himatismenida (suborder Parvamoebina, family Parvamoebidae) based on actin gene sequence analysis. We present further evidence that Parvamoeba belongs to the Himatismenida based on morphology, SSU rRNA and Cox1 gene sequences: (1) *P. rugata* shares morphological characters of the himatismenid morphotype producing a flat ventral hyaline cytoplasmic projection during locomotion. (2) Two new marine species of Parvamoeba that we isolated recently demonstrate a typical locomotive morphotype of Cochliopodiidae, but branch with *P. rugata* and *monoura* in the single-gene and concatenated phylogenetic trees based on both markers. The clade of Parvamoeba spp. is sister to Cochliopodiidae. These data confirm that Himatismenida is a proper taxonomic home for Parvamoeba and suggest that this genus retains the most ancestral morphological characters of the Himatismenida. Supported by the grant IZLR Z3_128338 from the Swiss-Russian Science and Technology Cooperation Programme and a RFBR grant 12-04-01835.

Intragenomic spread of plastid-targeting presequences in the coccolithophore *Emiliania huxleyi*

Fabien Burki, Yoshihisa Hirakawa and Patrick J. Keeling

Nucleus-encoded plastid-targeted proteins of photosynthetic organisms are generally equipped with an N-terminal presequence required for crossing the plastid membranes. The acquisition of these presequences played a fundamental role in the establishment of plastids. Here, we report a unique case of two non-homologous proteins possessing completely identical presequences consisting of a bipartite plastid-targeting signal in the coccolithophore *Emiliania huxleyi*. We further show that this presequence is highly conserved in five additional proteins that did not originally function in plastids, representing de novo plastid acquisitions. These are among the most recent cases of presequence spreading from gene to gene and shed light on important evolutionary processes that have been usually erased by the ancient history of plastid evolution. We propose a mechanism of acquisition involving genomic duplications and gene replacement through non-homologous recombination that may have played a more general role for equipping proteins with targeting information.

Is the replacement of a gene encoding plastid-targeted GAPDH on-going in the dinoflagellate genus in Karenia?

Euki Yazaki, Ryoma Kamikawa, Tetsuo Hashimoto and Yuji Inagaki

The vast majority of photosynthetic dinoflagellates has plastids containing a unique cartioind peridinin and encodes a single gene encoding plastid-targeted GAPDH (peridinin-type GapC1 or GapC1-p) in their nuclear genomes. On the other hand, the dinoflagellate genus Karenia is known to bear tertiary plastids derived from a haptophyte endosymbiont, and possess the endosymbiotically acquired 'gapC1-fd,' in addition to the endogenous gapC1-p. We here compared the primary structure and abundance of two types of gapC1 transcripts in *K. brevis* (Kb) and *K. mikimotoi* (Km). No significant expressional difference was detected between the two gapC1 genes in Kb cells, while gapC1-fd transcripts appeared to be much more abundant than gapC1-p transcripts in Km cells. As expected for dinoflagellate mRNAs, Kb gapC1-fd and gapC1-p, and Km gapC1-fd transcripts possessed the spliced leaders (SL) and poly-A tails at their 5′ and 3′ ends, respectively. However, only truncated gapC1-p transcripts without the SL or poly-A tail were isolated from Km cells, suggesting that Km gapC1-p is a recently established pseudogene. These results indicate that Karenia gapC1 genes are an excellent model to study orthologous gene replacements associated with tertiary endosymbiosis.

Localization of *Chromera velia* heme pathway enzymes by xenotransfection

Jitka Kručinská, Lilach Sheiner, Luděk Kořený, Boris Striepen and Miroslav Oborník

Apicoplast, relict plastid of apicomplexan parasites, is indispensable for the cell because likely essential biochemical pathways (isoprenoid synthesis, synthesis of fatty acids and heme pathway) take place here. Heme biosynthesis is very conserved through all three domains of life but it differs in the localization and origin of the particular enzymes of the pathway among eukaryotes. Heme synthesis in apicomplexan parasites is a mosaic of heterotrophic and phototrophic pathway. Apicomplexans synthesize 5-aminolevulinicacid (ALA) in mitochondria from glycine and Succinyl-coa (C4 pathway). Then the synthesis goes through apicoplast reflecting its phototrophic history and is finished in mitochondria again. In silico analyses of heme pathway genes from *Chromera velia*, the closest known photosynthetic relative of

Apicomplexa, revealed likely homologous origin of enzymes and also intracellular localization as in Apicomplexa. *Chromera velia* also seems to be the only known eukaryotic phototroph that synthesizes ALA by C4 pathway. We decided to experimentally test localizations of *C. velia* heme pathway enzymes by xenotransfection in *T. gondii* and *P. tricornutum*. Our results so far show that *C. velia* uses homologous targeting signal as *T. gondii* and *P. tricornutum* and that in vitro localizations so far reflect in silico analyses, further suggesting their common origin.

Microbial eukaryotes in the arctic marine ecosystem: Identity and seasonality

Miriam Marquardt, Archana Meshram, Anna Vader, Marit Reigstad and Tove M. Gabrielsen

Microbial eukaryotes are dominant primary producers for much of the year in the Arctic and also show both abundant and diverse heterotrophic populations. Nevertheless, information on the species level is limited due to difficulties of identifying them by traditional methods such as microscopy. The aim of this project is to identify the key species, investigate their seasonality and determine the environmental parameters that govern their existence, with special emphasis on polar night ecology. An intensive field campaign is currently taking place in Adventfjorden (Svalbard), where samples have been collected every week since December 2011. The station is equipped with a moored ocean observatory which allows us to describe the habitat of the microbes at great temporal resolution, even during the barely studied winter and early spring periods. We will characterize the biodiversity of the pelagic eukaryotes (size fraction: 0.45 – 10 μ m) throughout a year using amplicon pyrosequencing of the 18S rRNA and rDNA. In addition we will investigate the abundance of major microbial groups by epifluorescence microscopy using DAPI staining. Special focus will lie on the green alga *Micromonas pusilla* and the haptophyte *Phaeocystis pouchetii*, both species seem to dominate microbial communities in the Arctic Ocean.

Microtubular Organelles in *Euplotes patella* (Ciliophora: Hypotrichida) Revealed by fluorescent Labeling

Gu Fu-Kang, Lin Qin and Fan Xin-Peng

As show in the application direct fluorescence labeling of FLUTAX and immunofluorescence labeling using anti- α tubulin antibody, *Euplotes patella* microtubular organelles are composed of the cortical ciliature microtubules, base-associated microtubules and the dorsal cortical microtubule structure, as well as the morphogenesis of the cortical ciliature microtubules. The results indicate, the dorsal cortical network not only is consistent with the silver-line system network morphologically, but also is a microtubular cytoskeleton, Contrasting with other Euplotes, The proter AZM microtubules and dorsal bristle microtubules of *E. patella* have a different mode of morphogenesis, It can be used as the base of specie classification. Additionally, the comparison of the two fluorescently labeling shows that the tubulin element from the dorsal cortical surface may differ from other cortical location in the same ciliate. These differences come from morphogenesis. Keyword: Ciliate; *Euplotes patella*; Fluorescently labeling; Cortical Ciliature; Microtubule organelle (This project is supported by the National Natural Science Funds of China (No. 31172042)

Mitochondrial preprotein translocase of kinetoplastids is homologous to Tom40

Vojtěch Žárský, Jan Tachezy and Pavel Doležal

The mitochondrion of trypanosomatids was proposed to lack the essential Tom40 channel – a central subunit of the TOM complex (translocase of the outer mitochondrial membrane). This was suggested to be an ancient state, placing trypanosomatids to the root of the eukaryotic tree of life. Moreover Trypanosoma brucei was recently shown to contain an essential protein called archaic translocase of the outer mitochondrial membrane (ATOM), which was directly linked to bacterial Ytfm proteins, members of Omp85 superfamily. Hence, it was suggested that both ATOM and Tom40 represent mutually exclusive functional analogues of distinct origins. We re-examined the evolutionary origin and distribution of ATOM sequences among kinetoplastids. For distant homology detection we used sensitive Hidden Markov Model-based methods HMMER and HHsearch. Our results clearly refute the direct link between ATOM and bacterial Omp85-like proteins. Moreover, we show that ATOM is in fact diverged form of the 'classical' Tom40.

Molecular differentiation within *Paramecium dodecaurelia* from the *P. aurelia* spp. Complex reveals recent speciation process

E. Przybos, S. Tarcz, M. Prajer, M. Surmacz, N. Sawka, and M. Rautian

Species of the *P. aurelia* complex show different levels of intraspecific polymorphism, with *P. dodecaurelia* revealing a high level of intraspecific variation. Strains of *P. dodecaurelia* originating from distant localities in the Palearctic, North America (USA), and Oceania (Hawaii) were included into the present study concerning intraspecific differentiation and the degree of speciation. Sequences of the genes encoding the ITS1-5.8S-ITS2-5' end of LSU rDNA (1063–1097 bp) and cytochrome c oxidase subunit I mtDNA (638–644 bp) were obtained from 33 strains of *P. dodecaurelia*, other *P. aurelia* species, and another species of the genus Paramecium, with *Tetrahymena* sp. used as an outgroup. In phylograms, the majority of *P. dodecaurelia* strains from the Palearctic appear in one cluster, while strains from Japan, Hawaii, and the USA are grouped in another cluster together with some strains from Italy and representatives of the *P. aurelia* species complex. The resolution of the COI mtDNA tree is greater than that of the rDNA tree, as several groups can be seen within the main Palearctic clade of *P. dodecaurelia* strains. The studied strains of *P. dodecaurelia* are not monophyletic. Our results may suggest that a transition from genetic polyphyly to monophyly is observed in this species as far as the studied molecular markers are concerned. However, it is still an open question whether the revealed intraspecific differences within *P. dodecaurelia* are the result of

ongoing speciation, or perhaps they just indicate genetic differentiation.

Morphology and Molecular Analysis of six Antarctic Tintinnid Ciliates

Sun Young Kim, Joong Ki Choi, John R. Dolan and Eunjin Yang

Six tintinnid ciliates of Antarctic waters are characterized using molecular and traditional morphological analysis. Single-cell PCR was used to obtain new sequences of SSUrDNA,ITS1-5.8SrDNA-ITS2 region and partial sequences of LSUrDNA of 14cells. Morphometrics of lorica morphology were determined for all forms and the infraciliature structures of Codonellopsis gaussi and Laackmaniella prolonggata were analyzed from protargol preparations. Similarities of SSU and LSUrDNA ranged from 84.43 to 100%. The sequences were identical among individuals of a species and among all individuals of the different Cymatocylis species. The finding of 100% similarity in SSU and LSUrDNA sequences among Cymatocylis convallaria, Cy. calyciformis and Cy.drygalskii justifies previous suspicions of synonym. Co. gaussi and L. prolongata also showed high levels of similarity for SSU-ITS1-5.8S-ITS2-partial LSU (99.30%), SSU (99.83%), ITS1-5.8SrDNA-ITS2 (98.02%), partial of LSU (98.87%) and D2 domain (95.77%) as well as very similar infraciliature, implying that they are closely related, possibly phenotypes of a single species. Phylogenetic analysis placed Cymatocylis within the Metacylididae and Cyttarocyllidae, Amphorellopsis in the Tintinnidae and L. prolongata/ Co. gaussi in the Codonellopsidae. This work was supported by KOPRI and KRF grants.

Morphology and phylogeny of a new imbricatean flagellate (phylum Cercozoa)

Takashi Shiratori, Akiko Yokoyama and Ken-ichiro Ishida

Imbricatea is one of cercozoan classes including heterotrophic, parasitic, and photosynthetic amoebae and flagellates with various morphologies and lifestyles. Although Imbricatea currently consists of five orders, synapomorphy of this class remains uncertain. We established a culture of new heterotrophic flagellate from a seawater sample collected at a wharf in Tokyo bay, Japan, incubated with a centric diatom *Skeletonema* sp. as a food source. The cells of this flagellate was spherical with an anterior rostrum and two unequal flagella emerged from subapical deep flagellar pit. This flagellate resembled a poorly studied protist *Aboliffer prolabens* in cell shape and flagella pit morphology. A phylogenetic analysis using SSU rDNA showed that this flagellate was included in Imbricatea and branched as the sister lineage of order Marimonadida. Although general morphological observations using light and electron microscopy did not show any affinities to known marimonads, flagellar apparatus of this flagellate was similar to another imbricatean flagellate Thaumatomonas species in parallel basal bodies and the arrangement of microtubular root vp2. These results suggested that this flagellate is a new member of Imbricatea. Furthermore, conserved flagellar apparatus among imbricatean flagellates may be helpful for morphological definition of Imbricatea. Morphology and Ultrastructures of a New Frontonia Ciliate (Ciliophora, Peniculida) from Harbin, Northeastern China

Morphology and ultrastructures of a new frontonia ciliate (Ciliophora, Peniculida) from Harbin, Northeastern China

Ying Chen, Wenqiao Ding, Xuming Pan, Zijian Qiu and Weibo Song

The morphology, infraciliature of a frontonia ciliate, found in freshwater of Harbin, Northeastern China, were investigated by living observation, ammonia silver and silver impregnation methods. And a new scanning electron microscopy (SEM) technique were combined with transmission electron microscopy (TEM) to study the internal surface membrane ultrastructurses of this new specie. The new specie is recognized by the following features: large size 110～160 × 400～610μm, intensive somatic kineties 180～200 , single contractile vacuole located dorsal-right in posterior 1/2 of body. We also obtained clear SEM and TEM images of its subpellicular structures., found that there were three lays of fiber system under the pellicle playing a role in stabling the trichocysts and other kinds of extrusomes. And they are arranged in a manner consistent right-hand rule, should be the nature of dynamic fibrils. The trichocysts, tococists and mucocysts inter the pellicle were observed. The vestibular kineties (VK) in the oral were found that they were not alone and some woven fibers linked them. The nature and function of thick columnar structures on both sides of the vestibular kineties still needs further study. This work was supported by the National Science Foundation of China (No. 31101613)

Neospora caninum antibodies in cats from the Czech Republic

K. Sedlak and E. Bartova

Neosporosis is protozoan disease with clinical manifestation especially in dogs and cattle however *N. caninum* antibodies could be found in other animals. Sera of 286 cats coming from different parts of the Czech Republic (major part originated from Central Bohemia and North Moravia) were tested for *N. caninum* antibodies. Sera samples were collected during years 2002 - 2011. Antibodies against *N. caninum* were detected by a commercial competitive-inhibition enzyme-linked immunosorbent assay (cELISA) with cut off ≥30 % inhibition. Samples positive in cELISA were confirmed by an indirect fluorescence antibody test (IFAT); titre ≥50 was considered positive. Antibodies against *N. caninum* were found by cELISA in 130 of 414 (31%) cats with inhibition 30-40%, 40.1-50%, 50.1-60%, 60.1-70%, 70.1-80% in 75, 44, 5, 4, and 2 cats, respectively. *N. caninum* antibodies in IFAT were found in 16 of 130 (12.3%) cats positive in cELISA with titre 50 and 100 in 11 and 5 cats, respectively. This study was funded by the grant no. MSM6215712402 from the Ministry of Education, Youth and Sports of the Czech.

New anaerobic member of the jakobid genus Andalucia

T. Pánek, P. Taborsky and I Čepička

The genus Andalucia belongs to the tiny protist group Jakobida (Excavata: Discoba). The jakobids possess the most primitive mitochondrial genome of all known eukaryotes and are candidates for the most basal eukaryotic lineage. The genus Andalucia comprises two species. Interestingly, *A. godoyi* lives in soil, is aerobic and possesses normally developed mitochondrion, whereas *A. incarcerata* inhabits marine anoxic sediments, is anaerobic and its mitochondrion has lost cristae. In addition, phylogenetic analyses revealed a huge diversity of environmental SSU rDNA sequences belonging to Andalucia as well. We have isolated 10 strains of anaerobic members of the genus Andalucia from anoxic marine sediments. Phylogenetic analyses and light-microscopic morphology showed that most of our strains belong to *A. incarcerata*. However, two strains were morphologically and phylogenetically distinct from it representing an undescribed anaerobic species of Andalucia. Surprisingly, phylogenetic analyses based on three genes (SSU rRNA, α - and β -tubulin) were unable resolve its relationship to *A. incarcerata* and *A. godoyi*. It is therefore possible that the anaerobiosis arose not only once, but possibly twice independently within the single genus. This project is supported by grant from the Charles University Grant Agency 301711..

Newly isolated, branching and network-forming naked amoebae enhance the morphological, genetic and ecological diversity of class Variosea (Amoebozoa)

Cédric Berney, Stefan Geisen and David Bass

Large, branching and/or network-forming heterotrophic naked amoebae are very common in soils and in freshwater and marine sediments, and are suspected to play an important role in most microbial ecosystems. Such amoebae evolved convergently in many separate lineages among distinct eukaryotic supergroups (mostly Amoebozoa and Rhizaria). Yet they remain very poorly understood; for the majority of described species, molecular data is still missing, and very few lineages have been extensively studied morphologically and/or ecologically. Our current project focuses on the morphological, ecological and molecular diversity of these amoebae. In this poster we present several new isolates belonging to Amoebozoa (and likely to be new species). Using 18S rDNA, we show that most branch within class Variosea, but that the studied morphotype is polyphyletic within that class and interspersed with non-branching amoebae. To further investigate the ecological diversity of Variosea, we constructed environmental 18S rDNA libraries enriched in variosean sequences. We also screened the NCBI and BioMarKs databases by Blast to identify related sequences from available environmental DNA libraries and metagenomic surveys. We find variosean amoebae to be an important component of both marine and freshwater ecosystems, and significantly more diverse than previously assumed.

Parasitic protists (Syndiniales, Dinophyta) and fungi in sea ice and winter-time water in the Baltic Sea

Markus Majaneva, Janne-Markus Rintala, Maria Piisilä, David P. Fewer and Jaanika Blomster

The aim of this work was to study the diversity of the parasitic dinoflagellates (Syndiniales) and fungi in the winter-time water and sea ice in the Baltic Sea. This work is part of a larger study estimating the diversity of eukaryotic sea-ice organisms, and is the first time the diversity of parasitic protists and fungi has been studied in the winter-time Baltic Sea. We sequenced the small-subunit ribosomal RNA gene clone libraries from environmental samples of sea ice and winter-time water, and constructed phylogenetic trees using Maximum-likelihood and Bayesian analyses. We found both Syndiniales group I and II parasites from both sea ice and water. As previously postulated, the presence of Syndiniales sequences in clone libraries indicates active production of dinospores. Therefore, it is likely that parasitic Syndiniales are part of the wintertime water and sea-ice food web in the Baltic Sea. We also found a number of sequences of unicellular asco- and basidomycetous yeasts and Chytridiales, which are mainly parasitic, suggesting that the role of fungi in the wintery Baltic Sea may be more parasitic than saprophytic.

Persistent structures of trypanosomatid cryptogene editing domains

Alexander A. Kolesnikov and Evgeni S. Gerasimov

Trypanosomatid cryptogenes are known to be edited at different extent. For example, *Trypanosoma brucei* has 9 pan-edited and 3 partially edited cryptogenes while *Leishmania tarentolae* has 6 pan-edited 6 partially edited cryptogenes. In comparison with *T. brucei* three pan-edited cryptogenes of *L. tarentolae* became 5' edited. We obtained and analyzed various sequence data (mostly from previously unstudied homoxenous species) to show that the reduction of editing domain length is common among trypanosomatids. Besides we found that all known trypanosomatid cryptogene structures can be classified into few groups according to the location of gRNA genes and involvement of the cryptogene's mRNA in the process of alternative editing. In fact there are two major stabile evolutionary cases that can be described as: pan-edited cryptogene while it's product is alternatively edited and a gene with no editing when alternative product is redundant. Also we can see that some cryptogenes have short editing domain and do not lose it. Taking into account the retroposition model of editing domain reduction we suppose that such cryptogenes preserve short 5' domain due to maxicircle encoded gRNAs which can't be lost during cell division.

Phylogenetic analyses of *Discomorphella* sp. n. show that odontostomatids are polyphyletic

Bárbara do Nascimento Borges, Wallax Augusto Silva Ferreira, Maria Lúcia Harada, Inácio Domingos da Silva Neto and Thiago da Silva Paiva

The order Odontostomatida Sawaya, 1940, is one of the many ciliates groups with few information about

its phylogenetic position. Until now, only one study was able to assess the phylogeny of one odontostomatid representative, resulting in the unambiguously group of *Epalxella antiquorum* 18S rDNA sequence within the class Plagiopylea. Recently, we found a new species of the rare ciliate *Discomorphella* in a sapropelic artificial pond located at Rio de Janeiro, Brazil. Samples of *Discomorphella* sp.n. were isolated and used in morphological (protargol and *in vivo* observations) and in molecular analyses (DNA extraction and 18S rDNA sequencing). To assess the phylogenetic position of *Discomorphella* sp. n., the obtained sequence was aligned in a dataset composed of 195 sequences of ciliates and analyzed using Bayesian Inference, Maximum Likelihood and Neighbor-Joining methods. Our results consistently group *Discomorphella* sp. n. as a sister group of Metopidae + Nyctotheridae, and in a distant branch from *Epalxella antiquorum*. The close relationship between *Discomorphella* and armophorids is in agreement with old literature. Based on our data, we can suggest the polyphyly of odontostomatids. However, the molecular analyses of a higher number of representatives are needed to clarify this relationship.

Phylogeny and domain configuration of fatty acid synthases and polyketide synthase-like proteins in protists

Aika Yamaguchi, Maiko Tamura and Holger Jenke-Kodama

Fatty acids are essential components of all organisms. They are biosynthesized by fatty acid synthases (FASs), which use five enzymatic activities in an iterative manner: ketoacyl synthase (KS), acyl transferase (AT), keto reductase (KR), dehydratase (DH) and enoyl reductase (ER). The configurations of these activities are different among organisms. Type II FAS systems (FAS-II) have the activities located on distinct proteins and are typical for prokaryotes. Type I FAS systems (FAS-I) consist of long protein chains that comprise several of the enzymatic activities. They are characteristic for both animals and fungi but differ in their domain configuration. Polyketide synthases (PKSs) represent a similar enzyme system, which produce diverse secondary metabolites, while catalyzing the same reactions as FASs. The distribution of these enzyme systems in bacteria, plants, fungi and animals shows a clear pattern, but the situation among protists is less understood. We analyzed the distribution and domain configurations of FAS types and PKS-like proteins and reconstructed their phylogenies based on the KS domains. Our results show that FASs and PKS-like proteins in protists went through a long common evolutionary history and have unusual domain configurations. Distribution patterns seem to be more irregular than in bacteria and higher eukaryotes.

Phylogenetic Composition and Distribution of Picoeukaryotes in the Hypoxic Northwestern Coast of the Gulf of Mexico

Emma Rocke, Hongmei Jing, Takafumi Kataoka, Liangliang Kong and Hongbin Liu

In our study, the community structure and phylogeny of picoeukaryotes in the Gulf of Mexico, which are exposed to severe hypoxia in these areas was explored through a clone library approach. Both oxic surface waters and sub-oxic bottom waters were collected from three representative stations, located on the inner Louisiana shelf near the Atchafalaya and Mississippi River Plumes. A phylogenetic analysis of a total 175 sequences, generated from six 18S rDNA clone libraries demonstrated a clear dominance of parasitic dinoflagellates from MALV clades I & II in the sub-pynocline layer in the first two stations and in both layers at the more western station closest to the Atchafalaya River plume. The fact that species diversity was significantly higher at the most hypoxic sites and many novel species were present amongst the dinoflagellate and Stramenopile clades, suggests that picoeukaryotes could react to hypoxic stress by creating a unique biological species buffer. This study concluded that hypoxia in the Gulf of Mexico causes a significant shift in picoeukaryote communities, and that their metabolic functions and effects on the microbial food web and biogeochemical cycle need to be further investigated.

Phylogenetic relationships in Pyrenomonadaceae (Cryptophyta) inferred from nuclear and nucleomorph SSU rDNA and chloroplast rbcl genes

Iina Remonen, Markus Majaneva, Janne-Markus Rintala and Jaanika Blomster

The aim of this study was to examine the phylogenetic relationships in Pyrenomonadaceae (Cryptophyta). We sequenced the SSU ribosomal genes (both nucleus and nucleomorph), and the rbcl gene from 42 cryptophyte strains assigned to Pyrenomonadaceae, and constructed phylogenetic trees. All Pyrenomonadaceae and some Geminigeraceae and Chroomonadaceae sequences available in Genbank were included in the analyses. Our study confirmed that Rhodomonas is not monophyletic, as previously suspected. In the consensus tree of all three genes Rhodomonas was paraphyletic, but in two gene trees (nuclear SSU and rbcl) polyphyletic. We also show that the freshwater strain CPCC344 (*Rhodomonas minuta*) actually belongs to Geminigeraceae. In addition, *Rhodomonas marina, R. Salina* and *R. Baltica* strains used in this study were not monophyletic. Our study did not confirm previously suspected dimorphism in Rhinomonas and Storeatula, instead it indicated that Pyrenomonadaceae might be polymorphic: three morphologically different genera appeared mixed up in the phylogenetic trees. This study will continue with morphological analyses, so far only performed to a novel species *Rhodomonas nottbecki* sp. Nov., isolated from the Baltic Sea. Our study shows that the family Pyrenomonadaceae requires further examination with electron microscopy and sequencing prior to the revision of the family.

Phylogenomics reveals deep relationships of Rhizaria

Roberto Sierra, Mikhail Matz, Galina Aglyamova, Ioôc Pillet, Johan Decelle, Fabrice Not, Colomban de Vargas and Jan Pawlowski

Rhizaria is one of the six supergroups of eukaryotes comprising the majority of amoeboid and skeleton-

building protists. The deep phylogeny of rhizarians is unresolved mainly due to its overall lack of molecular data. The clade of Retaria includes two of the most important groups of microfossils: Foraminifera and Radiolaria, for which molecular data are particularly scarce. To bridge this gap, we have produced and sequenced EST libraries for 14 rhizarian species including seven foraminiferans, Gromia and six taxa belonging to traditional Haeckel's Radiolaria: Acantharea, Polycystinea, and Phaeodarea. A matrix was constructed for phylogenetic analysis based on 109 genes and 56 species, of which 22 are rhizarians. The phylogenomic data set presented here constitutes the largest and most complete available for Rhizaria to date. Our analyses confirm the hypothesis of polyphyly of Haeckel's Radiolaria providing the first multigene evidence for the branching of Phaeodarea within Cercozoa. We also confirm the monophyly of Retaria, a clade grouping Foraminifera with other lineages of Radiolaria.

Properties of the unique insert in the ribosomal stalk protein, phosphoprotein P0, shared by members of the Ciliophora

G. Pagano, R. King, L.M. Martin, J. Schumacher and L.A. Hufnagel

Our work with the *Tetrahymena thermophila* predicted ortholog of the 60S ribosomal protein, phosphoprotein P0, has revealed an insert exclusive to members of the protist phylum, Ciliophora. Using several bioinformatics methods, including sequence alignments, homology modeling and motif prediction tools, we have begun to characterize the insert in *T. thermophila* and other ciliates. The ciliate insert is between 15 and 17 residues long; with the exception of *Paramecium tetraurelia*, the nine species studied share a motif within the insert, with the consensus sequence (D/E)XX(Y/F)(D/E). Within this sequence, there is a predicted casein kinase II phosphorylation site in *T. thermophila* and some species of Euplotes, with the consensus sequence (S/T)XX(D/E). Homology modeling of the *T. thermophila* P0 (TtP0) shows that the insert has properties of a highly flexible loop on the surface of the folded P0, with potential ribosomal protein or rRNA contacts. We also report that this insert is absent in several apicomplexan species, so we can suggest that the insert represents an evolutionarily recent function unique to ciliates. We hypothesize that the insert may be used to distinguish ciliates from other protist lineages.

Protein import into mitosomes of *Giardia intestinalis*.

Eva Martincová, Ivana Fixová, Vojtěch Žárský, Jan Tachezy, Trevor Lithgow and Pavel Doležal

Due to the anaerobic lifestyle *Giardia intestinalis* reduced its mitochondrion to the mitosome – organelle which lost entire mitochondrial genome, ATP production and membrane potential. It contains about 20 different proteins, but the import pathway into mitosomes remains elusive. Giardia contains only bare bones of the mitochondrial protein import apparatus. While Tom40 channel is present in the outer membrane, Tim23 tranlocase in the inner membrane has not been identified. In classical mitochondria, the PAM complex and the membrane potential propels the transport through Tim23 channel. Given that the PAM complex is present in the inner mitosomal membrane, Giardia might employ unique translocase which cooperates with the PAM complex without the contribution of the membrane potential. Searching for the mitosomal proteins in Giardia, we turned our attention to Sec61 α - the endoplasmic reticulum translocase. In Giardia, unique N-terminal extension of Sec61 α is targeting chimeric proteins into mitosomes and yeast mitochondria. Single sec61 α gene is transcribed into two mRNAs – short one forming ER channel and long one potentially forming mitosomal inner membrane channel. Our research is focused on the identification of the mitosomal inner membrane translocase and on the possible role of Sec61 α in the mitosomal protein import.

Protist phylogeography: Effects of climate and geographic distance on the genetic diversity of the testate amoeba *Hyalosphenia papilio* (Amoebozoa: Arcellinida)

Thierry J Heger, Edward AD Mitchell and Brian S Leander

Microbial eukaryotes are essential for the functioning of soils and semi-aquatic habitats. In particular, they play fundamental roles in nutrient cycling and food web processes. However, little is known about their biodiversity and phylogeography. Here, we used partial sequences of the mitochondrial Cytochrome c Oxidase Subunit 1 (COI) gene to investigate the genetic diversity and phylogeography of the morphospecies *Hyalosphenia papilio* in 42 North American, European and Asian Sphagnum (moss) dominated peatbogs. Our results based on a single-cell PCR approach demonstrated an important genetic diversity within *H. papilio* morphospecies. From 301 sequenced individuals, 49 distinct haplotypes were found and 12 *H. papilio* genetic lineages were identified. Our data also showed a high degree of heterogeneity in genetic structure among different regions. We used variance partitioning based on partial redundancy analyses to evaluate the contribution of climate and geography on *H. papilio* genetic lineage distribution patterns. The largest fraction of the variance in genetic lineage distribution was attributed to purely bioclimatic factors, followed by the joint effect of spatial and bioclimatic factors and the purely spatial effect. Thus, these data suggest that *H. papilio* genetic lineage distribution patterns are more influenced by bioclimatic conditions than by dispersal rate.

PUF proteins in the biology of *Giardia intestinalis*

Vladimíra Najdrová and Pavel Doležal

Giardia intestinalis is an anaerobic protozoan pathogen, the surface of which is covered by variant surface proteins (VSP), which play an important role in the host immune response. VSP family contains about 200 proteins in a range from 20 to 200kda. There is only one type of VSP found on the surface of the cell but the mechanism of the antigen switching is still unknown. There are two different posttranscriptional mechanisms proposed. The first explains the antigenic variation by the RNA

interference, which specifically destroys all but one VSP mRNA, while the second by relies on the miRNA-mediated translation repression. In this project, we have decided to characterize the role of 3' untranslated region (UTR) of VSP mRNAs as well as the possible involvement of 3' UTR binding proteins called pumilio-Fem-binding Factor (Pufs). We have identified five representatives of PUF family in *G.intestinalis* genome and currently we endeavor to characterize their function in the biology and pathology of this important human parasite.

Purification and Characterization of Idfrataxin and Idisd11 proteins involved in Iron Sulfur Clusters (ISC) machinery of *L. donovani*

Amir Zaidi, Krishn Pratap Singh, Pradeep Das and Vahab Ali

Fe-S clusters are part of the active site of many enzymes and are essential for cell viability and its biosynthetic machinery is a fundamental component of cells. The Fe-S cluster assembled by three distinct machineries, viz NIF, ISC & SUF system. Leishmania genome databases search showed the presence of Frataxin and Isd11 homologue, components involved in Iron Sulfur Cluster (ISC) assembly in mitochondria. The frataxin and Isd11 genes were amplified from *L.donovani promastigotes* (Ag83 strains). Frataxin and Isd11 gene was cloned in pgex-4T1 vector containing the GST-tag at N terminal and respectively in pet-28a vector which possess N-terminal His-Tag. The Recombinant fusion proteins were expressed and purified using affinity chromatography. Protein-protein interaction between Idfrataxin and Idiscu (scaffold protein) which was identified by affinity chromatography followed by western blot analysis. These result shows stable complex formation of Idiscu & Idfrataxin protein involved in Fe-S cluster formation. The enzymatic activities of Fe-S proteins are found to be up regulated during drug resistance of the Leishmania parasites. The Isd11 protein enhances cysteine desulfurase (Idiscs) activity in Iron Sulfur Cluster assembly machinery of *L.donovani*. This protein also interact to stabilize Idiscs protein, will be discussed in the meeting.

Quantification and influencing factors of the protozoic Si pool in forested mature ecosystems

Daniel Puppe, Otto Ehrmann, Michael Sommer and Manfred Wanner

Biogenic silicon (Si) pools can be separated into phytogenic, microbial, and protozoic pools. While many researchers focus on phytogenic pools quantitative knowledge about protozoic Si pools represented by idiosomic testate amoebae (TA) using synthesized silicon platelets for shell construction is rare. For detailed analyses on protozoic Si pool size in soils and its driving factors 11 deciduous and coniferous forested mature ecosystems were sampled. We quantified amoeba communities in the upper 5 cm of soil directly using stained aqueous soil suspensions differentiating between xeno- and idiosomic taxa as well as living and dead TA. Soil samples were analyzed on abiotic factors comprising pH (ranging from 2.9 – 5.1), C:N (17 – 40), labile (60 – 960 mg m^{-2} 5 cm^{-1}), and pedogenic (20 – 220 g m^{-2} 5 cm^{-1}) Si fractions. Additionally, earthworm biomasses as a biotic factor of influence were determined. Our results of lab analyses up to now confirm the chosen sites to represent a broad range in earthworm biomasses and soil chemical properties, especially Si fractions. Here we would like to present our results on hand including calculated annual biosilicification (about 1.5 - 20 kg Si ha^{-1} 5 cm^{-1}) by living idiosomic TA and driving factors analyses.

Quantitative comparison and analysis on the differentially expressed genes of resting cyst and vegetative cell from *Pseudourostyla cristata*

Ji-wu Chen, Li-na Zheng, Bang-zheng Wang and Qiu-xia Gao

Ciliates resting is their favorite strategy of resisting adversity. The molecular mechanisms underlying the resting phenomenon are not quite clear. So based on previous experiments of suppression subtractive hybridization, this study continually used the resting cyst and the vegetative cell of *Pseudourostyla cristata* as experimental materials and investigated differentially expressed genes in the encystment processes by Real-time fluorescence quantitative-PCR. The products of quantitative-PCR was sequenced and the sequencing results were retrieved and compared using BLASTn and BLASTx . The results showed one gene down-regulation and fourteen genes up-regulation. Comparing with vegetative cell, the up-regulating genes in resting cyst include genes of calmodulin, Actin I, Actin binding protein, α-tubulin 3, HSP70, cathepsin B, etc., in witch calmodulin, actin I, actin binding protein, α-tubulin 3 relate with cellular structures, HSP70 relates with stress, phosphatidylinositol-4-phosphate 5-kinase, PAP2 superfamily phosphatase relate with signaling transduction, cathepsin B, glycosyl hydrolases relate with proteolysis. Therefore, relationship between these genes up-regulation and resting encystment of *Pseudourostyla cristata* was discussed and analysed. This study explored regulating mechanism of encystment of ciliates under adversity in molecular level. It helps insighting into metabolic activity and regulating mechanism of cell under adversity. (This project is supported by the National Natural Science Funds of China (No. 31071875)

Radiolaria: a reservoir for marine alveolates.

Anders K. Krabberød, Jon Bråte, Jane K. Dolven, Randi F. Ose, Tom A. Kristensen, Kjell R. Bjørklund, and Kamran Shalchian-Tabrizi

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. The poster 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.

Redescription of a poorly known *Metopus setosus* Kahl, 1927 (Armophorea, Armophorida, Metophidae) from Korea

Choon Bong Kwon and Mann Kyoon Shin

To clarify the definition of a poorly-known anaerobic ciliate, *Metopus setosus* Kahl, 1927 found in freshwater, Jeju Island, Korea, the morphology of it are examined and described using live observations and protargol silver impregnation. Based on the present studies, a new diagnosis is suggested: size is 55-85 ◊ 22-32 μm in vivo; preoral dome is more or less thick and very conspicuous. Postoral body portion gradually narrowed and rounded ends; irregular kidney shaped macronucleus with one micronucleus; cortical granules present; longitudinal somatic kineties is composed of 19-29 rows; caudal cirri present; Perizonal stripe is composed of five kineties; 15-27 adoral membranelles occupying about 60% of body length; Undulating membranes is consist of endoral and paroral membranes. The Korean population of *Metopus setosus* is well agree with original description in body size and shape, presence of caudal cirri, the number of somatic kineties and adoral membranes. The distance between perizonal kineties, arrangement of kinetids in the 5th perizonal kinety, and presence of endoral membrane are newly recognized in characterizing this species.

Seasonal dynamics of harmful algae in the outer Oslofjord monitored using microarrays, qPCR, and microscopy.

Vladyslava Hostyeva, Simon M. Dittami, Viljar Alain Skylstad, Wenche Eikrem and Bente Edvardsen

Monitoring of marine microalgae is important to predict and manage harmful algal blooms. MIDTAL (microarray Detection of Toxic algae) is an FP7-funded EU project aiming to establish a multi-species microarray as a tool to aid monitoring agencies. Here, we tested the suitability of a prototype version of this microarray for seasonal monitoring in outer Oslofjorden with samples collected monthly during a two-year period. Selected, potentially toxic species detected by microarray were compared to qualitative data generated by electron microscopy (e.g. *Pseudo-nitzschia* spp.), as well as light microscopical cell counts (e.g. *Dinophysis* spp.) And qPCR for *Pseudochattonella* spp. Cell counts generally correlated well with data from the microarray, but in several cases the latter was able to detect species that were otherwise only found using concentrated net hauls (e.g. *Alexandrium* spp.). Identification of phytoplankton to the species level using microarray was possible in several cases, but for some genera, most importantly Pseudo-nitzschia, additional work will be required before the assay is species specific. Overall our data demonstrate the potential of this new monitoring tool.

Seasonal succession of eukaryotic community detected by environmental sequences in Tokyo Bay, Japan

Akiko Yokoyama, Jun Kikuchi, Yuri Tsuboi, Shigeharu Moriya, Yuji Inagaki, Tetsuo Hashimoto and Isao Inouye

To elucidate the production mechanisms of the organic matter in aquatic ecosystem, the exact knowledge of the plankton biomass is required. Quantitative estimation by counting the numbers of organisms is the most common and effective method, but some alga and protists such as picoplanktons or heterotrophic organisms are hardly identified under the light microscopes caused by their size or the fragileness. Recently, the environmental sequence analyses have been applied because of its powerful performance for uncovering biodiversity of microbes. In this study, we monthly collected the coastal surface and middle seawater in Tokyo bay, Japan, from the late spring to the early winter in 2010, and determined 18S rDNA sequences via the PCR amplification using two universal primer sets and subcloning. At present, 2339 reads of eukaryotic sequences were obtained, and over 485 phylotypes were recognized. Consequently, we obtained the basic information concerning the monthly eukaryotic composition and the seasonal succession of each organism including the heterotrophic or the parasitic ones in this area. Furthermore, we found the sequences corresponding to picobilliphytes and many unclassified eukaryotes.

Seasonal variation in the picoeukaryote *Micromonas pusilla* in an arctic fjord, Svalbard, as revealed by quantitative PCR.

Lene Christensen, Wenche Eikrem, Anna Vade and Tove M. Gabrielsen

The seasonal variation of the picoeukaryote *Micromonas pusilla* was investigated in Billefjorden, Svalbard by real time quantitative Polymerase Chain Reaction (qPCR). Billefjorden is an arctic sill fjord with annually forming fast ice dominated by cold, locally produced water and limited influenced by the warmer and more saline water of the Isfjorden system. CTD profiles and water samples from four depths (5m, 15m, 35m, 150m) for qPCR, fractionated Chla biomass and nutrient analyses were collected monthly from April to August 2011. The Billefjorden pelagic system in 2011 was characterized by a massive advection of warmer water from outside the sill in early May, and a Phaeosystis-dominated bloom that peaked in mid-May. *Micromonas pusilla* was found to be an important picoeukaryote in the post bloom situation in June and July, with cell

numbers exceeding 6.5 x 105 cells ml-1. During the Phaeocystis-dominated bloom in mid-May, the cell numbers were much lower and *M. pusilla* contributed minimally to the phytoplankton. Although *M. pusilla* is known as an important picoplanktonic primary producer in arctic waters, it only accounted for a limited amount of the picoplanktonic fraction of the Chl a biomass, suggesting that other picophytoplankton species also play important roles in the Billefjorden ecosystem.

Seasonality of marine parasites of Syndiniales in the Isfjord system, Svalbard

Stuart Thomson and Tove Gabrielsen

Syndiniales (Alveolata) are commonly found in marine waters, including the arctic ecosystem. The importance of this group of marine parasites in arctic waters, particularly of the Group II lineage, is investigated in Isfjorden, western Spitsbergen. The abundance and temporal variability of distinct lineages within Syndiniales will be investigating using PCR and qPCR with group specific primers. Sea water samples at 4 depths were collected weekly from December 2011 to June 2012, and protists > 20 μm were additionally collected using phytoplankton net hauls and fixed in 1% acidic lugol. The sea water samples were filtered through a 10μm nylon mesh and collected on a 0.45μm filter for DNA extraction. The presence of Syndiniales lineages will be tested from the water samples and from capillary extraction of single protist cells, and the abundance of distinct lineages within Syndiniales will be investigated by qPCR on the sea water samples. We expect that our investigations will elucidate how the abundance of Syndiniales lineages changes in the winter to spring transition, and which protists are potential hosts in the arctic ecosystem. This information will provide valuable first steps to understanding the role of parasitism in the arctic microbial food web.

Simple repeat polymorphisms are not simply induced by DNA repair

John A Burns, Moinuddin Chowdhury, and David A Scicchitano

All living organisms have pathways in place to resist changes to their genome. DNA repair mechanisms do a superb job of maintaining the genome for the life of a cell while conceding sufficient variation within populations to permit change through evolution. How particular forms of genetic variation arise is a puzzle, however. Certain sequence repeats exhibit length polymorphism, and have a propensity to fold into non-B-DNA structures such as DNA stem loops. Looped out DNA, independent of any chemical modification to the DNA, could be recognized by the DNA repair machinery and altered. Frequent alteration of undamaged DNA, solely based on sequence, would increase the probability of mutation. The work presented here shows that, while DNA stem loops do interfere with cellular processes such as transcription, they are not of themselves subject to DNA repair. This suggests that without chemical modification, DNA is protected against promiscuous repair. It is interesting to wonder, however, what might happen if a DNA stem loop contained a chemically modified base. This could correctly lead to repair of the lesion, but due to the mis-folded DNA could also lead to DNA length polymorphism by removal or repetition of the stem loop sequence.

Strombidium paracalkinsi (Ciliophora: Oligotrichea: Oligorichida) Revised: Living Morphology, Infraciliature, and Small Subunit Ribosomal DNA

Eun Sun Lee, Dapeng Xu, Sun Young Kim and Young-Ok Kim

Thigmotactic membranelles (TMs) in oligotrich ciliates were considered to be a convergently evolved character to adapt benthic life and thus could not be used as a character to separate different genera, which leads to the transferring of Thigmostrombidium and Heterostrombidium into Strombidium. TMs-bearing species have been recorded among different oligotrich taxa, e.g. Strombidium, Spirostrombidium, Omegastrombidium, and Parallelostrombidium. *Strombidium paracalkinsi* which has three TMs on its dorsal side were discovered from Korean coastal waters recently and redescribed based on its living morphology, infraciliature and small subunit ribosomal DNA sequence. The Korean population showed some unique characters, i.e. its three TMs were composed of two rows of kinetosomes, respectively and clearly separated from the anterior membranelles (AMs) in protatgol impregnated specimens which makes it different from the other TMs-bearing species. In a dividing cell, the TMs looks like the natural extension of AMs in early-middle stage and the separation of TMs from AMs might happen in late stage of morphogenesis. Furthermore, we sequenced the SSU rDNA of *S. paracalkinsi* for the first time and in our phylogenetic tree, it grouped out of Strombidium clade which might indicate the potential separation with its congeners from genus Strombidium. Based on the morphological, morphogenesis and DNA sequence collected, we propose the necessity of re-establishment of the genus Thigmostrombidium.

Studies on the morphogenesis and phylogeny of various Hypotrichous (s. L.) Ciliates

Chen Shao, Weibo Song and Alan Warren

In the past several years, investigations on the morphogenesis of various ciliates have been performed. These have revealed much new data with implications for the phylogenetic relationships of a range of taxa which can be sumarised as follows. (1) Kiitricha marina, which is characterized by a unique combination of morphogenetic features, and probably represents an intermediate taxon between the heterotrichs (s. L.) And the Stichotrichia-Hypotrichia-complex and could be similar to the ancestral form of the latter. (2) Morphogenesis in the discocephaline *Prodiscocephalus borrori* has features that are typical both of hypotrichs (s. Str.) And euplotids. The balance of available evidence suggests that the discocephalines are probably more closely related to the former than the latter. (3) A non-oxytrichid pattern of morphogenesis was revealed in *Trachelostyla pediculiformis*. Cladistic analysis based on morphogenetic data suggests that

the family Trachelostylidae, represented by the genus Trachelostyla, is sister to the family Oxytrichidae. (4) Apokeronopsis is an intermediate form between Pseudokeronospis and Thigmokeronopsis as it has morphogenetic features common to both. (5) The pattern of morphogenesis in *Diophryopsis hystrix* is generally similar to that of other Diophrys-like species, with the exception of the marginal cirrus. The ontogenetic and ssRNA gene sequence data both support the conclusion that; (i) Diophryopsis represents a distinct clade separate from Diophrys, and; (ii) the Diophrys-complex is sister to Uronychia and should be considered as a distinct subfamily within the family Uronychiidae, i.e. Diophryinae Jankowski, 1979, comprising Diophrys, Diophryopsis, and Paradiophrys. Funded by the National Natural Science Foundation of China (Project 31172041; C. Shao).

Study on Subpellicular Ultrastructures of Six Ciliate Species

Zijian Qiu, Ying Chen, Jing Gao, Na Li, Wenwei Liang and Wenqiao Ding

In order to fully expose the inner surface of ciliate membrane, a new homemade method was used to repair samples for scanning electron microscopy (SEM). The SEM images of subpellicular structures were obtained in *Paramecium caudatum*, *Paramecium bursaria, Paraurostyla weissei, Urostyla* sp.,Dileptus sp. and *Frontonia* sp.. Subpellicular fiber system, inner surface of cytostome and cytopharynx, extrusomes and cortical granules were studied by SEM and transmission electron microscopy (TEM). Three types of trichocyst, one type of toxicyst and two types of mucocyst were observed and redescribed in *Paramecium caudatum, Paramecium bursaria*, *Dileptus* sp. and *Frontonia* sp.. Two new types of extrusome were found in *urostyla* sp. and *Dileptus* sp.We focused on the fiber systems that lying under the kinetosome of body area and in the area of cytostome and cytopharynx. Clear and complete SEM images of them were obtained and some special structures were found in *Paraurostyla weissei* and *urostyla* sp. We also illustrated other structures under the membrane including pellicular alveolus, pellicular pore, cortical granules similaring to red blood cell and other bubble-like structures, and studied their spatial location.

This work was supported by the National Science Foundation of China (No.30970311)

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Study on Trichodinidae phylogeny inferred from 18S ribosomal DNA, ITS and 5.8S rDNA sequences

Fahui Tang and Yuanjun Zhao

Based on 18S ribosomal DNA, ITS and 5.8S rDNA sequences, phylogeny analyses of Trichodinidae family were carried out in the present work. After sequencing 10 newly trichodinid species, we constructed phylogenetic ML and Bayesian trees in order to reveal the phylogeny of Trichodinidae family, the conclusion are presented below: 1) In the Mobilia ciliates phylogeny, the GC content of 18S rDNA plays an vital role and it can be concluded that the lower the GC content was, the earlier the species may differentiate. 2) Another important factor affecting the structure of trees is the denticle morphology especially the blade morphology which is involved with the phenomena of blade morphology dominance. 3) The phylogeny analyses suggests the genus Trichodina paraphyletic and the validity of the genus Trichodinella should be re-evaluated. 4) The present research has approved the central granules in the adhesive disc invalid as a generic character for taxonomy. 5) According to the phylogeny analysis on the basis of ITS and 5.8S rDNA sequences, especially the 5.8S rRNA gene, the result shows that the 5.8S rRNA gene represents highly conservative in the Trichodina species, which supports the research result that applying 5.8S rRNA gene as the conservative sequence for phylogeny.

Symbiosis between a euglenid and verrucomicrobial bacteria with extrusive structures. Extrusomes in the making?

Breglia S, Yubuki N, Leander B and Slamovits C.

Microeukaryotes in low oxygen environments are often enveloped with bacteria, but the diversity and function of these relationships are poorly understood. *Bihospites bacati* is an uncultivated euglenozoan flagellate recently described by us that was collected from a low oxygen zone in marine sand. The surface of *B. bacati* is covered by rod-shaped bacteria and spherical extracellular bodies. Transmission electron microscopy shows that the spherical bodies contain threads that are tightly wound around a core, and that are capable of rapid discharge through an apical pore. Comparative ultrastructure and 16S rRNA demonstrated that the spherical bodies are symbiotic verrucomicrobial bacteria that potentially extend their lifecycle through evasive evisceration. Highly similar verrucomicrobial episymbionts (i.e. “Epixenosomes”) Had been previously described in species of hypotrich ciliates. The features of these bacteria provide context for inferring lateral transfer of symbionts, convergent evolution over vast phylogenetic distances, and the origins of extrusive structures across the tree of eukaryotes.

Targeting into *Trichomonas vaginalis* hydrogenosomes is evolving towards a mechanism independent of cleavable N-terminal targeting sequences

Verena Zimorski, Peter Major, Kathrin Hoffmann, Xavier Pereira Brás, William F. Martin and Sven B. Gould

The anaerobic protist *Trichomonas vaginalis* harbors mitochondria-related organelles, the hydrogenosomes, which produce ATP via substrate level phosphorylation. In contrast to mitochondria they possess neither a genome nor a translation machinery and hence import an estimated amount of approximately 500 proteins from the cytosol. Little is known about the targeting and transport of proteins across the two hydrogenosomal membranes, but recent studies suggest internal targeting signals in Trichomonas hydrogenosomal proteins to be more common than in yeast. To further investigate the extent to which internal motifs play a role in hydrogenosomal protein targeting, we transfected the parasite with 24 individual hemagglutinin-fusion constructs, originating from 6 different proteins. Their

targeting properties were analyzed by subcellular fractionation and immunoblotting and include some of the most abundant hydrogenosomal proteins, fragments thereof, and an N-terminal targeting motif equipped murine dihydrofolate reductase in the absence and presence of its competitive inhibitor pyrimethamine. Our results show that hydrogenosomal targeting in *Trichomonas vaginalis* is evolving towards an N-terminal independent mechanism and the recognition of internal targeting modules within the mature, only partly folded, part of the protein.

The Acetabularia chloroplast genome

Jörn Habicht, Christian Wöhle, Gregor Christa, Katharina Händeler, William F. Martin and Sven B. Gould

The chlorophyte alga *Acetabularia acetabulum*, also known as the "Mermaid's Wineglass", is a member of the Ulvophyceae. The single-celled algae can reach a size of up to 6-8 cm and display a complex life cycle and morphology, which is reminiscent of higher plants. They are also the sole food source of our lab-grown sacoglossan sea slug *Elysia timida*, which sequesters only the plastids from their prey. The isolated plastids of *Acetabularia* then maintain their photosynthetic ability within the slugs for several months, despite their "nuclear mother genome" being absent. To elucidate their detailed phylogenetic position, and whether plastid longevity can maybe – at least in parts – be explained by the coding capacity of the chloroplast, we set out to sequence the genome. Previous work suggests the plastid genome to be at least 400 kbp of size and maybe contain repeats of unknown sequence. We enriched cpDNA combining Percoll density and CsCl gradients, and isolated DNA was screened for potential contaminations using a set of marker genes. Through GSL FX+ sequencing of the enriched cpDNA we have obtained 138285 kb of sequence information, commenced assembly and will present our latest results.

The community of ciliates forming on different substrates.

Ivan Mukhin

The formation of communities of periphyton ciliates on different types of substrates was investigated. It is shown that the species composition of ciliates which inhabit on the surface of natural and anthropogenic substrates is differed. At the same time the ciliates have similar structure of community on different types of substrates. Comparison of species diversity according to Shannon's index did not reveal any significant differences between emerging communities on different types of surfaces. We have identified three basic spatial niches that the ciliates depending on their morphological features can colonize. Since each type of substrate is characterized by formation of self-sufficient community, not similar structure to others, we proposed improve the standard method of bioindication by introducing the several types of substrates for the formation of periphyton ciliates. Our results confirmed that the ciliates may be adequate indicators of the quality of water in a natural waterbodies and in the laboratory experiment. The effectiveness of the using of community structure of ciliates for the monitoring of water's quality is discussed.

The complete mitochondrial genome sequence of the eustigmatophyte alga *Trachydiscus minutus*

Veronika Zbrankova, Jan Fousek, Cestmir Vlcek and Marek Elias

Eustigmatophytes are an interesting class of ochrophyte (stramenopile) algae with a high biotechnological potential yet with a poorly known biology and evolution. We sequenced and annotated the mitochondrial genome of *Trachydiscus minutus* representing a recently described early-branching group of eustigmatophytes, and annotated the mitochondrial genome of a distantly related eustigmatophyte *Nannochloropsis oceanica* sequenced but ignored by another team. A comparative analysis of the eustigmatophyte mitochondrial genomes revealed several notable features. First, they have kept a nearly identical gene arrangement, whereas the gene order in other ochrophyte classes appears less evolutionarily stable. Second, all genes, except tatc, are on the same strand, whereas all other stramenopiles exhibit several blocks of genes on both strands. Third, eustigmatophytes are the first ochrophyte class identified to have retained the apt1 gene in the mitochondrial genome. Fourth, a novel protein-coding gene lacking discernible homologs outside eustigmatophytes is conserved in their mitochondrial genomes. Fifth, uniquely among the taxa compared, the eustigmatophyte gene rps3 harbours a putative intron. Finally, eustigmatophytes exhibit a split form of the nad11 gene with the part encoding the C-terminal region of the Nad11 protein retained in the mitochondrial genome and the part encoding the N-terminal region apparently relocated to the nuclear genome.

The differentiation of cortical ciliature microtubules of *Oxytricha platystoma* under different physiological conditions

Bing Ni, Jian Guo, Ji-wu Chen and Fu-kang Gu

Abstract: The cortical ciliature microtubules of one hypotrichous ciliate named *Oxytricha platystoma* were visualized by confocal laser scanning microscopy. During the process of asexual reproduction, when old ciliature microtubules disintegrated, new adoral zone of membranelles (AZM), undulating membranes (UM), frontal-ventral-transverse cirri(FVTC) and left and right marginal cirri(L-and RMC) were differentiated in order. And one cell divided into one proter and one opisthe, both which had one set of ciliature microtubules. During the process of physiological reorganization, adoral zone of membranelles(AZM), undulating membranes (UM), frontal-ventral-transverse cirri(FVTC) and left and right marginal cirri(L-and RMC) were dedifferentiated first and then redifferentiated. And the cortical microtubular organelles of the cell were renewed to be a new cell with one set of ciliature microtubules. The results show that during the different processes of asexual reproduction and physiological reorganization, the regulation mechanism for forming and renewing ciliature microtubules of *Oxytricha platystoma* is same and the old ciliature might play a role of

inducing location and physical contribution to the new ones during the processes of morphogenesis. Key words: *Oxytricha platystoma*, ciliature microtubules, asexual reproduction, physiological reorganization (This project is supported by the National Natural Science Funds of China (No. 311720429

The diversity in the Vischeria/Eustimatos complex (Eustigmatophyceae): morphological and molecular perspectives

Katerina Prochazkova, Lira A. Gaysina, Martina Pichrtova, Alena Lukesova and Marek Elias

Vischeria spp. and *Eustigmatos* spp. are closely related coccoid algae common in terrestrial habitats. The two genera were distinguished by relatively subtle morphological features (the cell surface raised into projections or ridges, or smooth, respectively). Three species in Eustigmatos were recognised, but their discrimination proved difficult in practise. Twelve species were described in Vischeria, but nine of them have been rarely, if ever, observed since the original description. To reassess the diversity and taxonomy of the Vischeria/Eustigmatos complex, we studied a wide set of strain from public algal collections, including type strains of two Eustigmatos and three Vischeria species, and of strains newly isolated from places distributed all over the globe. Sequencing of the nuclear ITS rDNA region and the plastid rbcL gene showed that: 1) maintaining Vischeria and Eustigmatos as separate genera is not tenable; 2) the five species represented by type strains are indeed genetically distinct from each other; 3) there is a large number of additional lineages of a similar degree of phylogenetic separation, few of which can, however, be identified as some of the remaining Vischeria/Eustigmatos species described previously. Our results thus indicate that the morphological species concept cannot be easily applied in this algal group.

The genus Tetracystis (Chlamydomonadales, Chlorophyceae): another highly polyphyletic taxon of coccoid green algae

Tereza Hasíková, Alena Lukešová, Eva Sýkorová and Marek Eliáš

Brown and Bold (1964) established a new genus Tetracystis to accommodate twelve species (nine newly described and three transferred from the genus Chloroccocum) of coccoid zoospore-producing algae. Nine species were subsequently added by various authors. So far, only the type species, *Tetracystis aeria*, has been studied by molecular taxonomy and shown to belong to the order Chlamydomonadales, leaving the phylogenetic position of the remaining species uncertain. We determined 18S rDNA sequences for 13 additional species and found that Tetracystis is a highly polyphyletic taxon represented by at least seven separate groups distributed in five major primary clades of Chlamydomonadales. However, the delimitation of the separate groups reflects the diversity of some delicate morphological and ultrastructural features that were thus previously not given the proper taxonomic significance. Strikingly, four species (*T. Excentrica, T. Pulchra, T. Texensis,* and *T. Intermedia)* turned out to be nested in a clade of fusiform flagellates recently described as the genus Gungnir. Despite their morphological disparity (coccoid versus flagellate vegetative stage), the relationship of Gungnir to these four Tetracystis species is corroborated by shared ultrastuctural features of the pyrenoid. Analyses of the ITS2 region are underway to aid further assessment of Tetracystis species. Financial support: GACR521/09/1912; GACRP506/10/0705

The plastid genome of Eutreptiella provides a window into the process of secondary endosymbiosis of plastid in Euglenids

Stepanka Hrda, Jan Fousek, Jana Szabova, Vladimir Hampl and Cestmir Vlcek

Euglenids are a group of protists that comprises species with diverse feeding modes. One distinct and diversified clade of euglenids is photoautotrophic, and its members bear green secondary plastids. In this paper we present the plastid genome of the euglenid Eutreptiella, which we assembled from 454 sequencing of Eutreptiella gDNA. Comparison of this genome and the only other available plastid genomes of photosynthetic euglenid, *Euglena gracilis*, revealed that they contain a virtually identical set of 57 protein coding genes, 24 genes fewer than the genome of *Pyramimonas parkeae*, the closest extant algal relative of the euglenid plastid. Searching within the transcriptomes of Euglena and Eutreptiella showed that 6 of the missing genes were transferred to the nucleus of the euglenid host while 18 have been probably lost completely. Euglena and Eutreptiella represent the deepest bifurcation in the photosynthetic clade, and therefore all these gene transfers and losses must have happened before the last common ancestor of all known photosynthetic euglenids. Our results show that the early secondary plastid of euglenids was much more susceptible to gene losses and endosymbiotic gene transfers than the established plastid, which is surprisingly resistant to changes in gene content.

The role of algae in assessing the water quality of Hulan River wetland.

Yawen Fan, Hongkuan Hui and Nnaemeka Emmanuel Okpala

Hulan River wetland is a nature reserve located in the south of Hulan District, Harbin City, Heilongjiang Province, China. Algae play a vital role in water bodies and are widely used in water quality assessment. In order to assess the water quality of Hulan River wetland, algae assemblages were investigated at eight sampling sites in the wetland from May to October 2009 and 2010. A total of 216 algae were found in the study area of the wetland, however, their seasonal composition and abundance changed greatly. The result of the quantitative analysis revealed that the total average cell density of the algae was 2.35x107ind/L. The dominant species in the study area are *Cyclotella meneghiniana, Melosira granulate, Navicula cryptotenella* and *Euglena acus.* Their individual

abundance and environment parameters were examined by canonical correspondence analysis (CCA). They include; water temperature, pH (6.5-7), conductivity (175-509 μS/CM), dissolved oxygen (DO, 4.15-16.38mg/L), total nitrogen (TN, 2.86-4.18mg/L), phosphorus (TP, 0.06-0.14mg/L), chemical oxygen demand (COD,17.60-20.88mg/L) and biological oxygen demand (BOD, 1.93-2.60mg/L). The result revealed that different physical and chemical indicators affected different sites. The CCA showed that DO, WT, TN and TP were important environmental parameters affecting algae community of the wetland. The result of this study suggests that the relationship between algae and environmental parameters can be used to ascertain the water quality and habitat conditions in a wetland.

Ultrastructural and molecular characterization of cyanobacterial endosymbionts in polycystine radiolarians

Osamu Takahashi, Tomoko Yuasa and Takeo Horiguchi

Bacterial endosymbionts have been reported in natural populations of radiolarians (Foster et al. 2006). They revealed that the bacterial symbionts of the radiolarian *Dictyocoryne truncatum* might belong to the cyanobacterial genus Prochlorococcus based on the 16S ribosomal RNA gene sequences and the ultrastructural features. Although a wide range of algae have been reported as endosymbionts of radiolarians, the diversity of cyanobacterial symbionts is still poorly understood. In this study, cyanobacterial endosymbionts were detected in the extracytoplasm of polycystine radiolarian *Dictyocoryne profunda*. The bacterial symbionts were observed as numerous spherical bodies ~0.5–1.0 μm in diameter under transmission electron microscopy. They were found in a very restricted location close to the periphery of the host radiolarian shell adjacent to the central capsular wall, and they were not enclosed within the host vacuoles. The bacterial symbionts had thylakoid-like structures, which ran along the cell periphery in two or three concentric layers. Our sequences of the symbiotic bacteria of *D. profunda* clearly belong within the cyanobacterial radiation. The cluster including the sequences we obtained was composed of the members of the Synechococcus clade II
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Ultrastructure and molecular phylogeny of dinoflagellate symbiont from solitary polycystine radiolarian

Tomoko Yuasa, Takeo Horiguchi and Osamu Takahashi

Polycystines (Radiolaria) generally possess algal symbionts within their bodies. Various types of algae occur as intracellular symbionts in the polycystine radiolarian, including dinoflagellates, prasinophytes, and prymnesiophytes. The most conspicuous and well-studied symbiotic algae of radiolarians are dinoflagellates. However, the nomenclature of generic name of the radiolarian symbiotic dinoflagellates has been very confused. In this study, we report some new findings on molecular phylogeny and fine-structural studies of a symbiotic dinoflagellate in the polycystine radiolarian *Didymocyrtis tetrathalamus*. The dinoflagellate from *D. tetrathalamus* in culture had the cell shape typical of small peridinioid dinoflagellates and the 18S rDNA phylogenetic analysis revealed that the dinoflagellate formed a single clade with radiolarian specific symbiont *Scrippsiella nutricula*. However, this clade was separated from the clade of the Scrippsiella group including the type species (*S. sweeneyae*) and also from other peridinioid genera. Moreover, the thecal plate pattern differs from those of the peridinioid genera.

What can phenotypic plasticity tell us about coccolithophore evolution?

Andrea Gerecht, Bente Edvardsen, Ian Probert and Jorijntje Henderiks

The microalgal group of the coccolithophores (Prymnesiophyceae, Haptophyta) are covered by tiny calcite plates, called coccoliths, which are produced internally in specialized vesicles. Previous studies have demonstrated deleterious effects of decreasing ocean pH on coccolithophore calcification. However, less is known about other environmental parameters likely to change under future climate scenarios such as nutrient availability. Therefore, the effect of phosphorus availability on two species of coccolithophores differing in cell size and evolutionary history, *Emiliania huxleyi* and *Coccolithus pelagicus*, was studied. Cultures were grown as continuous and batch cultures at various phosphorus levels. The phenotype was characterized by polarized light microscopy (cell and coccolith size) and scanning electron microscopy (coccolith morphology). As a rule, phosphorus limitation led to an increase in cell size. Also, malformed coccoliths became more abundant in phosphorus limited compared to nutrient replete cultures. These results are tested against two hypotheses. First, that nutrient availability is a possible driver of cell size evolution, taking into account the different cell sizes in the evolutionary histories of the species as evident in the fossil record. Furthermore, calcification rates and percentage of normally formed coccoliths under nutrient limitation are evaluated as indicators of the adaptability of different species to changing environments.

Social program

Banquet Thursday, August 2 from 19:00

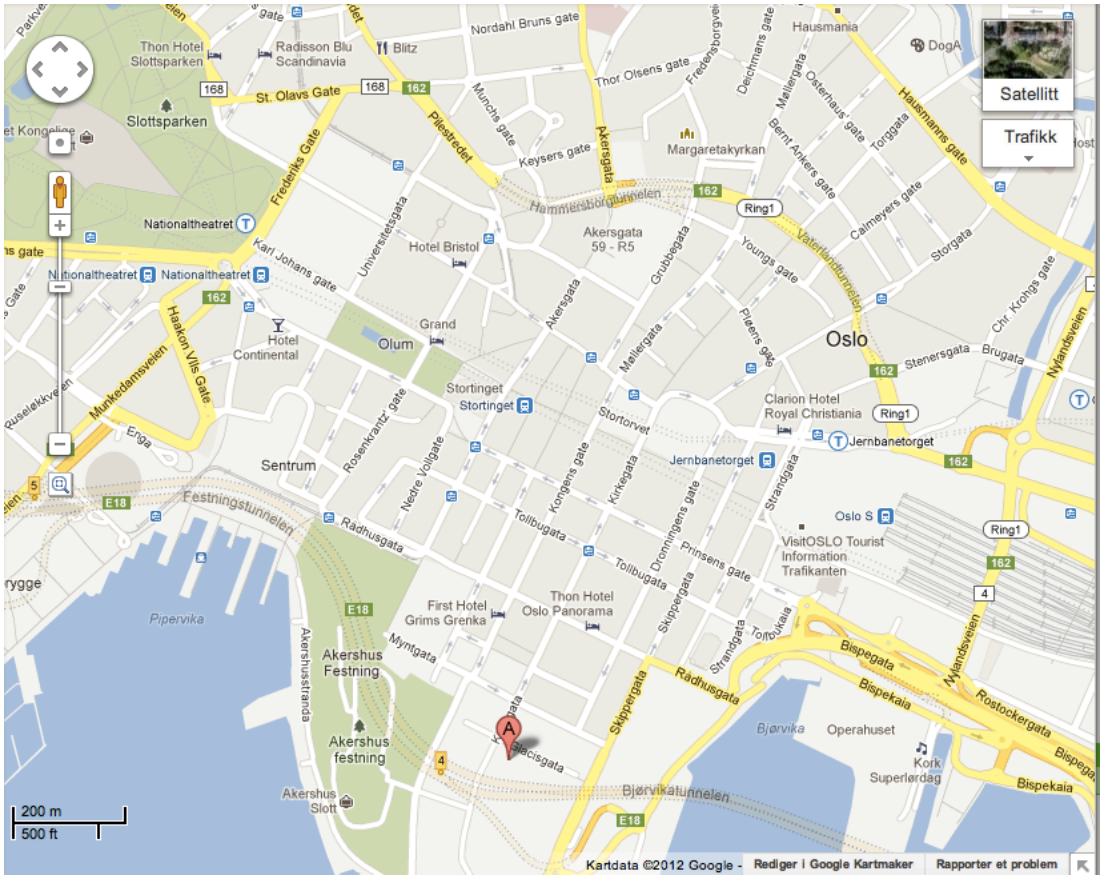
The banquet dinner will be held in *Gamle Logen* downtown Oslo in walking distance from the main street Karl Johan and Oslo Central Station (Jernbanetorget).

When: Thursday, August 2 from 19:00

Where: Grev Wedels plass 2, 0151 Oslo

Dinner is included in the conference fee.

For accompanying persons please pay the fee of NOK 1000 at the registration on July 29



Guided Sightseeing August 1

Program
A guided sightseeing tour on Wednesday will take you to the Viking Ship Museum (http://www.khm.uio.no/vikingskipshuset/index_eng.html) followed by a guided tour through the sculpture park in Vigelandsparken.

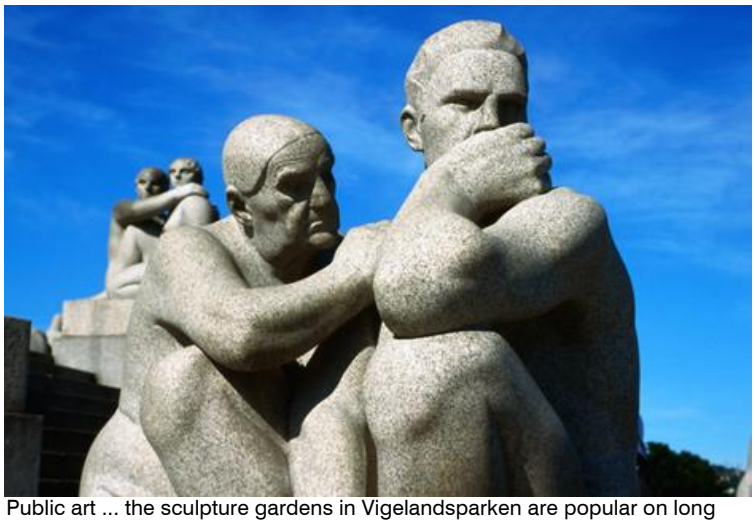
The guided tour is included in the conference fee.

When
Wednesday, August 1, 15:45 – 18:45,

The busses will leave from Motlke Moes vei, precisely at 15:45. Follow sign from Vilhelm Bjerknes (building 13)

Please register for the sightseeing before 12:00 on Monday, July 30. Make a tick mark by your name on the poster by the information desk at Vilhelm Bjerknes (building 13) to let us know that you are joining the tour.


Vikingship museum, Photo: UiO


Public art ... the sculpture gardens in Vigelandsparken are popular on long summer evenings.
Photo: Lonely Planet

Conference discount at the Viking Ship Museum
If you want to visit The Viking Ship Museum outside the guided tour you will get a discount as a participant of the conference. Protist2012 entrance fee to the Viking Ship Museum is NOK 30 (Regular ticket price is NOK 60)

Bring your name tag to show that you are a conference participant.

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