

## Abstracts of the International Scientific Forum “PROTIST-2016”

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### ULTRASTRUCTURE AND PHYLOGENY OF NEW SPECIES OF *GLUGEA* INFECTING THE INTESTINAL WALL OF *CEPHALOPHOLIS HEMISTIKTOS* IN SAUDI ARABIA

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A new microsporidian species of the genus *Glugea* Thélohan, 1891 parasitizing the marine teleost fish *Cephalopholis hemistiktos* Rüppell, collected from the Red Sea in Saudi Arabia, is described on the basis of microscopic and molecular procedures. Spherical and whitish xenoma were observed adhering to the intestinal wall. The numerous spores contained within these xenoma, were ovoid to pyriform and measured 5.1 (4.3–6.0) µm in length and 2.2 (1.8–2.9) µm in width. The spore's wall was composed of two thick layers, which were thinner in the area contacting the anchoring disk. The latter appeared at the spore's anterior pole, in an eccentric position to the longitudinal axis. A lamellar polaroplast surrounded the uncoiled portion of the polar filament projected to the basal region of the spore, giving rise to 26–29 turns with winding from the base to the anterior zone of the spore. The posterior vacuole, located at the spore's posterior pole, and surrounded by the polar filament coils, was irregular and composed of light material. Molecular analysis of the rRNA genes, including the ITS region, was performed using maximum parsimony, neighbour-joining and maximum likelihood methods. The ultrastructural features observed, combined with the phylogenetic data analyzed, suggest this parasite to be a new species of the genus *Glugea*.

### BIODIVERSITY AND COMMUNITY STRUCTURE OF SOIL CILIATES COLLECTED FROM THREE DIFFERENT SITES OF DELHI, INDIA TO ASSESS SOIL QUALITY

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Soil is a natural body of mineral and organic material differentiated into horizons, which differ among themselves as well as from underlying materials in their morphology, physical make-up and chemical composition. Biological components of the soil can vary from one site to another site depending upon the inorganic and organic make up of the soil. Soil ciliates are an integral part of the soil community. They play an important role in nutrient recycling by feeding on bacteria. Also, they are known to stimulate ammonification and nitrification that can later be used by plants and other members of the food web. Although they play an influential ecological role, information on soil ciliate diversity is still rudimentary. Particularly from India, very few data are available on soil ciliates. In this present study, the ciliate diversity was investigated from three different areas, that is, from human inhabitant land [Acharya Narendra Dev College (ANDC) campus], an agricultural land (Karnal) and a sewage treatment plant (Rithala) within Delhi, India. Physicochemical analysis of soil samples was conducted for its pH, nitrogen content, organic carbon content. Ciliate abundance was correlated with physicochemical properties. In total ciliates belonging to 5 Classes, 7 Orders, 15 genera and 20 species were found with maximum number

belonging to Spirotrichs. Maximum number of oxytrichids were found in Rithala sewage site though ciliate diversity was less. Maximum ciliate diversity was found in ANDC whereas ciliate diversity was less in Karnal. Colpodids were present in all the sites examined but most abundant in sewage site. Variation in soil ciliate diversity can be correlated with soil quality of studied habitats.

#### SOIL PROTIST BACTERIA CONSUMPTION IS CENTRAL TO NUTRIENT CYCLING

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Protists are the most abundant consumers of bacteria in soils. Their diversity covers most lineages making them the most diversity group of organisms in soils. Communities assemble along abiotic microgradients, such as  $pO_2$ ,  $pCO_2$ , pH, temperature, etc. Species of protist bacterivores are not homologous or interchangeable. Species in functional groups have differing ecological preferences, occupying distinct niches. There are significant measurable species specific differences regarding prey ingestion rates and prey preferences. It has been well recognised in both soil and aquatic ecology that ignoring inter-species variability in behaviour and consumption rates is problematic. The top-down grazing pressure on bacteria was calculated for a variety of species representing a variety of feeding types, from functional response curves. We calculated threshold prey levels that sustained growth and initial rate slopes for ingestion rates, to compare competitive ability at low food conditions. Together with maximum growth rates and maximum ingestion rates inter-specific competitiveness and niche preferences could be distinguished. Data will be presented for several well established cercozoa cultures, amoebae cultures, ciliates, and other protists. This information helps to improve our calculations of global bacteria biomass turnover rates in soils. It also helps to understand how soils can support dozens of bacterivorous species in each gram of soil, in a seemingly similar habitat.

#### BIODIVERSITY STUDIES IN LORICATE PROTISTS: THE CASE OF TINTINNIDS (ALVEOLATA, CILIOPHORA, SPIROTRICHA)

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Species identification is crucial in biodiversity research (ecology, taxonomy, barcoding etc.). In tintinnids, taxonomy and classification are almost exclusively based on features of their vase-shaped loricae (houses). The lorica-based species circumscriptions are, however, problematic as loricae might show a considerable intraspecific variability and interspecific similarity and might be influenced by the cell cycle and physico-chemical factors. To overcome the difficulties with deviating species limitations suggested by “lumpers” or “splitters” in revisionary treatises, it is recommend to “go back to the roots”, i.e., to use exclusively the original descriptions or authoritative redescriptions for identification. Additionally, good documentation of the identified loricae by means of illustrations and morphometric data is advisable. Depending on the aim of the tintinnid study, further procedures are proposed to generate at high rates long-lasting high-quality species re-/descriptions and/or DNA barcodes, which are essential for reliable phylogenetic analyses and thus for the establishment of a natural tintinnid classification. These suggestions represent a compromise between data quality and work effort and can most easily be followed by collaborations of molecular biologists and morphologic taxonomists. Financially supported by FWF project P28790.

#### MICROBIAL EUKARYOTES IN OILSANDS-ASSOCIATED ENVIRONMENTS OF NORTHERN ALBERTA

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The oil sands in Northern Alberta are the second largest bitumen deposit in the world, and comparable in magnitude to world’s reserves of conventional petroleum. They have global significance as energy reservoirs. However, some aspects of their exploitation are also cause for environmental concern. Fluid wastes from bitumen extraction are retained in enormous tailings ‘ponds’ under a policy of no release to the environment. The estimated surface area of tailings ponds in Alberta ranges from 77-176

km<sup>2</sup>. Thus, since their commissioning approximately five decades ago, the ponds have accumulated anoxic, brackish, hydrocarbon and heavy metal containing sediments overlain by a thinner layer of process-affected water that may be oxic near the surface. Microbial processes are anticipated to play a major role in remediation of these environments, and, although the prokaryotic communities are increasingly well-characterized, little is known about the microbial eukaryotes present in the oxic and anoxic environments. We have recently reported the first NGS-based exploration of protists in tailings ponds. We found that, despite the anoxic and hydrocarbon-enriched nature of the environment, the tailings ponds harbour complex communities of microbial eukaryotes indicating that these organisms should be taken into account when studying the microbiology of the oil sands.

#### NOVEL PICOPLANKTONIC GROUPS FROM LAKE BAIKAL REVEALED BY MASSIVE SEQUENCING

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Compared to the ocean, freshwater systems offer multiple ecological niches in terms of oxygen and DOC concentrations, light accessibility, temperature variability. However, we are still limited by the data of freshwater microbes biodiversity. Studies are highly biased because correspond mainly to Europe and North America. In particular, studies of Northern Asian protists are very limited, though this region extends on about 8000 km in length and has a big impact on microorganisms' migrations. Lake Baikal is the oldest and deepest lake in the world, being located in Northern Asia. It is cold, oxygen rich, and has one of the world richest endemic freshwater biotas, belonging both to very ancient lineages and to recent immigrants. Our goal was to explore small planktonic protists populating the lake. The genetic diversity (V4 region of the 18S rDNA) of planktonic microeukaryotes (< 8 µm fraction) was analyzed in 48 samples using the Illumina MiSeq platform. We determined 1,461 protist OTUs, with 9.6% of them having less than 90% similarity with sequences from SILVA database. We suggest that they belong to endemic Baikal protists. We analyzed the phylogenetic relationships of groups such as MAST, Telonemiidae, Perkinsiidae, Chitridiomycota which were not previously described in Lake Baikal. Interestingly, we found Amoebophrya-like 18S rDNA in Baikal, even though Syndiniales were not reported from freshwaters. Overall, the most diverse

group was stramenopiles. This study contributes to improve our understanding of the diversity of protist communities living in ancient lakes as well as to comprehend better intra-lake evolutionary diversification processes.

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#### MODELLING THE LOCAL-TO-GLOBAL DIVERSITY FOR MICROBES AND MULTICELLULARS: COULD WE ESTIMATE THE NEAR-IMPONDERABLE?

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In the current discussion around either “protist diversity is different” or not, the ratios of local:global diversity and relative endemism are often treated as arguments. This approach has, however, been flawed by the fact that actual global diversity of protists is still largely unknown, especially for the rare species. I use simple models to simulate distribution of species on the 400-cell lattice. Three models have been considered:

- 1) “Everything is everywhere” (equal probability for a particular species to occur at every cell);
- 2) “Environmental selection” (a species can occupy only a fixed portion of randomly distributed “suitable localities”); and
- 3) “Endemism” (most of species are restricted in its potential distribution to a few regions).

To parameterize the models, several world-wide datasets on ciliates, flagellates and harpacticoid copepods were used. The local:regional and regional:global diversity ratios and percentage of endemics were estimated by simulating the equal-effort sampling across the lattice, with varying the full number of species (“actual diversity”) and number of samplings per cell (“sampling effort”). All parameters, predicted by every model, strongly depended on the sampling effort but were only slightly, if ever, influenced by the observed:actual global diversity ratio. The predictions of the first two models were generally close to each other, but differed noticeably from those of the third one. These results indicate that the above-mentioned parameters are heavily influenced by undersampling. Nevertheless, they can serve as informative characteristics in comparative biodiversity studies, even if the true number of species can hardly be estimated overall.

#### GLOBAL DIVERSITY AND DISTRIBUTION OF MARINE BENTHIC HETEROTROPHIC FLAGELLATES

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Protists are ubiquitous, but the factors influencing their diversity and biogeography are poorly understood. We use a comprehensive database on the marine benthic heterotrophic flagellate (HF) morphospecies to explore the worldwide patterns in their diversity and distribution in comparison with predictions of the Ubiquity model (UM) and Moderate Endemicity model (MEM). Number of known HF morphospecies was limited (even if considering the rates of descriptions), and local-to-global diversity ratio was relatively high (10-25%). Regional diversity was highly correlated with the investigation effort, indicating considerable under-exploration. Regional endemics were few (not over 19% of total richness), and many morphospecies were widespread or even cosmopolitan. No obvious latitudinal trend in HF diversity was detected. By species composition, the regions were distinctly arranged into three groups according to cold, temperate and warm waters, but not in accordance with geographical distances. This distribution pattern was most likely explained by contemporary climate (temperature) but did not suggest clear geographical barriers for dispersal. Therefore, the HF morphospecies are less concordant with the MEM predictions but closer to the UM than other (larger) protists. (In)consistency between the distributional patterns obtained from genetic- and morphology-based data are briefly discussed.

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#### MOLECULAR DIVERSITY OF SUMMER PLANKTON PICOEUKARYOTES IN THE WHITE SEA

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Picoeukaryotes (protists < 2 µm) play an important role in marine ecosystems, although knowledge of their diversity and ecosystem functioning is limited. The White Sea is located in the sub-Arctic zone.

However, its abiotic conditions are characterized by a number of features typical of the Arctic seas. In this study, the molecular diversity of picoeukaryotes in July 2014 in Kandalaksha Bay (the White Sea) was examined using conventional filter fractionation and Illumina sequencing. In < 2 µm size fraction 186 taxa were revealed that included some nanoplankton (2–20 µm) and even microplankton (>20 µm) taxa. Plankton assemblage was prevailed by autotrophs (>57% of all sequences). Putative picoeukaryotic autotrophs were mostly dominated by three genus: *Micromonas* sp., *Bathycoccus* sp. and *Ostreococcus* sp. (35% of all sequences). Putative heterotrophic picoeukaryote assemblage was more diverse and was presented by marine stramenopiles (MAST, Labyrinthulomycetes), Amoebozoa, Cercozoa and picrozoa. Illumina sequencing revealed some new groups and genus of nano- and microplankton organisms which have been never detected in the White Sea. Our study revealed that diversity of picoeukaryotes in summer in the sub-Arctic White Sea corresponds to that of the other Arctic seas, such as the Beaufort and Norwegian Seas.

#### NEW *PARAMECIUM* SPECIES “CANDIDATUS *PARAMECIUM OSSIPOVI*”

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Among *Paramecium* species *P. putrinum* is one of the best identifiable. Most important discriminative features are small cell size (70-140 µm), single big micronucleus (MI) and very special contractile vacuole (CV) represented as set of small vacuoles. We have studied several clones from different collecting sites that were characterized by all features mentioned above but with unusual habitus. Detailed analysis revealed several very specific characteristics of these clones. Usually the cells are bigger up to 120-140 µm. Their single MI has got “chromosomal” structure. Contractile vacuole differs from typical *P. putrinum* one. It has got short channels and is similar to CV of *P. nephridiatum*, or *P. polycarium*. *Holospora*-like bacterium found in *P. putrinum* do not infect “atypical” strains. Finally the sequence of cytochrome c oxidase subunit I gene has only 86% identity with the one of *P. putrinum*, which is much higher than difference between different *P. putrinum* syngens. Taking in account all above, we propose new species “Candidatus *Paramecium ossipovi*”. Scientific research was performed at the Center for Culturing Collection of Microorganisms and Center for Molecular and Cell Technologies of Research park of St. Petersburg State University.



#### NUCLEUS-ASSOCIATED ACTIN IN DIFFERENT STAGES OF *AMOEBA PROTEUS* CELL CYCLE

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Despite of plenty of the reports and reviews there are still a lot of blank spots in the matter of the organization and functioning of *Amoeba proteus* nuclear apparatus. According to our 3D-reconstruction of its chromatin compartment, it has a complicated and highly dynamic structure (Demin et al., 2016). Actin was shown to be a key protein actor in providing different nuclear processes. Immunocytochemical study has shown an actin meshwork strongly colocalized with chromatin fibrils. On the other hand, the amoeba nucleus appears embraced by a basket-like structure formed by F-actin that in turn is connected with cytoplasmic actin filaments. 3D-observations of the nucleus during the cell cycle indicate a regular process of the chromatin extrusion/elimination of the part of the chromatin “excess” to the cytoplasm. Based on the 3D-reconstruction of phalloidin-stained cells, we suppose the existence of specific chromatin-binding sites interacting with actin filaments meshwork. We also propose a hypothesis that the chromatin extrusion is provided by actin filaments which may pull out the chromatin fibrils from the nucleus. Funded by the Russian Foundation for Basic Research, projects 15-04-03451, 15-04-01857, and the granting program “Molecular and Cell Biology” of the Presidium of RAS.

#### INFLUENCE OF SALINITY STRESS ON DNA SYNTHESIS AND CHROMOSOME FINE STRUCTURE OF DINOFLAGELLATES *PROROCENTRUM MINIMUM* (PAVILLARD) SCHILLER

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*Procentrum minimum* is a common planktonic potentially toxic bloom-forming dinoflagellate. Being widely distributed, this species demonstrates high adaptive capacity to different factors, particularly salinity. We studied cellular and biochemical aspects of physiological adaptation of this dino-

flagellate. The basic culture of *P. minimum*, isolated from the Black Sea, was grown in 17 psu f/2 medium (cultivation conditions described in Pozdnyakov et al., 2014). Experiments were carried out by inoculation of cells into 4, 8, 35 psu f/2 medium and 17 psu as a control for 30 min. Then cells were returned to the basic medium, incubated for 24 h and fixed for flow cytometry or transmission electron microscopy. We detected DNA concentration value (1.2 pg/cell) and fine structure of chromosomes in control series. After inoculation of cells into 8 psu f/2 medium we observed the highest DNA concentration (1.5 pg/cell) and the lowest cell mortality rates. Chromosomes became more condensed, local unwound sites increased in number and appeared more distinct. Transfer into the medium with native for these organisms salinity 35 psu (the World ocean level) did not caused any reliable differences in DNA concentration (1,3 pg/cell). At the ultrastructural level, we observed total splitting of chromosomes in most cases. Inoculation of cells into 4 psu f/2 did not caused any significant changes in DNA concentration value, but led to high cell mortality rates. Our results are in agreement with the protistan species maximum concept for the horohalinicum. Funded by the Russian Science Foundation, project 16-14-10116.

#### FINDING AND ANALYSIS OF AMOEBOTAXA-SPECIFIC GENES TO STUDY ENVIRONMENTAL DIVERSITY OF AMOEBAE

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Amoebozoa is a one of the supergroups of eukaryotes, which includes naked and testate lobose amoebae, pelobionts, mycetozoa, and several groups of flagellated organisms. In environmental DNA surveys done on traditional DNA barcodes (SSU-rDNA, Cox I gene) amoebozoan genes normally constitutes a minor part of the total gene diversity and represent only the most abundant lineages. To resolve this problem, we attempted to find Amoebozoa-specific genes and gene families with low level of paralogy appropriate for the application as a DNA barcodes for this group of protists. We analyzed the Amoebozoa RNA-Seq data which were available from MMETSP (<http://www.moore.org/>). First, we analyzed assembled transcriptomes from MMETSP and found transcripts with unusually big size (more than 50 000 bp) in several assemblies. This led us to the decision to repeat data assembly *de novo*.

During this work we developed pipeline, based on publically available bioinformatics tools and our own scripts written in Python for transcriptome assembly and annotation. We have found 300 groups of genes, which not found outside Amoebozoa or were highly derived within this group of protists. Among them we selected 15 groups of genes with low level of paralogy and performed phylogenetic analysis and primers construction. These genes are promising DNA barcodes for studies of environmental diversity of Amoebozoa. Supported with MK-4853.2015.4 President grant, RFBR 16-34-60111 and SPSU grant 1.38.251.2014.

#### BURIED BUT NOT DEAD: INSIGHTS INTO THE DIVERSITY, PHYSIOLOGY, FUNCTIONS AND ECOLOGICAL ROLES OF DEEP SUBSEAFLOOR FUNGI USING AN INTEGRATED APPROACH

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Bacteria and Archaea are the most commonly studied microorganisms in the marine environment, and habitats such as deep seafloor ecosystems are no exception. However, recent studies strongly support the idea that deep seafloor microbial communities include Fungi, which seem to dominate those micro-eukaryotic communities. Using sediment samples from the IODP Expedition 317 as a model, our aims were (i) to better understand the diversity, physiology and functions of deep seafloor fungi and (ii) to provide clues about how they interact with other microbial populations in those communities. Using a record depth sediment core, fungal molecular signatures and fungal cultures were obtained from samples as deep as 1740mbsf (Rédou et al. 2014) and 1884mbsf (Rédou et al. 2015), respectively. In spite of the fact that those complementary approaches revealed low diversity of higher fungal lineages, DNA and rRNA signatures as well as almost 200 cultured isolates provide direct evidence that fungi persist in this challenging habitat. Consistent with this idea, physiological analyses indicate some deep seafloor fungal isolates appear well-adapted to *in situ* conditions.

Metatranscriptome analysis provided an examination of the functional repertoire of deep seafloor fungi. Gene expression was assigned to metabolic and biosynthetic processes, responses to stress, cell and membrane functions, conidiogenesis and biosynthesis of secondary metabolites (Pachiadaki et al., in revision). These results all provide further support for the notion of fungal presence and activity in the deep seafloor biosphere, with the ability to interact with other microbial populations by synthesizing antimicrobial compounds (Navarri et al. 2016).

#### THE GREEN ALGA AND THE SALAMANDER: A SUFFOCATING LOVE STORY

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The recently discovered endosymbiosis between the green alga *Oophila amblystomatis* and the salamander *Ambystoma maculatum* is a unique relationship among the chloroplastida and vertebrates. Using a dual RNA-seq approach, we assembled novel transcriptomes of these two organisms and identified differentially expressed transcripts between ecto- and endo-symbiotic algae as well as between salamander cells with and without endosymbiotic algae. The results offer a glimpse at the changes in both organisms that take place during this novel endosymbiosis. We found that the intracellular algae downregulate nutrient transporters related to phosphate and nitrogen acquisition from the environment. They also exhibit hallmarks of cellular stress, especially related to osmotic stress, sulfur starvation, and hypoxia. Further, the results suggest that the alga undergoes a large scale metabolic shift from oxidative metabolism to fermentation with the potential evolution of hydrogen gas. The salamander cells exhibit milder differences, including changes in gene expression indicating the initiation of an innate immune response to the alga, and alterations in nutrient sensing related to insulin sensitivity. The salamander cells do not exhibit large scale stress or apoptotic responses suggesting that intracellular algae are not a big drain on the salamander cell's resources.

#### GENOME AND TRANSCRIPTOME OF *HEMISTASIA PHAEOCYSTICOLA*, A FLAGELLATE RELATED TO A NOVEL HYPER-DIVERSE CLADE OF MARINE PROTISTS

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*Diplonemea* (Euglenozoa) is an understudied group of heterotrophic flagellates, related to euglenids and kinetoplastids. Diplonemids are abundant in the deep ocean and, surprisingly, represent the most diverse clade of pelagic eukaryotes, according to our metabarcoding data. There are four major clades within diplonemids: i/ clade of ‘classic’ diplonemids, *Diplonema* and *Rhynchopus*; ii/ small clade of *Hemistasia*; iii/ small clade of environmental sequences (DSPD II, deep-sea pelagic diplonemids II); iv/ large clade of environmental sequences (DSPD I). The DSPD I clade accounts for >97% of diplonemid abundance and diversity in the oceanic plankton, and *Hemistasia phaeocysticola*, a recently isolated marine flagellate that feeds on diatoms and dinoflagellates, appears to be the closest relative of DSPD I according to some phylogenetic analyses. It not clear whether *H. phaeocysticola* is a predator, a parasite, or a scavenger of dead cells. An axenic culture was obtained from *H. phaeocysticola* growing with *Thalassiosira cf. rotula*. The genome and transcriptome of *H. phaeocysticola* were sequenced using 300 and 250 nt paired-end Illumina MiSeq reads, respectively. A draft genome assembly was obtained using CLC Genomics Workbench v.8.1 and has the length of ~260 Mbp and N50 of 1.5 kbp. The results suggest that the genome of *H. phaeocysticola* is similar to that of *Diplonema papillatum* in size and has a very high repeat content. Additional genome sequencing using PacBio will be performed and the metabolism of *H. phaeocysticola* will be studied using a transcriptome assembly.

MIXOTROPHIC CILIATES AS SPECIAL ECOLOGICAL GROUP IN WATERBODIES OF CENTRAL AND LOWER VOLGA AND KAMA  
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In plankton of polytypic waterbodies from Cent-

ral, Lower Volga and Kama 39 species of the mixotrophic ciliates are revealed. Their structure is heterogeneous: there are “epilimnic” aerophylic myxotrophs and microaerophylic or anaerobic myxotrophs. The first group appears in the low trophic state reservoirs; reservoirs with unstable stratification; in the epilimnium of the stratified waterbodies; in acid, colored, with level fluctuation waterbodies. The second group prefers microaerobic and anaerobic conditions of stratified eutrophic and hypertrophic lakes with high sulfides concentration. The absolute maximums of abundance (206540 ind./l) and biomass (7264 mg/m<sup>3</sup>) of mixotrophs are registered during summer stratification in a highly trophic meromictic pond, and the maximum contribution to biomass - in the small polyhumic lakes (98%). The ciliates’ mixotrophic biomass (6,1 g/m<sup>3</sup>) is comparable with the anoxic phototrophic bacteria biomass (9,6 g/m<sup>3</sup>) and phytoflagellates (2,6 g / m<sup>3</sup>) in chemocline and adjacent layers. Their contribution to the overall “phototrophic” component biomass reaches about 37%, while in other waterbodies the total biomass of ciliates several orders of magnitude less than the biomass of other protists and bacteria. The scheme of different mixotrophs species confinement to various waterbodies and ecotopes in gradient of major abiotic factors has been drawn. An inverse relationship between environmental indicators saprobity and mixotrophic ciliates percentage was shown on example of oligo-mesotrophic lake. This can be used when assessing the state of aquatic ecosystems. It was proved that the species composition and the nature of their distribution are determined by specific abiotic and biotic conditions and weakly dependent on the zonal factors.

THE ROLE OF PROTISTS IN THE PLANKTON COMMUNITY OF FRESHWATER LAKE IN THE PERIOD OF ITS EUTROPHICATION

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The Lake Kandry-Kul is one of the largest natural lakes of the Middle Volga basin. It is a popular resting place and therefore experience significant recreation load. From 2010 to 2012 the trophic level of the lake has increased from a predominantly oligotrophic to mesotrophic. We studied the following groups of protists: ciliates, dinoflagellates, chrysophytes, cryptophytes and euglenids. Their biomass in 2012 compared to 2010 has increased 2.4 times. Their contribution to the total biomass of plankton community and to the total unicellular



plankton biomass has also increased 1.9 and 1.3 times, respectively. Along with these changes, a redistribution of the total biomass of the plankton community has occurred between the pelagic and littoral parts. In the pelagic zone of the lake the increase was most significant, 3.8 times, and in the littoral zone it amounted 1.3 times. The share of protists in the unicellular plankton biomass in the pelagic zone remained unchanged, while in the littoral zone it increased 2.3 times. Protist's contribution into the total plankton biomass in the pelagic zone increased 1.3 times, and in the littoral 3.4 times. These changes were mainly related to the increased role of the cryptophytes, dinoflagellates and ciliates, especially mixotrophic. In the context of eutrophication the most significant correlations of individual groups of protists were registered with the concentrations of phosphorus, ammonia nitrogen and chlorophyll a. The influence of environmental factors on the peculiarities of protist distribution and ratios of their groups are discussed.

***IOTANEMA SPIRALE* GEN. ET SP. NOV., A NEW ENDOBIOTIC LINEAGE OF FORNICATA WITH STRIKINGLY SIMPLIFIED MORPHOLOGY AND ULTRASTRUCTURE**

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Fornicata is a lineage of excavates living in low oxygen environments and lacking a conventional mitochondrion. Three main morphological/ecological subgroups of Fornicata are recognized: free-living *Carpediemonas*-like organisms (CLOs) from marine habitats and two parasitic/commensal lineages living in animal hosts, retortamonads and diplomonads. The research of Fornicata centers on evolutionary perspectives such as transition from free-living lifestyle to parasitic one and mitochondrion transformation. Nonetheless, current modest knowledge of the biodiversity of Fornicata limits our ability to draw the whole picture of evolutionary history of this group. The free-living CLOs are particularly important due to the plesiomorphic traits of excavates. In this study, we cultured a novel member of Fornicata, *Iotanema spirale* gen. et sp. nov., isolated from fresh feces of a gecko. *I. spirale* exhibits several unique features not seen among fornicates. It possesses a single flagellum and a highly reduced cytoskeletal system with a single microtubular root, two fibers, and

dorsal fan; the excavate ventral groove is missing. SSU rRNA gene analyses demonstrated that *I. spirale* branches as a sister lineage to the free-living CLO *Hicanonectes teleskopos* and its relatives, which means that it represents the third known endobiotic lineage of Fornicata.

**SPATIOTEMPORAL DISTRIBUTION OF SPECIES DIVERSITY AND COMMUNITY STRUCTURE OF CILIATES IN INTERTIDAL SANDY SEDIMENTS OF HUOKUN'AO BEACH, NANJI ISLANDS (EAST CHINA SEA, CHINA)**

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In intertidal sediments, ciliates are a highly diverse and quantitatively dominant group, whose community structure, in some sense, may reflect the condition of environmental quality. We investigated the spatiotemporal distribution of species diversity and community structure of ciliates in the intertidal sandy sediments of Huokun'ao beach, Nanji Islands National Marine Natural Reserve based on sample collections in November 2013 (autumn), February (winter), May (spring) and August (summer) 2014. The results showed highly diverse and abundant ciliates in the intertidal sediments of the beach, where a total of 148 species representing 19 orders and over 70 genera were identified. The abundance and species composition of benthic ciliates showed significant correlation with seasons and sediment depths. Bacterivorous ciliates constituted the most abundant group, followed by carnivores and algivores. The predominance of bacterivores indicated that the ciliate community in the intertidal sediment of Huokun'ao was not a typical interstitial ciliates fauna, but a pattern similar to those in muddy sand sediments with high level of organic matter. This is likely a subsequent effect of long-term human activities. In spite of the impact, the high diversity of ciliates indicates that the benthic environment in the Huokun'ao beach and its surrounding area is in fair condition, which is attributed to the biodiversity conservation measures adopted, e.g. the relocation of surrounding residents and facilities.

**PROKARYOTIC ENDOCYTOBIONTS OF PELOMYXIDAE**

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At present the taxonomy of Archamoebae is based on molecular phylogeny, and it is problematic to find non-molecular synapomorphies for different lineages within this group. The family Pelomyxidae includes two archamoebal genera: *Pelomyxa* and *Mastigella*. All pelomyxae and most mastigellae have various prokaryotic endocytobionts. Host-symbiont composition could be useful for the phylogeny reconstruction, but information about such associations is controversial. We analyzed consortia of prokaryotic symbionts in *Mastigella nitens* and 11 *Pelomyxa* spp. There are two kinds of such consortia within pelomyxid cells: binary and ternary ones (with 2 and 3 symbionts correspondingly). The composition of these associations revealed to be species-specific. According to morphological characters, autofluorescence ability and results of Gram staining 4 main types of cytobionts can be distinguished. One of them is common to all investigated pelomyxae and *M. nitens*. Morphologically similar prokaryotes can be also found in the cytoplasm of other *Mastigella* spp. Distribution of the remaining types of cytobionts among hosts species is not correlated with the molecular phylogeny of Archamoebae based on 18S rRNA gene. We propose the ability to form symbiotic associations with prokaryotes as a shared feature of all Pelomyxidae.

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THE GENOME EVOLUTION OF THE THERMOACIDOPHILIC CYANIDALES RED ALGAE  
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The Cyanidiales is an early diverged red algal group that comprised of seven species with numerous cryptic species. They are asexual, unicellular photosynthetic eukaryote, which usually thrive in

extremophilic environments (pH 1.5~3, 35~55°C). Cyanidiales species are reported from hot springs around world including Yellowstone National Park (US), Italy, Iceland, New Zealand, Indonesia, and Japan. Some Cyanidiales species are also found in acidic bogs, mesophilic caves, interlithic or endolithic environments. In addition, they show trophic differences being either autotroph or mixotroph. From previous researches, the genomes of Cyanidiales are highly reduced, but horizontally acquired from bacteria to adopt in extreme habitats. Here we report two nuclear genomes of *Galdieria maxima* and *Cyanidium caldarium* and conduct comparative genome analysis with three available genomes of *Cyanidioschyzon merolae*, *Galdieria sulphuraria*, and *Galdieria phlegrea*. We will discuss gene contents, mutation rates, and unique horizontal gene transfer that related to the environmental adaptation.

EVOLUTION OF HEME BIOSYNTHESIS PATHWAY IN ALGAE WITH COMPLEX PLASTIDS  
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Tetrapyrroles are organic compounds essential for life. Organisms are either synthesizing tetrapyrroles or they have to obtain them from their environment, host or prey. We showed that the composition of the biosynthetic pathway is shaped by passed endosymbiotic events in eukaryotes. We map the pathway in phototrophic eukaryotes, particularly in algae with secondary or other advanced plastids, by investigating origins of involved enzymes and predicting their location in the cell of the euglenophyte *Euglena gracilis*, the chlorarachniophyte *Bigeloviella natans*, the cryptophyte *Guillardia theta*, the dinoflagellate with green secondary plastid *Lepidodinium chlorophorum*, and dinoflagellates bearing diatom endosymbiont (also called “dinotoms”) – *Glenodinium foliaceum*, *Kryptoperidinium foliaceum* and *Durinskia baltica*. Chlorarachniophytes and euglenophytes still possess two independently operating tetrapyrrole pathways with the first common precursor  $\delta$ -aminolevulinic acid synthesized either by the C4 pathway in two steps using the mitochondrially located ALA synthase (ALAS), or by the plastid located C5 pathway by consecutive enzymes glutamyl-tRNA reductase (GTR) and glutamate-1-semialdehyde 2,1 aminomutase (GSA-AT). We propose that such arrangement of the pathway was ancestral for all

phototrophic eukaryotes. Other algae show partial (cryptophyte) or total (dinoflagellates) reduction of the redundant mitochondrially-cytosolic pathway. In dinotoms, two redundant plastid located pathways are present suggesting a presence of the two plastids of different origins in the dinotom cell. Although *L. chlorophorum* and *B. natans* contain chlorophyte-derived plastids, enzymes involved in tetrapyrrole biosynthesis are dominantly of rhodophyte origins. This may suggest an early acquisition of the rhodophyte-derived secondary plastid through the secondary endosymbiotic event on the root of SAR group.

#### MICROBIAL EUKARYOTES FROM THE DEEP: REVEALING THE DIVERSITY OF PROTISTS AND THEIR TROPHIC RELATIONSHIPS AT HYDROTHERMAL VENTS

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Protists in the deep-sea are a diverse mixture of taxa that are either transported there by sinking or occur there as endemic species. It is not uncommon for the closest genetic relatives of deep-sea protists to be found in samples from other deep-sea habitats thousands of kilometers away. In other cases, the nearest relatives of deep-sea protists are from equally distant, but shallow-water habitats with similar characteristics to the deep-sea locales (e.g., low-oxygen or high-temperature sediments). Many of the most conspicuous protists at hydrothermal vents are ciliates and small flagellates that rely on the copious bacterial production occurring in the proximity of vent ecosystems. We investigated the diversity and activity of marine protists from two deep-sea hydrothermal vent ecosystems including those at Guaymas Basin (Gulf of California, N. Pacific) and 9-North (Eastern Tropical N. Pacific). Complementary molecular techniques were employed to reveal the full breadth of protistan diversity, the diversity of the active fraction, and the taxonomic identities of some of the most conspicuous and active deep-sea ciliates. Clone libraries based on RNA extractions revealed a substantially greater proportion of the protists that appeared to be the most active via microscopy, while libraries based on DNA captured the diversity of both endemic organisms and those that sank into the vent eco-

system. Single ciliates were hand-isolated and subjected to whole genome amplification, prior to cloning and sequencing. This approach identified specific protistan taxa that were likely some of the most active members of the deep-sea community of microbial eukaryotes.

#### FIRST RECORDS OF MOSS CILIATES IN MEXICO

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Mosses (Bryophyta) are not vascular gregarious plants that require some environmental characteristics to grow, i. e. the presence of some moisture degree, doing possible the establishment of ciliate communities like those that occur in peatlands. Mexico has a diversity of 984 species of mosses and for ciliates about 959 species have been recorded in several habitats, however the study of ciliates associated to these plants remains unknown until the present. The aim of this work was to record the ciliate community in *Campylopus pilifer* Brid during the summer of the year 2015 in the Sierra Juárez, Oaxaca. Samples were manually collected and observed alive using bright field and DIC microscopy. We also employed silver impregnation techniques for species identification. We recorded five species of ciliates: *Colpoda inflata*, *C. aspera*, *Colpoda* sp., *Vorticella infusionum* and *Vorticella* sp. in *C. pilifer* belonging to habitats with different environmental characteristics. These findings represent the first moss-ciliate record for Mexico.

Keywords: Ciliates, Bryophytes, México, Moss, Oaxaca.

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#### POPULATION STRUCTURE OF PLANKTONIC MICRO-ORGANISMS (TINTINNID CILIATES OF THE MICROZOOPLANKTON) IN THE SOUTH PACIFIC OCEAN: COMPARISON OF A HIGH PRODUCTIVITY BLOOM AND A TYPICAL OLIGOTROPHIC SITE

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We hypothesized that the population structure of planktonic grazers in a high productivity site differs from that known for typical oligotrophic sites. We expected the high productivity site to harbor more species. We compared a high productivity site with dense populations of N-fixing *Trichodesmuim*, and a distant oligotrophic site. We examined patterns of species abundance distribution and the abundance distribution of ecological types, that is species of similar shell or lorica oral diameter, analogous to gape size. We also examined species packing within ecological types, defined as lorica oral diameter size-classes. Population abundance differed between the sites by a factor of 3, and the productive site population was highly dominated by a single species (not found at the oligotrophic site) but species richness and turnover were very similar. Species abundance distributions for both sites on all dates most closely fit a log-series or lognormal distribution. Abundance distributions of ecological types, forms of distinct lorica oral diameter, were the typical geometric for the oligotrophic site. In contrast, at the high productivity site, lognormal or log-series distributions provided equally good fits. In the oligotrophic site there was a positive relationship between the number of individuals in a lorica size-class and the number of species but not in the productive site. Despite large differences in population size and dominance, the two populations were surprisingly similar by most measures. Our results suggest that overall food web structure is likely similar as well.

#### ARE SPECIALIZED TROGLOBIONT CILIATES EXIST?

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The organisms inhabiting in subterranean waters can be separated into two groups. One of them is represented by species, which for any reasons being situated in the underground habitats and have adapted to them. As a rule, these are the same species as in epigeal waters or soils and possible has originated from mentioned habitats. Thus, the group might denoted as epigeal. Another group of organisms is represented by specialized troglobiont species. The list of ciliated protozoans living in subterranean waters comprises about 150 species, but most of them can be defined as representatives of epigeal group. Only a few ciliate species, which are host-specific commensals or parasites of subterranean animals (especially crustaceans) possibly, should be considered as troglobionts. In

such a way, the list of specialized troglobiont ciliates must include suctorians *Spelaeophrya troglacaridis* from shrimps, *Tokophrya bathynellae* from syncarids, *Echinophrya stenaselli* and *T. microcerberi* from subterranean isopods. The analysis of literary and own data on distribution and host prevalence of suctorian ciliate *T. niphargi* from amphipods and peritrich ciliate *Ballodora marceli* from wood louses permit to define these ciliates to epigeal group of species. As for apotomes *Gymnodinioides* sp. from cavernicolous amphipods and shrimps, status of these ciliates remains to be seen.

#### SPECIES DIVERSITY AND BIOTOPICAL DISTRIBUTION OF HETEROTROPHIC FLAGELLATES IN SMALL RIVER FLOODPLAIN Dubrovsky Yu.V.<sup>1</sup>, Mylnikov A.P.<sup>2</sup>

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Twenty-seven species and forms of heterotrophic flagellates have been found in the result of analysis of 8 water and 4 soil samples (downstream of river Ros', Ukraine, 49°37'N; 31°30'E) during summer 2014. Observed species belongs to Opisthokonta (2 species), SAR (17), Excavata (8) and incertae sedis group (2). The most flagellates were bacterivorous. *Allantion tachyploon*, *Kathablepharis* sp., *Alphamonas edax*, *Colponema vietnamica*, *Diphyleia rotans*, *Rhynchobodo armata* belonged to predators and *Goniomonas truncata* and *Paraphysomonas* spp. were omnivorous. The relationship between locality of the sample and species composition has not been revealed. This fact indicates the random character of species distribution. The most abundant (9-13 species) were overgrown river floodplain creeks and ponds, as well as wet soil deciduous forest. The silted ponds and creeks without flow, as well as coastal soils, are characterized by the average species richness (6-7 species). River watercourse, dried puddle, bedrock at a depth of 12 m and the soil under field crops possess the lowest species richness (2-4 species). The vast majority of species is common for all investigated biotopes and occurs both in water and in the soil. Among the 11 species occurring in more than three samples, 7 species are inhabited both water and soil, and 4 species were found only in water samples. The average number of species for water and soil samples (7 and 6 correspondingly) was not significantly differed. The specificity of species composition within habitats is not observed. This study was supported by the Russian Foundation for



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#### THE CHLAMYDOPHRYIDAE REVISITED WITH SPECIAL FOCUS ON THE GENUS *LECYTHIUM*

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Although testate amoebae have attracted interest of protistologists for more than 150 years, some groups especially those with a hyaline test are still poorly known. One of those fairly understudied groups is the family Chlamydrophyidae. They are difficult to culture and therefore only old, sometimes obscure, species descriptions are available. Further, no molecular data was obtained and therefore in modern mass sequencing studies they are only recognized as OTUs of unknown affinity. Nevertheless, they are widely distributed, can occur in high abundances in freshwater and terrestrial habitats, show interesting feeding behavior and have a high diversity in morphology. This talk will give an overview of the up to date knowledge on the Chlamydrophyidae and will further focus on one of its genera, *Lecythium* that was first described by Hertwig & Lesser in 1874. We (a) established 7 cultures of 5 *Lecythium* species and provide detailed morphological as well as ecological observations (b) obtained SSU sequences and conducted phylogenetic analyses of the Tectofilosida, showing that *Lecythium* is closely related to the Pseudodiffugiidae and splits into a terrestrial and freshwater clade and (c) performed food choice experiments that underline the mycophagous and algivorous feeding behavior of *Lecythium*. Our talk will show that the Chlamydrophyidae are a group of protists that are highly fascinating.

#### THE BASE OF CERCOMONAD RADIATION IS STILL IN FOR A SURPRISE, KRAKEN GEN. NOV.

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The term ‘filose amoebae’ describes a highly polyphyletic assemblage of protists whose phylogenetic placement can be unpredictable based on gross

morphology alone. We isolated 6 filose amoebae from soils of two European countries and describe a new genus of naked filose amoebae, *Kraken* gen. nov. comprising one new species *Kraken carinae* sp. nov. We provide a morphological description based on light microscopy and small subunit rRNA gene sequences (SSU rDNA). In culture, *Kraken carinae* strains were very slow-moving and preyed on bacteria using a network of filopodia. Phylogenetic analyses of SSU sequences reveal that *Kraken* are core Cercozoa, branching weakly at the base of the cercozoan radiation, most closely related to *Paracercomonas*, *Metabolomonas*, and *Brevimastigomonas*. *Kraken* sequences are >99% similar to an environmental sequence obtained from a freshwater lake in Antarctica, indicating that *Kraken carinae* is not exclusively soil dwelling, but also inhabits freshwater habitats.

#### FIRST RECORD OF *BROMELIOPHYA BRASILIENSIS* FOISSNER, 2003 (CILIOPHORA: GLAUCOMIDAE) FROM TANK BROMELIADS IN MEXICO WITH SOME NOTES ON ITS DISTRIBUTION AND ECOLOGY

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*Bromeliophrya brasiliensis* is a glaucomid bacterivorous ciliate described from terrestrial tank bromeliads in the Atlantic Coast of Brazil, and recorded in Paraná, Brazil and Ecuador from rupicolous bromeliads. The reduction of somatic ciliature and the presence of two kinetofragments, which migrate to the left margin of the buccal cavity, are the main cytological characteristics as compared with *B. quadristica* which presents four kinetofragments and it has been recorded in the Antilles. The objective of this work is to describe the ciliate diversity from tank bromeliads in Mexico. We sampled 30 individuals of epiphytic tank bromeliads including five species of genera *Catopsis* and *Tillandsia* in three different temperate forest sites from Central and Southern Mexico, during the year 2015. The sites were located from 1336 to 2426 m a.s.l. Observation and identification of *B. brasiliensis* was performed using bright and DIC microscopy,



and with silver impregnation methods. Cellular specific characteristics were analyzed and *B. brasiliensis* was recorded in 23% of the samples with temperature values between 18 to 22 °C, and pH values from 3 to 6, demonstrating a wider range of temperature and pH tolerance, and a broad geographic distribution but only in Neotropical tank bromeliads. This is the first record for the species in these microecosystems at different altitudes in Mexico. Financial support was provided by National Council of Science and Technology (CONACYT) fellowship number 224627. Biol. Margarita Reyes and M.A. Aldi de Oyarzábal from Facultad de Ciencias, UNAM, are thanked for technical assistance and scientific illustration respectively.

#### PROTIST DIVERSITY AND INTERACTIONS WITHIN DEEP-OCEAN SEDIMENT AND WATER-COLUMN MICROBIAL COMMUNITIES

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Protists are integral members of marine food webs and exhibit complex relationships with other microbial taxa. Phagotrophic protists contribute significantly to carbon turnover in the sunlit ocean and evidence suggests grazing in the dark ocean can be significant as well. The molecular signatures of parasitic protists comprise significant fractions of many high-throughput sequencing datasets, suggesting a potentially important role in controlling populations of their host(s). Prokaryotic symbionts of free-living protists can be numerous, and, particularly in low-oxygen to anoxic marine habitats their collective metabolisms may contribute significantly to biogeochemical cycling. Particular groups of protists are abundant and common inhabitants of marine sediment and water column oxyclines and very deep halocline habitats where physical associations with prokaryotes are frequently observed. Using open ocean oxygen minimum zones, deep hypersaline anoxic basins of East Mediterranean Sea, and the permanently stratified water column of Cariaco Basin, Venezuela as natural laboratories to link biogeochemistry and diversity, we report evidence of niche partitioning and specialized communities. Sediment communities seem to be dominated by different key players but exhibit similar niche

partitioning. Projects supported by NSF OCE-0849578, OCE-1061391, and OCE-1336082.

#### THE MARINE CARAVAN INVADING THE TWO BIG SEAS OF THE RED SEA AND THE MEDITERRANEAN THROUGH THE MARITIME SUEZ CANAL: I- CILIOPROTISTS

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Suez Canal is the main connecting link between the Red Sea in the south and Mediterranean in the north. It crosses many lakes, which in its turn represent different habitats. 28 plankton ciliate taxa and species were collected from the canal waters during the present study. Much interest was focused on determining from which end of the canal these organisms were invading the opposite sea. Planktonic ciliates appear to enter the Suez Canal from the south via water currents; to do so it needs to be carried over a distance of 25 km along the canal from the Gulf of Suez into the Bitter Lakes, then pass across the Bitter Lakes before being carried a further 15 km along the canal into Lake Timsah. Plankton ciliate affinities and differences between the eastern Mediterranean and the Red Sea were discussed in the light of the results of distribution studies of the twenty eight species of plankton carried out. These observations point to the role of the canal as a selective barrier and/or as a link in the process of marine bio-invasions of planktonic organisms, while cases of invasion from one sea to the other are more likely to occur in either direction, those concerning species of Indo-Pacific origin are more successful and numerous (23 species). Meanwhile, the Suez Canal acts as a local endemic habitat by itself (5 species). Thus, the opening of the Suez Canal during the year of 1869 caused marine bio-invasions in the Mediterranean Sea and rarely in the Red Sea. Accordingly Red Sea species invaded the Mediterranean ecosystem and not vice versa. Finally, it would be concluded that, a continuous monitoring programme must be launched especially after the new expansion of the canal and opening the new one to record the recent invasive plankton species and follow up the distribution and abundance of those previously recorded as invasive or alien species to assess their impacts on the native biodiversity of the Suez Canal, Red Sea and Mediterranean.

## THE STABILITY OF THE MARINE CILIATE COMMUNITY TO ENVIRONMENTAL CHANGES

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A complete study of processes that occur during the formation of marine communities and their stability to factors of the environment requires information for a sufficient period of time that would cover a succession of many hundreds or even thousands of generations of organisms. Studies like these have been conducted by a team of biologists at the White Sea for a several decades. In field experiments on the transplantation of fragments of natural communities of marine ciliates into an estuary, restoration of the initial or modified species structure was observed after a short period of adaptation of the organisms. Their responses to new conditions included both species-specific reactions and group reactions (the synergistic effect). In a group, species are able to sustain larger amplitudes of variations in environmental factors than if they were separate and they can even occupy extreme biotopes. Under the conditions of the experiment, as well as in nature, a multitude of structural variants of the psammophile community (multivariability of structure) were created from the united pool of species through the recombination of their abundances. In total, the formation and maintenance of communities of unicellular organisms is determined by a complex mechanism that includes physiological (tolerance), population (reproductive properties), cenotic (interspecific interactions), and stochastic (reaction to environmental fluctuations) processes; their relative roles depend on the level of organization of the community (succession stage) and on the range of variations in environmental factors.

## AGGREGATIVE BEHAVIOR, CELL SIGNALING AND MORPHOMETRICS IN ENTAMOEBA DISCRIMINATION STUDIES

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Studies on clone- and kin-discrimination in protists have proliferated during the past decade. We report

clone-recognition experiments in seven *Entamoeba* lineages (*E. invadens* IP-1, *E. invadens* VK-1:NS, *E. terrapinae*, *E. moshkovskii* Laredo, *E. moshkovskii* Snake, *E. histolytica* HM-1:IMSS and *E. dispar*). We demonstrate that amebas themselves can discriminate self (clone) from different (themselves versus other clones). In mix-cell-line cultures between closely-related (*E. invadens* IP-1 versus *E. invadens* VK-1:NS) or distant-phylogenetic clones (*E. terrapinae* versus *E. moshkovskii* Laredo), amebas consistently aggregated with same-clone members. Additionally, we identified six putative cell-signals secreted by the amebas (RasGap/Ankyrin, coronin-WD40, actin, protein kinases, heat shock 70, and ubiquitin) and which known functions in *Entamoeba* spp. included: cell proliferation, cell adhesion, cell movement, and stress-induced encystation. Each clone was characterized morphometrically (length, width, and cell-surface area) and documented how they differed statistically from one another (as per single-variable or canonical-discriminant analyses). This is the first multi-clone characterization of *Entamoeba* spp. aggregative behavior, cell-signaling secretion, and morphometrics in the context of clone-recognition. Because unicellular eukaryotes belong to ancient and highly diverse phylogenetic lineages, occupy all environments on Earth, and participate in complex interactions with other organisms (as hosts, symbionts or parasites), they can be robust model systems to study the implications of taxa, clone and kin discrimination/recognition in ecological and evolutionary contexts, and with emphasis on basic or applied sciences.

## DIVERSITY AND ABUNDANCE OF KINETOPLASTIDS IN THE WORLD OCEAN

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Kinetoplastids include important protozoan parasites of humans, other mammals and plants. The key genera, *Trypanosoma* and *Leishmania*, belong among the best-studied unicellular eukaryotes. However, our knowledge of diversity and distribution of basal, mostly free-living heterotrophic lineages is rather limited, despite their potential importance in understanding the evolution of parasitism in kinetoplastids. We have analyzed kinetoplastid 18S rDNA barcodes (V9 region) in a large global metabarcoding dataset, combining 123 stations of the Tara Oceans. According to our results, the majority of planktonic kinetoplastids belonged to Neobodonida: about 90% reads and 70% OTUs. Compared to their sister-clade, diplomonads, kinetoplastids show similar distribution patterns: both are more abundant in the mesopelagic than in the photic zone, in the picoplankton (<5 µm in size), and demonstrate no apparent biogeography. Both groups are dominated by a few abundant cosmopolitan OTUs: neobodonids and *Bodo sorokinii* in the case of kinetoplastids. However, kinetoplastids were much less abundant in all depth zones (on average 0.2% of eukaryotic reads per sample, and ranging from 0% to 10.8%), and much less diverse (~650 OTUs vs. >50,000 OTUs for diplomonads). Also, unlike diplomonads, planktonic kinetoplastids show only weak vertical structure with 23% OTUs present in all sampled depth zones: surface, deep chlorophyll maximum, and mesopelagic. Rarefaction curves revealed that kinetoplastid diversity was saturated in the whole dataset, as well as in separate depth zones, size fractions, and oceanic provinces. Our results suggest kinetoplastids are rare but ubiquitous component of the global plankton.

#### FISH TECHNIQUE AS A POSSIBLE TOOL FOR CORTEX INVESTIGATION IN ARMOPHOREAN CILIATES (CILIOPHORA, ARMOPHOREA)

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Fluorescence *in situ* hybridization (FISH) is nowadays common molecular technique. One of possible application of this technique is *in situ* identification and phylogenetic position of uncultured bacterial endosymbionts (Amann et al., 1991). For this purpose rRNA-targeted oligonucleotide probes were developed for different groups of bacteria. In protistological studies alphaproteobacterial and

eubacterial probes which able to visualize wide range of most common bacterial endosymbionts are used more often. During long time of using (Fokin et al., 1996), I have found that sometimes, using the same protocol, these probes specifically can bind also to several cortical structures of ciliated protists, namely: kinetosomes, extrusomes, and cortical granules. The reason of such unusual FISH results is not so clear from molecular point of view. However, this “impregnation technique” could be useful for morphological and taxonomical investigations of ciliates. In many cases (representatives of Nassophorea, Plagiopylea, Heterotrichea, and Spirotrichea) such decoration could be found only sporadically, but for Armophorea members the cortical granules always impregnated by both oligonucleotide probes. In majority of heterotrichs in which presence of cortical granules is very common feature I could not able to get such a FISH impregnation. Phenomenon discovered not only allows to use FISH technique in some cases for the ciliate’s kinetome investigation, but puts a question about origin of cortical granules in Armophorea and in other ciliate lineages. Unusual labelling are illustrated by FISH images of different *Metopus* spp., *Brachanella spiralis*, *Copemetopus* sp., *Blepharisma* sp., *Euplotes crassus*, *Sonderia vorax*, and *Pseudomicrothorax dubius*.

#### AN ALVEOLATE ALGA *VITRELLA BRASSI-CIFORMIS* FORMS ZOOSPORES BY BUDDING

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The ease of cultivation and availability of genomic data promoted intense research of free-living relatives of apicomplexans, *Chromera* and *Vitrella*, to understand evolutionary processes leading to the emergence of parasitism. Recently the ultrastructure was revealed of an apically located structure of *Chromera velia*, supposedly homologous to the apical complex essential for host invasion in Apicomplexa. Yet, *Chromera* and *Vitrella* differ significantly in their physiology, morphology, phylogenetic position and genomic features, and *Chromera* is likely the more evolutionarily advanced of the couple. Hence, in an expectation of more ancestral features, we inspected in further

detail the ultrastructure of *Vitrella brassicaformis*, concentrating on the apical region of the flagellar stage. A previous work described a lifestage, surrounded by roughly structured matter of the containing sporangium, with an intracytoplasmic axoneme structure suggested to develop into mature flagellum. Here we describe a novel stage with cells developing a flagellum extracellularly. These cells concurrently bud off the center of the sporangium, surrounded by smooth matter, in a process reminiscent of ectomerogony as seen in *Eimeria*. We suggest that the former process generates gamete-like flagellate progeny, while the latter process yields zooflagellates. We failed to find apical structure similar to the pseudoconoid in *C. velia*. We propose that the common ancestor of Apicomplexa and “chrompodellids” exhibited a complex lifecycle, while extant descendants underwent gradual reduction of these processes as forced by their environment.

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#### DIFFICULTIES IN ASSESSING THE GLOBAL DISTRIBUTION OF A MODEL ORGANISM - THE BIOGEOGRAPHY OF *FAVELLA PANAMENSIS* (ALVEOLATA, CILIOPHORA)

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Accurate circumscriptions and determinations of species are essential for biodiversity and biogeography assessments; traditionally, the morpho-species concept was employed. Tintinnid ciliates are exceptional as - in contrast to the vast majority of ciliates - the whole taxonomy and classification of the more than one thousand marine planktonic species are almost exclusively based on characteristics of their vase-shaped loricae (houses). Because it is relatively easy to collect, preserve, examine, and classify, the lorica has allowed the accumulation of invaluable diversity and distribution data for more than two centuries. However, lorica-based taxonomy is problematic because of the high intraspecific variability and interspecific similarity of the loricae; so, the species limitations are currently unknown. Besides misidentifications, the diversity and geographic ranges perceived are especially affected by revisionary taxonomic treatises differing in their species circumscriptions from the original descriptions due to synonymisation (range of morphologic features became wider) and splitting (range of features became smaller) of species. Despite these difficulties, Montagnes (2013)

suggested the tintinnid genus *Favella* as a model for planktonic ciliates. In the present study on the biogeography of *Favella panamensis*, the species records from about one hundred of taxonomical and ecological studies were classified according to their quality: (i) reliable records from the type and neo-type localities mentioned in the original description and authoritative redescription; (ii) more or less reliable records supported by descriptions, measurements, and/or illustrations that fit the original description and redescription; and (iii) unsubstantiated records (mostly simple species lists) based on uncertain identifications. The comparison of the data provided by substantiated records with the original description revealed false positive and negative identifications. Since currently the species circumscriptions are uncertain, the usage of the original descriptions or authoritative redescriptions for the identification of tintinnid ciliates is strongly recommended. Only later, when the cell features and barcodes of the morphotypes are known, we might be able to perform justified synonymisations. References. Montagnes (2013): Ecophysiology and behavior of tintinnids. In: Dolan J.R., Montagnes D.J.S., Agatha S., Coats W.D. & Stoecker D.K. (2013): The Biology and Ecology of Tintinnid Ciliates: Models for Marine Plankton. Wiley-Blackwell: 85-121.

#### THE BEGINNING: INFERENCE OF THE TINTINNID ANCESTOR'S MORPHOLOGY (ALVEOLATA, SPIROTRICHA, TINTINNINA)

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Tintinnids are a speciose group of ciliates, whose taxonomy and systematics are almost exclusively based on lorica features. Physico-chemical factors and the cell cycle, however, influence these taxonomic characters, resulting in an artificial classification with several non-monophyletic groupings. The sparse cytological data and the more comprehensive molecular phylogenies indicate that the tintinnids developed in the marine environment and entered freshwater several times independently. The tintinnidiid genera *Tintinnidium* and *Membranicola* are typical representatives of the limnetic plankton and are characterized by a simple somatic ciliary pattern and a strong synapomorphy, namely, the two de-novo-originating ventral organelles. The family is sister to the remaining, mainly marine species. As yet, only a single member of the genus *Tintinnidium*, *T. mucicola*, is known to occur in marine and brackish coastal waters, suggesting that it might provide clues pivotal to



infer the morphology of the tintinnid ancestor's morphology. Especially, the ventral organelles of the Tintinnidiidae require a closer inspection owing to their uncommon de-novo-origin which contrasts the intrakinetical proliferation of basal bodies in the other somatic kineties of the Tintinnidiidae and the somatic ciliature in all other tintinnids. Accordingly, the morphology of lorica and cell of *T. mucicola* are described based on live observations and protargol-impregnated material and the cell division pattern is analysed with special emphasis on the development of the ventral organelles. The study indicates that all somatic ciliary rows of *T. mucicola* are exclusively dikinetidal and develop by intrakinetical proliferation of basal bodies and thus demonstrate the plesiomorphic character states already present in the dorsal rows of the euplotids and hypotrichs as well as in the girdle and ventral kineties of the Oligotrichida. The species has two obliquely orientated kineties resembling the ventral organelles in the other *Tintinnidium* species and the monotypic genus *Membranicola*. Hence, the ancestor of the tintinnids apparently had exclusively dikinetidal somatic kineties all reproducing by intrakinetical proliferation of basal bodies, and the de-novo-origin of the ventral organelles in the remaining Tintinnidiidae necessitates confirmation. The study was financially supported by the Austrian Science Fund (FWF): Project P28790.

#### THE BLACK SEA TINTINNIDS (SPIROTRICHEA, CHOREOTRICHIA, TINTINNIDA): CURRENT STATE OF BIODIVERSITY AND SPECIES COMPOSITION

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According to literary and own data considering recent nomenclature revision there were 20 tintinnid choreotrich species in the Black Sea: *Codonella lagenula*, *Helicostomella subulata*, *Tintinnopsis campanula*, *T. compressa*, *T. lobiancoi*, *T. rossolimi*, *T. baltica*, *T. karajacensis*, *T. cylindrica*, *T. tubulosa*, *T. beroidea*, *T. meunieri*, *T. urnula*, *T. minuta*, *Tintinnidium mucicola*, *Metacylis jorgensenii*, *M. mereschkovskii*, *Favella ehrenbergii*, *Stenosemella ventricosa* and *S. nivalis*.

It should be noted that against the background of stable level of tintinnid diversity we observed the significant changes in the species composition of the group. Thus from 2001 to 2015 we registered 8 invasive species: *Eutintinnus lususundae*, *E. tubulosus*, *E. apertus*, *Eutintinnus* sp., *Salpingella decurtata*,

*Tintinnopsis tocantinensis*, *Rhizodomus tagatzi* and *Amphorellopsis acuta* replacing the indigenous species as *Codonella lagenula*, *Helicostomella subulata*, *T. compressa*, *T. lobiancoi*, *T. rossolimi* and *Metacylis jorgensenii*. However, the indigenous *Tintinnopsis campanula*, which was not observed during 10 years (since 1999) again, began to be present in the plankton from 2009 constantly.

The finds of alien species are not rare, during certain seasons they reach high abundances. For example, such species as *Eutintinnus lususundae* and *E. tubulosa* are often dominating in the plankton community.

The reasons for mentioned changes are not known exactly. It is possible that these related with invasion of ctenophore *Mnemiopsis leidyi* into the Black Sea, which caused significant changes in the plankton community structure. Resulting the current list of the Black Sea Tintinnida consist of 23 species including invasive.

#### MITOCHONDRIAL TRANSCRIPTOME OF *LEPTOMONAS PYRRHOCORIS* H10 - CLOSE MONOXENOUS RELATIVE OF *LEISHMANIA* CLADE

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Mitochondrial genome of trypanosomatids have several unusual traits both in it's structural organization and in it's expression. About 22 mitochondrial genes are compactly grouped in coding region of maxicircle - major coding component of the mt-genome. Some transcripts undergo extensive RNA-editing process before they become mature mRNAs and this is mediated by small gRNAs coded in minicircles. Biological advantages of such complex expression system remains unclear but it definitely plays crucial role in mitochondrial genome expression. Editing is well-studied in some dixenous species like *Trypanosoma brucei* and *Leishmania tarentolae* but less data exists for monoxenous species. In this work we present a full mitochondrial transcriptome map of *Leptomonas pyrrocoris* H10 generated using RNAseq data. The overall transcription profile appeared to be similar to profile of *Leishmania tarentolae*.

Optimized U-insertion\deletion search software TAligner and usage of NGS technology enabled us to focus on molecular intermediates of editing process and build detailed editing graphs for all maxicircle cryptogenes. Reconstruction of editing process in cryptogenes showed that in most cases editing can proceed in a variety of alternative ways yielding ORFs with lots of aminoacid substitutions or even with alternative protein product. Our results suggest that editing can be the major driving force of rapid adaptation to environment changes through the generation of proteins diversity.

#### GENETIC DIVERSITY OF CENTROHELID HELIOZOA IN BRACKISH INLAND WATERS OF RUSSIA

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Centrohelid heliozoa are ubiquitous, unicellular, non-ciliate phagoheterotrophs with slender radiating axopodia for trapping prey. Centrohelids have a cosmopolitan distribution; inhabit benthos and periphyton in both marine and freshwaters ecosystems, in communities they play role of predators grazing on other microorganisms. At present both genetic and morphological approaches for their taxonomy and classification are used. Morphological and genetic diversity of heliozoa in environmental saline and brackish waters has been studied very poorly. Genetic diversity of centrohelids with group-specific originally designed primers was studied by NGS with MiSeq (Illumina). Fifteen samples from brackish inland water bodies of Russia with mineralization 2–20‰ were examined. As a result 40 OTUs were obtained. Most of the environmental OTUs (32) were aligned with *Polyplacocystis contractilis* (AB196984, NCBI) with support 99% and were placed inside Pterocystina clade. This fact indicates that the most common habitants in brackish waters are related to this particular genetic variant of centrohelida. Other OTUs with 26% and 28% support grouped with environmental centrohelidian sequences from hypersaline habitats and occupied a basal position in Pterocystina A clade.

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#### BIOGEOGRAPHY AND THE GENETIC STRUCTURE OF MORPHOSPECIES IN THE GENUS *MAYORELLA* (AMOEBOZOA, DISCOSEA, LONGAMOEBIA)

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Due to the lack of biogeographical data for the majority of naked lobose amoebae genera, the genetic structure of amoebae morphospecies have been previously observed only in several species from Flabellinia group (Amoebozoa, Discosea). Further extensive study of amoebae biogeography including species from different phylogenetic lineages is clearly required given the assumption of amoebae significance for freshwater microhabitat function based on their abundance. In this study Cox I and 18S rDNA genes were used as DNA barcodes in order to identify strains of the genus *Mayorella* (Discosea, Longamoebia) in addition to morphological data, that allowed to elucidate the morphospecies genetic structure and general principles of their distribution in a global scale and to evaluate resolving power of various identification approaches. The samples were collected from distant freshwater habitats of North America, Europe and Russian Far East. Results contributed to the amoebae morphospecies conception which represents it as a limited set of genetic lineages. Supported with SPSU project 1.38.251.2014

#### DEEP SEQUENCING OF ANCIENT 18S RDNA IN SEDIMENT CORES FROM CONTINENTAL SHELVES OF CHINA

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Protists/phytoplankton in overlying waters and terrestrial biomaterials could be transported, deposited and buried in sediments on continental shelves of marginal seas. Some of these ancient DNA could be preserved, serving as a powerful proxy for reconstructing past regimes of the ecosystems. We obtained three sediment cores each about 2.5 meters in length from three sites at the Bohai Sea (the mouth of Yellow River, shorted as YR) and Yellow Sea (the northern basin, NYS, and southern basins, SYS). The DNA was extracted and quantified and subjected to high throughput sequencing of eukaryotic 18S rDNA fragment using Illumina MiSeq. Classification using pipelines indicated the

presence of molecular signals of eukaryotic groups including Dinophyta, Stramenopiles, Ciliophora and Fungi. The relative proportions of these major groups were different among the three sites, with NYS and SYS were more similar to each other than to YR. Here we will briefly report the community composition and structure of microbial eukaryotes, and hope to link these signals in the near future with geochemical information to reflect the ecosystem conditions and changes of the north China Seas during the past thousands of years.

#### FEEDING SELECTION OF MIX-TROPHIC FLAGELLATE, *POTERIOOCHROMONAS MALHAMENSIS*, ON THE GREEN ALGAE OF *CHLORELLA*

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*Poterioochromonas* is a very common mix-trophic flagellate in most freshwater environments, which can grow in autotrophic way or by feeding bacteria and other protists. Several species of algae have been reported to be the prey of *Poterioochromonas*, however little is known about whether and how *Poterioochromonas* has feeding selection on the prey. In this study, two strains of *Chlorella sorokiniana* showed significantly different defense ability on the grazing of *Poterioochromonas*. Both morphological and molecular approaches were combined to discover the factors which contributed to the defense ability on grazing. Light microscopic showed that the two strains didn't have much difference on the cell size and shape, however transmission electron microscopic observations and protein composition analysis indicated that the two strains have different cell wall composition and biochemical composition. Our research suggested that probably some protein in the cell wall of *Chlorella* played an important way to defense the grazing of *Poterioochromonas*.

#### THE GENOME OF *UNDARIA PINNATIFIDA* AND *UNDARIA PETERSENIANA*: INSIGHTS INTO KELP EVOLUTION

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The genus *Undaria* consists of large multicellular brown algal kelps. In Korea, *U. pinnatifida* and *U. peterseniana* are extensively cultivated (~500,000 tons/year) for human food and commercial extracts. Although phylogenetically related, the two species are ecologically distinct and have strikingly different distributions, morphologies and reproductive strategies. Spores of *U. pinnatifida* are produced in a specialized sporophyll that is localized on the lower part of the stipe whereas in *U. peterseniana* the sporophylls differentiate directly from the blades, which is a process similar to that for species of the genus *Saccharina*, another common kelp genus. We produced draft genomes for *U. peterseniana* and *U. pinnatifida*; with the previously released draft genome of *Saccharina japonica*, they represent the only genomic resources available for the kelps. We examined gene content, genome organization, and transposable elements dynamics. We will discuss the genomic differences between *U. pinnatifida* and *U. peterseniana*, and we will describe new insights into their evolutionary history; most notably, we will comment on the origin of multicellularity in the brown algae. Finally, the genome sequences for the two edible algae should be a new, major resource for kelp crop improvement and biotechnology.

#### NEW GENOMES OF UNICELLULAR HOLOZOANS SHED LIGHT ONTO THE ORIGINS OF COMPLEX ANIMAL GENE ARCHITECTURE

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The origin of animal multicellularity is a major event in eukaryotic evolution. Metazoans share many novelties in genome content and structure related to their multicellular lifestyle, like gene families (transcription factors and specific signaling pathways) and regulatory mechanisms (alternative splicing or enhancer-enabled introns). Since many of these traits predate metazoans, the study of their unicellular holozoan relatives is key to understand animal origins. We analyse the evolution of gene architecture using new ichthyosporean genomes, choanoflagellates and the filasterean *Capsaspora owczarzaki*, plus 40 other eukaryote genomes. This two-fold analysis focuses on the evolution of 1) intron/exon structure and 2) protein domain architectural rearrangements (which define gene families' function and diversification). Animals



have complex gene architectures, intron-rich and with high protein domain diversity. We find that such enrichments occur in differentially-timed innovation bursts. For example, the most important premetazoan intronization event occurred in the last common ancestor of choanoflagellates and animals (>80% homologous introns when compared to the urmetazoan). Conversely, animal-specific protein domain arrangements often appear before, in the urholozoan (~12% of novel arrangements, compared to frequent contractions in later-branching lineages). Our results support a scenario of sudden innovation processes within holozoans followed by gradual simplifications in extant protistan lineages, while Metazoa retain most novelties and develop more of their own. This is observed for both intronization and protein rearrangements. Yet, these aspects of gene architecture are decoupled in time and mechanisms: non-coding and coding gene elements are under different evolutionary pressures, and innovation bursts are identified in different ancestors.

THE ROLE OF *CAEDIBACTER*-INDUCED KILLER TRAITS IN *PARAMECIUM* INTER-STRAIN AND INTERSPECIES COMPETITION  
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Some species of *Paramecium* (Ciliophora) present killer traits when inhabited by bacterial endosymbionts belonging to the genus *Caedibacter*. These bacteria are occasionally released by killer strains to the environment, get engulfed by other paramecia, and secrete a not yet characterized toxin, which kills symbiont-free ciliates. We studied three *Paramecium biaurelia* strains bearing cytoplasmic symbionts *Caedibacter caryophilus*. The killer strains originated from St Petersburg region, Russia, and from Sicily, Italy. All strains were characterized by different rate and dynamics of killer effect manifestation; sensitive paramecia displayed various lethal and malfunction symptoms. The killer cells were resistant to their own killer trait even after antibiotic-induced removal of symbionts, thus allowing to assume that resistance is genetically determined. Also killer strains appeared to be relatively reciprocally resistant for the other killers. We made a series of tests to reveal resistance or sensibility to killer effects for a number of strains

representing several *Paramecium* species. The results indicated that the strains of the *P. aurelia* species complex and *P. caudatum* were generally rather sensitive, while the strains of phylogenetically distant species were almost always resistant. Thus, *Caedibacter* might give a significant advantage for the host, providing it with a powerful weapon for competition with uninfected paramecia normally present in the same environment. Several strains of symbiont-free *P. biaurelia* also appeared to be resistant, thus allowing to speculate that this species might be genetically adapted to be the host of *C. caryophilus*. Supported by RFBR 16-04-01195.

HIDDEN DIVERSITY IN *MALLOMONAS MATVIENKOA*E SPECIES COMPLEX

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*Mallomonas matvienkoe* B. Asmund & Kristiansen is considered as a widespread taxon (Kristiansen & Preisig 2007) from the order Synurales, class Chrysophyceae. This species have been described from Ukraine under the name *Mallomonopsis elliptica* Matv. (Matvienko 1941) and later redescribed and transferred into the genus *Mallomonas* (Asmund & Kristiansen 1986). *Mallomonas matvienkoe* is a polymorphic species; it includes five varieties and forms. An investigation of the clonal cultures of *M. matvienkoe* representing populations from subtropics resulted in descriptions of three new species using both morphological features and molecular data (Jo et al. 2013). The authors also described a fossil species of this group. Our studies of this species complex in the tropics (Vietnam and Indonesia) revealed at least four new taxa for science confirmed by molecular methods. One of them has been already described as *M. paragrandsis* E.S. Gusev (2015). Additionally, five new morphotypes of scales have been found which can be considered as new species for science. Three taxa, recently described by Jo et al. (2013), were also found in Vietnam (*Mallomonas hexareticulata*, *M. sorohexareticulata* and *M. pseudomatvienkoe*). Thus, *Mallomonas matvienkoe* species complex is a rather diverse group of pseudocryptic taxa, which needs further studies using polyphasic approach. This study was supported by RFBR grants 15-04-04181 and 16-34-60099.



THE PARASITIC ASSIMILATION FROM DIATOMS BY *APLANOCHYTRIUM* (THRAUSTOCHYTRIIDAE, LABYRINTHULEA, STRAMENOPILES)

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Thraustochytrids inhabit the ocean all over the world and have been recognized as important eukaryotic decomposers in the marine ecosystem. The monitoring works have suggested that they obtain nutrients from the terrestrial organic matter at estuarine areas. On the other hand, the habitat of the thraustochytrids is spread over the coastal areas, in which there are almost no influences from terrestrial fields. This suggests that the nutrition source of the thraustochytrids in the coastal areas is the detritus of phytoplankton. In this study, we investigated the assimilation of the thraustochytrids from diatoms, which are important primary producers in the coastal areas. First, we examined growth tests of the thraustochytrids under the 2-membered culture condition with *Skeletonema* diatoms. As the result, *Aplanochytrium* strains have quite higher growth than 8 other genus-level groups of the thraustochytrids. Observation under light microscopy showed that the vegetative cells of *Aplanochytrium* extended the pseudopod-like ectoplasmic nets to the diatom cells, and then chloroplasts of the diatoms shrank and bleached. It was suggested that the thraustochytrids play the role as not only decomposers but also “predators”. Moreover, *Aplanochytrium* cells pulled the diatom cells and made aggregates, which are suited to be baits for juvenile fishes. It is possible that the food chain pathway of the *Aplanochytrium* has a large impact on the marine ecosystem because there are few steps in the food chain between the producers and the higher consumers.

A RE-INVESTIGATION OF THE PELAGOPHYTE GENUS *SARCINOCHRYSIS* USING MATERIAL COLLECTED FROM THE TYPE LOCALITY

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Modern systematists use molecular markers to identify and distinguish species; however, many microalgae were described before gene sequencing was invented and the holotype was often an ink drawing. Cryptic speciation and biogeographic isolation are potential problems when anchoring an old species name with a modern gene sequence. Therefore, to avoid possible confusion caused by a misidentified microalgal culture from a different locality, the best approach is to sequence genes from the alga re-collected from the type locality. The marine alga *Sarcinochrysis marina* was described in 1930 by Geitler from the Canary Islands, Spain; Geitler used two cultures in his study, but these cultures no longer exist. *Sarcinochrysis marina* is the oldest genus in the Pelagophyceae. We re-isolated *S. marina* from the type locality near Las Palmas, Gran Canaria. Furthermore, we included additional Pelagophyceae strains that were obtained from culture collections. We produced a total of 109 sequences, representing the nuclear-encoded SSU rRNA and the plastid encoded *rbcL*, *psaA*, *psaB*, *psbA*, and *psbC* genes. The sequences were used to infer maximum likelihood phylogenetic trees. We anchored the genus *Sarcinochrysis* using the Las Palmas isolate, and we discovered several undescribed genera and species that are morphologically similar to *Sarcinochrysis*.

UNEXPECTED DIVERSITY OF THE PECULIAR GENUS *CRENEIS* (EXCAVATA: HETEROLOBOSEA)

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*Creneis* is a recently (2014) discovered genus of marine anaerobic heteroloboseids. Its only species, *C. carolina*, displays several uncommon features including amoeboid flagellates with a single flagellum, a multiflagellate form with ca. 14 flagella and the unique structure of its flagellar apparatus. Therefore, its affiliation to Heterolobosea is recognizable only thanks to the results of molecular-phylogenetic analyses. *Creneis* was described on the basis of a single isolate and has never been observed again. We have established six marine *Creneis* strains in culture. According to the morphology and SSU rRNA gene

sequences, our strains represent five novel species of *Creneis*. The species morphologically differ from each other as well as from *C. carolina*; the diagnostic features include the cell size, character of the flagellum, type and arrangement of pseudopodia, and character of the uroid and uroidal adhesive filaments. At least three new species are able to form the fast-swimming “multiflagellate” form, which, however, possesses only four or five flagella. Our results show that *Creneis* is a widespread and diverse lineage of anaerobic protists. Because six known species (including *C. carolina*) are represented by only seven strains, its true diversity is certainly much higher.

#### PREDATORY CAPABILITIES OF THE FILASTEREAN *CAPSASPORA OWCZARZAKI* REVEALS ITS POTENTIAL FOR A FREE-LIVING LIFESTYLE

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*Capsaspora owczarzaki* is one of only two known members of Filasterea – a group of protists closely related to animals. Due to its phylogenetic position, complex life cycle, and wide gene repertoire, it has become an important non-model organism in evolutionary studies, especially on the origin of animals. *C. owczarzaki* was isolated from several strains of freshwater snail *Biomphalaria glabrata*. However, it remains unclear whether it is an obligate symbiont or it can have a free-living lifestyle. In order to answer this question, we tested the interaction of *C. owczarzaki* with bacteria (*Enterobacter aerogenes*), with other unicellular eukaryotes (*Dictyostelium discoideum* and *Acanthamoeba castellanii*), and with *B. glabrata* embryonic (BGE) cells. The interactions were observed and documented by microscopy. In addition, we followed the growth of *C. owczarzaki* in the presence of these organisms. We found that *C. owczarzaki* is able to grow on a diet consisting

only of bacteria. Furthermore, it efficiently kills and consumes *Dictyostelium discoideum* and BGE cells, and ingests material from live *Acanthamoeba castellanii*. We performed RNAseq of *C. owczarzaki* fed by the organisms mentioned before and found distinct expression profiles for each food type. Our results strongly suggest that *C. owczarzaki* is not an obligate snail symbiont, but rather an opportunistic predatory organism able to feed on a variety of food types. This research increases the knowledge on the diversity of lifestyles among unicellular holozoans, and has implications on our understanding of the origin of animals.

#### MORPHOLOGY OF TWO SPECIES OF THE THECATE DINOFLAGELLATE GENUS *BLEPHAROCYSTA* EHRENBERG (DINOPHYTA) FROM THE TROPICAL MEXICAN PACIFIC

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Species of the thecate dinoflagellate genus *Blepharocysta* Ehrenberg are usually globose, spherical to ovoid, and lack a depressed cingulum and sulcus, typical of most dinoflagellates. The five recognized *Blepharocysta* species are truly marine planktonic forms and are mainly distributed in tropical and subtropical areas. During an oceanographic cruise along tropical coasts of the Mexican Pacific, net phytoplankton samples yielded a number of tropical forms of dinoflagellates, including two *Blepharocysta* species: *Blepharocysta paulseni* and *B. splendormaris*. Both species were studied by LM and SEM and the general morphology previously described in the literature was confirmed, although the theca ornamentation was observed in detail. The cell shape, more ovoid in *Blepharocysta splendormaris* and more spherical in *B. paulseni*, the development of sulcal and theca ornamentation lists, with *Blepharocysta paulseni* having more reduced lists and a higher pores density, appear as the morphological characters separating both species. Additionally, *Blepharocysta splendormaris* has been found not only in tropical areas, but also in Antarctic waters. These two species (and probably all *Blepharocysta* species) showed no chloroplasts and are supposedly heterotrophic. Comparison among all species of the genus was made, emphasizing the shape and relative size of the cells, the development and ornamentation of the sulcal lists, tabulation and ornamentation of the theca, and the general distribution.

## TWO NEW SPECIES OF THE DINOFLAGELLATE GENUS *PHALACROMA* STEIN (DINO-PHYTA) FROM THE TROPICAL MEXICAN PACIFIC

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Species of the thecate dinoflagellate genus *Phalacroma* Stein are common in the marine phytoplankton all over the world, but especially diverse in tropical areas. Some species are considered to produce Okadaic Acid or Dinophysistoxin, which cause diarrhetic shellfish poisoning (DSP). The genus *Phalacroma* includes mixotrophic and heterotrophic species with an elevated epitheca, visible in lateral view and narrow horizontally projected cingular lists. During surveys of phytoplankton from the tropical Mexican Pacific, two undescribed species of *Phalacroma* were found, and in this paper they are depicted following detailed observations by LM and SEM. *Phalacroma ornamentatum* sp. nov. is a new species, superficially similar to species of the *Phalacroma rotundatum* (Claparède et Lachmann) Kofoid et Michener group, but its main distinctive characteristics are: (1) thick theca, (2) ornamentation of the theca and sulcal lists, and (3) relatively narrow sulcal lists and junction close to the cingulum. The other new species is *Phalacroma palmatum* sp. nov., which is relatively similar to *Phalacroma bipartitum* Kofoid et Skogsberg, but is characterized by (1) shape and extension of the left sulcal list to the posterior part of cell, (2) absence of R2 in the left sulcal list, (3) sulcal lists joined at the cingulum level, and (4) ornamentation of the theca and both sulcal lists. *Phalacroma ornamentatum* was fairly common in the Gulf of California, whereas *Phalacroma palmatum* was very rare.

## ORGANELLE DNA REPLICATION IN CHLORARACHNIOPHYTE ALGAE

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Plastids evolved by multiple endosymbiotic events where photosynthetic organisms became fully integrated with host eukaryotic cells. Chlorarachniophyte algae possess complex plastids acquired by the uptake of a green alga, and the plastids harbor

a relict nucleus of the endosymbiont, the so-called nucleomorph. Therefore, chlorarachniophyte cells possess three different endosymbiotically-derived genomes in the plastid, mitochondrion, and nucleomorph. These organelle genomes do not encode essential DNA replication components (i.e., DNA polymerases), and it remains unclear whether nucleus-encoded counterparts are imported into respective organelles. To gain insight into the evolution of complex plastids, we investigated nucleus-encoded DNA polymerases related to the chlorarachniophyte organelles. We found that chlorarachniophytes evolved two phylogenetically distinct DNA polymerases for the plastid and mitochondrion, although a single DNA polymerase is dually targeted to both organelles in Archaeplastida. Our phylogenetic analyses suggest that the mitochondrion-targeted one is originated by the host, and the plastid-targeted one seems to be derived from a red algal lineage via lateral gene transfer. We also discovered a nucleomorph-targeted DNA polymerase that was phylogenetically related to viral sequences. Our data imply that organelle DNA polymerases of chlorarachniophytes have been replaced by a counterpart of other organisms during the evolution.

## PRIMARY AND SECONDARY ENDOSYMBIOTIC GENE TRANSFER OF BACTERIAL RECA IN EUKARYOTES

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Recombinases promote DNA repair by homologous recombination and these genes occur in all known domains of life: virus and bacteria (RecA), Archaea (RADA, RADB) and Eukaryotes (RAD51X, DMC1). The bacterial recombinases (RecA) are present not only among bacteria, but also in several eukaryotes: Chlorophyta, Rhodophyta, Amoebozoa, Peronosporomycetes (Oomycetes), Bacillariophyta and other SAR group lineages. The eukaryotic *recA* genes have two different origins, a mitochondrial and a chloroplastic. The acquisition of *recA* genes by eukaryotes was possible by means of endosymbiotic gene transfer (EGT) in the form of primary endosymbiosis (bacteria-eukaryote) and secondary endosymbiotic gene transfers (eukaryote-eukaryote). The RecA proteins present a transit peptide and are imported by the organelles, where they act in homologous recombination. Several major eukaryotic lines lost the bacterial recombinases, as seen in Opisthokonta, where the bacterial ho-



mologues were probably replaced by eukaryotic specific homologue or in the secondarily amitochondriate groups where it is not necessary anymore. In the other hand, *recA* genes were subject to further duplication events in green plants, where they present several forms. The reconstruction of the RecA phylogeny with its EGT events retells the very evolutionary history of the eukaryotes and also bacteria and enables a further understanding of endosymbiosis. We showed a group-wide presence of mitochondrial *recA* genes in Amoebozoa, Oomycetes, green plants and minor groups giving support to an ancestral EGT acquisition of mitochondrial *recA* prior to eukaryotic diversification, as well as chloroplastic *recA* in all major photosynthesizing groups.

#### UNVEILING SSU rDNA INTRAGENOMIC POLYMORPHISM IN DEEP-SEA FORAMINIFERA USING SINGLE-CELL HIGH THROUGHPUT SEQUENCING

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The SSU rRNA genes are commonly used for the assessment of protist diversity. It is assumed that the rDNA copies are identical within the individual cells. However, previous studies, using cloning and Sanger sequencing approach showed a high level of intra-individual polymorphism in shallow water benthic foraminifera. Here, we present the results of single cell high-throughput analysis of SSU rDNA in about 200 specimens of abyssal foraminifera. Our data confirm the presence of intragenomic polymorphism in foraminifera but levels of intra-individual divergence are different among taxonomic groups. Our approach further allows us a quick sorting of foraminifera for barcoding purposes by distinguishing unknown foraminiferal sequences from those that are already present in our database. In the case of unknown sequences the longer SSU fragment is Sanger sequenced afterwards to phylogenetically characterize the putative new species.

#### ANCIENT MITOCHONDRIAL PROTEIN SECRETION

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The bacterial origin of mitochondria has been evidenced by a number of shared features with current bacteria, including some of the protein transport components. However, most of the original bacterial protein transport pathways have been lost from the mitochondria and replaced by the protein import apparatus. To some detail, mitochondria of *Discoba* represent an evolutionary intermediate stage as they carry the largest mitochondrial genomes encoding bacterial SecY and TAT translocases. By a multi-phylome approach we have analyzed eukaryotic proteomes for nuclear encoded genes, which are exclusive to *Discoba*. We show that their nuclei encode for about forty genes not found in other eukaryotic lineages. These include eight components of bacterial type II secretion system (T2SS). We show that mitochondria of *Discoba* express minimalist T2SS, which includes the pore forming secretin in the outer mitochondrial membrane and pseudopilin in the intermembrane space. Using the bacterial and yeast two hybrid assays, we are currently looking for the putative substrate of the ancient mitochondrial protein secretion pathway.

#### OTHER-COLORED GRANULES IN THE CILIATE *CYCLOTRICHUM* SP. ARE EVIDENTLY DIATOM CHLOROPLASTS

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As a crucial part of our exploration of the diversity of animal protists associated with algal endosymbionts, we have examined the ultrastructure and molecular characteristics of a ciliate of the genus *Cyclotrichium* collected from Lake Biwa, the largest and oldest lake in Japan. The ciliates, which are nearly spherical and about 100 µm in diameter, are filled with hundreds of 2–3 µm ocher-colored granules that impart their color to the whole cell. The internal structure of these granules is indistinct by light microscopy, but examination by SEM shows them to be chloroplasts consisting of three-layered thylakoid membranes. Many of the chloroplasts seem to be at different phases of digestion in food vacuoles (from

intact-looking to half-digested), but there are also some naked chloroplasts with three surrounding membranes in the ciliate cytoplasm. To identify the origin of these chloroplasts, we attempted to analyze PCR products obtained from isolated whole ciliate cells using various specific primers. The diatom-targeted primers yielded a probable nuclear SSU rDNA, and phylogenetic analyses based on Neighbor Joining showed that this sequence pertains to the centric diatom genus *Discostella* (Stephanodiscaceae). It was especially close to that of the diatom symbiont of the dinoflagellate *Peridiniopsis penardii*. We are now carrying out a detailed study of the ciliate and its symbiont from taxonomic and life history points of view.

EVOLUTION OF THE MITOCHONDRIAL PROTEOME, FROM THE LARGE-SCALE PATTERNS TO THE NITTY-GRITTY DETAILS  
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I will review what has been inferred about the changes at the level of the proteome that accompanied the evolution of the mitochondrion from an Alphaproteobacterium, quantifying the amount of gene loss, gene replacement and gene gain. Most of the loss and replacements that separate current day mitochondria from Alphaproteobacteria took place before the radiation of the eukaryotes. Detailed analyses of the evolution of the mitochondrial complexes like the ribosome and oxidative phosphorylation show that also the acquisition of new proteins occurred mainly before the radiation of the eukaryotes, supporting an early acquisition of mitochondria in eukaryotic evolution. Secondly I will analyze the accumulation of new supernumerary subunits and assembly factors from pre-existing protein families for one protein complex in detail: complex I. There is a spectrum of protein function conservation between the complex I representatives and their non-complex I homologs. In general, the new complex I proteins appear to have been recruited from proteins that are active in mitochondria: proteins one expects to be expressed when and where complex I is active. Within complex I and its assembly there are many cases of neo-functionalization after gene duplication, one case of sub-functionalization, and one case in which a complex I protein itself appears to have been the source of the evolution of a new protein in another complex. Complex I and its assembly can therewith be regarded as a treasure trove for pathway evolution.

CONDITIONAL EXPRESSION SYSTEM IS NOT SUITABLE FOR DEVELOPMENTAL STUDIES IN *LEISHMANIA*

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The genus *Leishmania* unites parasitic protists of the family Trypanosomatidae causing leishmaniasis, several closely related diseases that affect human and animal populations mainly in the tropical and subtropical regions. The clinical manifestations vary from spontaneously healing skin lesions to progressive and potentially fatal visceral infections. Leishmaniasis represent a global health problem with over 350 million people at risk and an annual incidence rate of 2–10 million worldwide. Conventional and conditional systems allow for a controlled activation or repression of gene expression in time and space. Such systems are nowadays widely used to analyse a variety of cellular processes in numerous parasites including *Leishmania*. A T7-driven, tetracycline-inducible system for protein expression was established in a human pathogen *Leishmania mexicana*. The gene expression in this strain is tightly regulated and dose- and time dependent. We believe that it can be widely used by the parasitology community to analyse effects of genes of interest on biology, physiology and virulence of parasites causing cutaneous leishmaniasis. This system was used to analyse gene expression profiles during *L. mexicana* differentiation (procyclics, metacyclics, and amastigotes). The transcription/translation of the gene of interest was severely decreased upon *Leishmania* differentiation into metacyclic and amastigotes. However, the same expression profile was documented for the T7 polymerase. The expression was demonstrated to be not locus-specific but dependent on untranslated regions flanking open reading frames of studied genes. We concluded that the previously established conventional gene expression systems might have certain limitations in their common applications.

## OBSERVATION OF ZOOSPORE SETTLEMENT FOCUSING ON THE DEVELOPMENT OF ACTIN FILAMENTS (LABYRINTHULEA, STRAMENOPILES)

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The Labyrinthulea is characterized by an ectoplasmic net (EN) system. The nets are superficially similar to narrow pseudopods of many protists but different in the following points: their origin from a unique organelle named the bothrosome, membrane invagination along EN, and absence of mitochondria and ribosomes. The bothrosome is located near the cell surface and is a complex of the electron dense material and the endoplasmic reticulum. Previously we observed, by immunofluorescence staining, that the actin filaments are located in EN. In this study, we investigated the process from a zoospore to a vegetative cell of *Schizochytrium aggregatum*, especially focusing on the development of the bothrosome and behavior of actin filaments. After the flagella were drawn into the cell, the bothrosome appeared *de novo* at the anterior-ventral region of the cell. After the zoospore settled and rounded, the actin appeared as a spot in the same position of the newly appeared bothrosome by immunofluorescence staining. It was expected that the electron dense material of the bothrosome is the organizing center of the actin filaments. However, under immunoelectron microscopy, reaction by the anti-actin antibody was observed in EN and cytoplasm around the bothrosome, not in the electron dense material of the bothrosome. It was suggested that the function of the bothrosome is not nucleation of actin filaments. We expect that actin relates to morphogenesis of EN, that is, the actin filaments probably play an important role of pulling

the endoplasmic reticulum to the bothrosome and stretching the membrane invagination along EN.

MULTIGENE PHYLOGENY OF *SYNURA* (SYNUROPHYCEAE) AND DESCRIPTIONS OF FOUR NEW SPECIES BASED ON MORPHOLOGICAL AND DNA EVIDENCEJo B.Y.<sup>1</sup>, Kim J.I.<sup>1</sup>, Škaloud P.<sup>2</sup>, Siver P.A.<sup>3</sup>, Shin W.<sup>1</sup><sup>1</sup> - Department of Biology, Chungnam National University, Daejeon 305-764, Korea<sup>2</sup> - Department of Botany, Faculty of Science, Charles University, Benátská 2, Prague 2, CZ-12801, Czech Republic<sup>3</sup> - Department of Botany, Connecticut College, New London, Connecticut 06320, USA

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We used phylogenetic analyses based on multiple gene sequences (partial nr SSU and LSU rDNA, partial pt LSU rDNA, *psaA*, and *rbcL*) from 148 strains (including three outgroups) and scale ultrastructure to examine phylogenetic relationships among species of the colonial genera *Synura* and *Tessellaria*. The phylogenetic tree based on the combined dataset was congruent with ultrastructural characteristics of the scales. *Synura* was divided into three major clades, two including species in section *Synura*, and one representing section *Peterseniae*. One clade, consisting of seven strains of *S. uvella* (section *Synura*), diverged at the base of the genus. The second clade consisted of the remaining species belonging to the section *Synura*. The third clade, containing organisms in the section *Peterseniae* and characterized by scales possessing a keel, was monophyletic with strong support values. Based on our findings, *S. uvella* needs to be in a separate section from other spine-bearing species, and we therefore propose new sectional ranks; *Synura*, *Peterseniae*, *Curtispinae* (presence of body scales with slender spines, tubular scales, and caudal scales). We further propose four new species based on phylogenetic analyses and unique scale characters; *S. longitubularis* sp. nov., *S. sungminbooi* sp. nov., *S. soroconoepa* sp. nov., and *S. lanceolata* sp. nov.

## CORRELATION BETWEEN FRESH WATER CILIATED PROTIST COMMUNITIES AND THEIR MICRO-ECOLOGY

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River Yamuna maintains a reasonably good quality from its origin in the Himalayas to its entry into the National Capital Region (NCR) of Delhi, India. 18 sewage drains carrying industrial and domestic wastes flow into the river in the 22 km stretch (1.6% of total length) as it flows through the NCR. 80% of the pollution in the river's total length of 1375 km occurs as it passes through the NCR, severely compromising its water quality. As free-living ciliated protist species differ widely in tolerance to pollutants, the present study was conducted to catalogue ciliate communities (with appropriate morphological, morphometric and morphogenetic descriptions) at two selected locations along the river, namely the point where the river enters the NCR (S1) and 500 m downstream of the site where the largest sewage drain empties into the river (S2). Physico-chemical parameters including heavy metal concentrations were analyzed in order to assess the water quality at these two locations. Compared to S1: the Biochemical Oxygen Demand and phosphate concentrations increased 5X at S2; total hardness, total dissolved solids, conductivity, turbidity and nitrates doubled at S2; total coliforms and faecal coliform increased 50X at S2; lead concentration increased 2X times and arsenic 3X at S2. There was a significant fall in the species richness and abundance of ciliated protists at S2. Of the hypotrichous ciliates, there was a significant loss of members of the family Oxytrichidae, whereas euplotid populations were maintained. The changes in ciliate community will be discussed with respect to water quality.

#### NEW DATA ON THE ULTRASTRUCTURE OF THE GENUS *PARADERMAMOEBEA*: UNUSUAL FEEDING MODE AND PRESENCE OF CYTOPLASMIC MTOCS

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Naked amoebae engulf food objects via phagocytosis, and during this process the portion of cell membrane covered with the glycocalyx appears inside newly formed food vacuoles. Further cell has to restore membrane and reconstruct respective portion of the glycocalyx. It may be an energetically costly process for species possessing thick and highly differentiated glycocalyx. Genus *Paradermamoeba* (phylum Amoebozoa, class Discosea) includes two species – *P. levis* and *P. valamo*. Both possess highly differentiated cell coat composed of tightly

packed helical glycostyles, ca. 220 nm in thickness in *P. levis* and ca. 520 nm in *P. valamo*. Our study reveals that during formation of the food vacuole *P. valamo* resorbs cell coat at the area of contact with food object, thus avoiding digestion of glycocalyx elements. The similar feeding mode is known in other thick-coated amoebae, like *Pellita* and *Dermamoeba*. This observation confirms our earlier hypothesis, stating that thick-coated amoebae have to adopt special ways to avoid energetically costly digestion and further reconstruction of glycocalyx elements. We provide the first description of the cytoplasmic microtubule-organising centres (MTOCs) associated with dictyosomes of the Golgi complex in genus *Paradermamoeba*. This finding confirms the hypothesis that presence of cytoplasmic MTOCs is a synapomorphy of the phylogenetic lineages forming the subclass Longamoebia. Detailed study of the ultrastructure of *P. levis* reveals that so-called “supernumerary nucleus” of *P. levis* noted in the initial description is not an individual structure but an outgrowth of the cell nucleus containing its own nucleolus. Supported with RSF grant 14-14-00474.

#### ISOLATION OF FREE-LIVING AMOEBAE FROM SOUTHERN HAN-LIVER IN KOREA

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Pathogenic *Naegleria fowleri* and *Acanthamoeba* spp., free-living amoebae exist in the natural environment, are causing agents of an acute and lethal primary amoebic meningoencephalitis (PAM) and amoebic keratitis (AK) in humans, respectively. To ascertain the existence of free-living amoebae in Korea, in late August 2015, water samples of eight sites were collected in Korean hydrosphere where water skiing and recreation have been actively performed, and then the non-nutrient agar culture and PCR-based detection technique were carried out. The surface waters were collected and filtered, and then final samples were cultured

on non-nutrient agar medium with inactivated *E. coli* and subjected to PCR with various primer pairs (amplify mainly the 18S-small ribosomal RNA). Free-living amoebae were intensively detected by PCR in two collection regions (Yeosu and Yanggeong city around Southern Han-Liver). PCR products obtained from water samples of Yeosu and Yanggeong city were subjected to gene sequencing. The similarity of 18S-rRNA sequences were compared with various reference amoebae in GeneBank, and they showed 86-99% homology with *N. gruberi*, *N. philippinensis*, *N. clarki*, *Acanthamoeba polyphaga* and *Vermamoeba vermiformis*. A Korean isolate (confirmed by PCR as *A. polyphaga*) was isolated from Yeosu sample and have been subcultured in Nelson's and PYG liquid medium with 10% FBS at 30 °C incubator. In the *in vitro* cytotoxicity test, Korean isolate (tentative *A. polyphaga*) showed high cytotoxicity as much as reference amoebae, *A. polyphaga* and *A. castellanii*. This study will be useful, in the further study, for the detailed seasonal detection of free-living amoebae from Korean hydrosphere.

#### MORPHOLOGICAL AND GENETIC DIVERSITY OF OPISTHOSPORIDIA: NEW APHELID *PARAPHELIDIUM TRIBONEMAE* GEN. ET SP. NOV

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Aphelids are a poorly known group of parasitoids of algae that have raised considerable interest due to their pivotal phylogenetic position. Together with Cryptomycota and the highly derived Microsporidia, they have recently re-classified as the Opisthosporida, which constitute the sister group to the fungi within the Holomycota. Despite their huge diversity, as revealed by molecular environmental studies, and their phylogenetic interest, only three genera have been described (*Aphelidium*, *Amoebophilidium*, and *Pseudaphelidium*), from which 18S rRNA gene sequences exist only for *Amoebophilidium* and *Aphelidium* species. Here, we present the molecular phylogeny, life cycle and ultrastructure of new freshwater aphelid, which is a new genus and species

*Paraphelidium tribonemae*. Molecular phylogenetic analysis of 18S rRNA gene sequences of this parasite indicates that *P. tribonemae* defines a distinct cluster which is only distantly related to *Amoebophilidium* and *Aphelidium*, what confirms the wide genetic diversity of the aphelids. *P. tribonemae* has amoeboid opisthokont zoospores which are twice smaller than morphologically similar zoospores of *Aphelidium* aff. *melosirae*. Nonetheless, although the morphology of *Paraphelidium* species is generally similar to that of *Aphelidium* representatives, molecular phylogenetic analyses unambiguously show, that the two clades of strains are distantly related and must define two distinct genera.

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#### AN EVOLUTIONARY TRANSITION OF CHLOROPLAST DEGRADATION IN EU-GLENIDS: HETEROTROPHIC DIGESTION TO SECONDARY PLASTID SENESCENCE

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Establishments of regulatory mechanisms for the integrated endosymbiont must have been key steps in organelle acquisitions, where both endosymbiont and host processes cooperated to evolve new functions. Specifically important for the chloroplast is to establish regulatory mechanisms for chlorophyll-related metabolisms since the phototoxicity of chlorophyll would cause fatal damages. In particular, regulated degradation of chloroplasts or chloroplast dismantlement is critical not only for recycling nutrients but also for disposing the phototoxic chlorophylls safely. Unlike land plants, little has been understood for algae on chloroplast dismantlement and associated chlorophyll catabolism. We previously reported that the phototrophic euglenoid accumulates 13<sup>2</sup>,17<sup>3</sup>-cyclophosphoribide enols (CPEs) within their cells; CPEs are non-phototoxic catabolites

of chlorophylls. A phototrophic euglenoid *Euglena gracilis* exhibited that chloroplasts underwent dismantled when incubated in the dark, leaving brown-colored granules. The brown granules were massive structures of membranes generated after degraded thylakoids. Chlorophylls are converted to CPEs in an early stage of dismantling, and proteins were removed during the dismantling. Macroautophagy of chloroplasts was not apparent, and the dismantling chloroplasts seem not to undergo acidification; instead, large acidic vacuoles commonly formed beside the dismantling chloroplasts. This would be comparable to digestion of chloroplasts by phycophagic euglenoids, where also accumulated CPEs. However, the digestive degradation proceeds within acidified phagosome hence being apparently non-homologous to the dismantlement in the phototrophs. We infer that the CPE-metabolism, which has not been reported for green algae, might have been inherited from ancestral heterotrophs to phototrophs, and is now adapted into a novel mechanism to dismantle the secondary chloroplasts.

#### COMPARISON OF FRESH WATER CILIATED PROTIST COMMUNITIES FROM TWO LOCATIONS ALONG NAJAFGARH DRAIN, DELHI, INDIA, AND THEIR CORRELATION WITH PHYSIO-CHEMICAL PARAMETERS

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There are 18 sewage drains traversing the National Capital Region (NCR) of Delhi, India, that flow into the River Yamuna. Najafgarh Drain is the largest with a catchment area of around 400 km<sup>2</sup>. It picks up domestic and industrial wastes from 38 secondary drains coming from over 2/3 of the city. It carries a total flow of over 2100 million litres per day of which 30% is treated. Sewage treatment plants and effluent treatment plants discharging into the drain are inadequate and not working to full capacity. Ciliated protist communities were assessed (with appropriate morphological, morphometric and morphogenetic descriptions) at two locations along the drain: the mid-point of the drain (D1) and at the end of the drain before it empties into the river Yamuna (D2). Various physico-chemical parameters were also

measured. Sampling was carried out weekly for 3 weeks during the pre-monsoon season. Compared to D1, substantially higher levels of free CO<sub>2</sub>, chloride, phosphates and hardness were observed in D2 indicating the water here is more polluted. The ciliate communities at the two sites differed significantly. The results of detailed analyses, showing correlations between physico-chemical parameters (including heavy metals), and ciliate communities, will be presented. The significance of such data collected over a period of time along the drains, the river, and at water treatment plants, shall benefit ecologists in developing strategies to help mitigate river pollution as ciliates are known to clarify waste water and act as bio-indicators of specific pollutants.

#### OLIGOTROPHIC LAGOONS OF THE SOUTH PACIFIC OCEAN ARE HOME TO A SURPRISING NUMBER OF NOVEL EUKARYOTIC MICROORGANISMS

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The diversity of microbial eukaryotes was surveyed by metagenomic sequencing from tropical lagoon sites of the South Pacific, collected through the American Museum of Natural History (AMNH)'s Explore21 expedition to the Solomon Islands in September 2013. The sampled lagoons presented low nutrient concentrations typical of oligotrophic waters, but contained levels of chlorophyll *a*, a proxy for phytoplankton biomass, characteristic of meso- to eutrophic waters. Two 18S rDNA sites that include the V4 and V8-V9 regions were amplified from the total of eight lagoon samples and sequenced on the MiSeq system. After assembly, clustering at 97% similarity, and removal of singletons and chimeras, a total of 2,741 (V4) and 2,606 (V8-V9) operational taxonomic units (OTUs) were identified. Taxonomic annotation of these reads, including phylogeny, was based on a combination of automated pipeline and manual inspection. 18.4% (V4) and 13.8% (V8-V9) of the OTUs could not be assigned to any of the known eukaryotic groups. Of these, we focused on OTUs that were not divergent and possessed multiple sources of evidence for their existence. Phylogenetic analyses of these sequences revealed more than ten branches that



might represent new deeply-branching lineages of microbial eukaryotes, currently without any cultured representatives or morphological information.

#### THE PLASTID GENOMES OF THE CRYPTOMONAD ALGAE REVEAL LINEAGE-SPECIFIC GENE LOSS

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Cryptophytes are an enigmatic group of photosynthetic unicellular eukaryotes with plastids derived from red alga through secondary endosymbiosis that contains four different genomes (host nuclear, mitochondrial, plastid, and nucleomorph) in a cell. They are comprised of brown-, red-, or blue/green photosynthetic species, as well as colorless nonphotosynthetic species. Although its complex evolutionary history, the origins of the host and endosymbiont components in cryptophyte algae are poorly understood. Here, we report a comparative analysis of plastid genomes from six cryptophyte genera. Three newly determined cryptomonads plastid genomes of *Chroomonas*, *Cryptomonas*, and *Storeatula* share a number of features including gene arrangement and gene content with previously reported ones of *Rhodomonas*, *Teleaulax* and *Guillardia*. The plastid genomes of cryptomonads reveal lineage-specific gene loss and intron insertion in eight significant gene syntenic positions. The chlL/chlN/chlB genes, which is the light-independent (dark active) protochlorophyllide oxidoreductase (LIPOR), show the evolutionary changes across the cryptomonad lineages. We will discuss phylogenetic relationships of the red-derived lineages using 93 plastid genes showing a strong monophyletic group of the cryptophytes and haptophytes.

#### A NEW SOIL CILIATE, *BIROJIMIA SOYAENSIS* NOV. SPEC. (CILIOPHORA: UROSTYLIDA) FROM SOUTH KOREA

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A new soil urostyleid ciliate, *Birojimia soyaensis* nov. spec., was discovered from Soya Island, Incheon, South Korea. It is described the species based on live and stained specimen observation, and 18S ribosomal RNA gene sequence analyses. *Birojimia soyaensis* nov. spec. is characterized by the following features: body slender elongate and somewhat twisted; 170–200 μm × 40–50 μm body size *in vivo*; contractile vacuole located at middle of left cell margin; cortical granules present; 37–48 adoral membranelles; 3 frontal and 2 frontoterminal cirri present; III/2 and buccal cirrus present; midventral pairs only; pretransverse ventral and transverse cirri present; 1 left and 4 right marginal rows, including 3 compound rows; 5 complete dorsal kineties with additional 3 shortened kineties which are anteriorly of compound rows; 8–11 caudal cirri; 53–69 macronuclear nodules; and 2 or 3 micronuclei. *Birojimia soyaensis* nov. spec. is similar to *B. terricola*, but is distinguished by cortical granules (present vs. absent), number of caudal cirri (8–11 vs. 2–7) and number of dorsal bristle rows (8 vs. 6, on average). *Birojimia muscorum* is separated from *B. terricola* and *B. soyaensis* n. sp. by midventral row present (vs. absent, midventral pairs only) which is an important key of the Bakuellidae. Furthermore, in phylogenetic tree, *Birojimia soyaensis* has widely distance from the bakuellids. Thus, we agree with Berger (2006) that the new genus needs to establish for *B. muscorum*. Phylogenetic tree suggests this new species is the most closely related to the genus *Hemicycliostyla*.

#### FIRST MARINE PHOTOSYNTHETIC TESTATE AMOEBA CONTAINING THE CHROMATOPHORE: *PAULINELLA LONGICHROMATOPHORA*

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Of 10 formally described species in the genus, all species are marine heterotrophic species, except for one freshwater species, *Paulinella chromatophora* which is the sole species to have plastids termed the “chromatophores” of a *Synechococcus/Prochlorococcus*-like cyanobacterial origin. Here, we report the first marine phototrophic species, *Paulinella longichromatophora* sp. nov., using light and electron microscopy and molecular data. This new species contains two blue-green U-shaped plastids reaching up to 40 μm in length. Further, the new *Paulinella* species is characterized by having five oral scales

surrounding the pseudostomal aperture. All trees generated using three nuclear rDNA datasets (18S rDNA, 28S rDNA, and the concatenated 18S + 28S rDNA) demonstrated that three photosynthetic *Paulinella* species congruently formed a monophyletic group with robust bootstrap and Bayesian supports ( $\geq 99\%$  RAxML and 1.0 Bayesian support), but their relationships remained unresolved within the clade in all trees. The *P. longichromatophora*, nevertheless, clustered consistently together with *Paulinella* strain FK01, but with very poor supported. Phylogenetic analyses inferred from plastid-encoded 16S rDNA and the concatenated dataset of plastid 16S+23S rDNA demonstrated that chromatophores of all photosynthetic *Paulinella* species formed a monophyly and fell within cyanobacteria clade with a close relationship to  $\alpha$ -cyanobacterial clade containing *Prochlorococcus* and *Synechococcus* species with very robust supports of 100% bootstraps and 1.0 Bayesian posterior probabilities. Additionally, phylogenetic analyses of nuclear 18S rDNA and plastid 16S rDNA showed divergent evolution within the photosynthetic *Paulinella* population after a single acquisition of the chromatophore. After the single acquisition of the chromatophore, ancestral photosynthetic *Paulinella* appears to diverge into at least two distinct clades, one containing marine *P. longichromatophora* and freshwater *Paulinella* strain FK01, the other *P. chromatophora* CCAC 0185.

#### PREY SPECIFICITY AND MOLECULAR PHYLOGENY OF THE THECATE MIXOTROPHIC DINOFLAGELLATE *FRAGILIDIUM MEXICANUM*

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Feeding mechanism and prey specificity of the mixotrophic thecate dinoflagellate *Fragilidium mexicanum* (strain Fm-LOHABE01) were examined using the culture isolated from Masan Bay, Korea in 2011 during summer blooms of the toxic dinoflagellate *Alexandrium pacificum*. We also used novel 18S and 28S rDNA sequences for *F. mexicanum* to explore inter-species relationships within the genus *Fragilidium* and to examine its phylogenetic relationships with morphologically similar species (*Alexandrium*, *Goniodoma*, and *Pyrophacus*). *F. mexicanum* (strain Fm-LOHABE01) fed on species belonging to three dinoflagellate genera (i.e. *Alexandrium*, *Ceratium*, and *Heterocapsa*) when

separately offered a variety of prey including dinoflagellates, raphidophytes, cryptophytes, and a ciliate. In addition, *F. mexicanum* displayed different levels of specificity for species of *Alexandrium*. While *F. mexicanum* consistently fed on *A. fundyense* and *A. pacificum*, feeding on *A. affine* was observed only once. *F. mexicanum* ingested prey by direct engulfment through the sulcus, after capturing the prey by a tow filament. Phylogenetic analyses of 18S and 28S rDNA datasets demonstrated that *Fragilidium* sequences formed a monophyletic group with high statistical supports and diverged into four distinct clades. The first clade consisted of seven *F. cf. duplocampanaeforme* strains, *F. subglobosum* from New Zealand, and an unidentified *Fragilidium* sp. from Florida, USA. The second clade branched as a single sequence for *F. subglobosum* from Denmark and formed a sister lineage to *F. cf. duplocampanaeforme*, with weak statistical support. The third clade included *Fragilidium* sp. EUSK D from Angola, as well as Korean strains of *F. mexicanum* and *F. fissile*, and was very strongly supported. The last clade contained the five most divergent sequences of *F. subglobosum* strains. Further, phylogenetic analyses revealed that the genera *Fragilidium* and *Pyrophacus* were sister to a clade that included *Alexandrium* and *Goniodoma*. *Pyrophacus* was a sister to a clade containing members of the genus *Fragilidium*.

#### TWO ENIGMATIC GENERA ARE ONE? COMPARISON OF *BELONOCYSTIS MARINA* KLIMOV, ZLATOGURSKY, 2016 WITH *LUFFISPHAERA* SPP

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*Belonocystis* Rainer, 1968 and *Luffisphaera* Belcher and Swale, 1975 are enigmatic genera, which currently lack supergroup affiliation. The genus *Belonocystis* was studied mainly on the light-microscopy level, while the genus *Luffisphaera* was studied only with electron microscopy. Recently provided ultrastructural data on *Belonocystis* gave an opportunity to compare these taxa using one and the same method. This analysis discovered many similarities in the organization of the cell structure as well as in morphology of the coverings. It was clearly shown that surface structures of *Belonocystis* represent not a solid capsule, but giant scales, which are similar to those of *Luffisphaera*. Comparison of the scales has revealed that some *Belonocystis* species were similar to *Luffisphaera* species more than inside the genus. Each scale of *B. marina* was

associated with a short cytoplasmic outgrowth and the same was shown for all of *Luffisphaera* spp., studied for that matter. All organelles, described for *Luffisphaera* were found in *Belonocystis* and shown to have almost identical organization. Both have tubular mitochondrial cristae and vacuoles associated with stacks of rough endoplasmic reticulum cisternae and neither of them was shown to have kinetosomes. *B. marina* was the first record of marine species for *Belonocystis*, which again put it closer to *Luffisphaera*, initially recorded in marine and freshwater habitats. It's possible that the subsequent study of *Luffisphaera* and extra *Belonocystis* isolates in future may lead to a fusion of both or to transfer some species from one genus to another. Study was supported with RFBR grants 15-04-18101\_a, 15-29-02749-ofi\_m.

#### HIGH ARCTIC BENTHIC FORAMINIFERANS DURING THE POLAR NIGHT: DORMANCY OR ACTIVE FEEDING?

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Benthic foraminiferans are the vast meiobenthic group of protists widely represented in all marine habitats including the high Arctic. Although some foraminifera species are known to feed on bacteria, the majority of these protists keep to an algal diet, preying on diatoms and dinoflagellates. Some species are also capable of capturing prey chloroplasts and maintaining them functionally active, thus performing kleptoplasty, which also results in a change of the host cytoplasm coloration. Given the seasonal transitions to long periods of darkness performed in high latitudes and leading to scarceness of primary production, one would expect foraminiferans to face absence of prey algae and starvation, the latter resulting in either death or dormancy of the most part of population. Nevertheless, our results contradict such assumptions. In January 2015 and 2016, during the Marine Night cruise with RV Helmer Hanssen, we collected live specimens in the Kongfjorden area, Svalbard. All major species (*Nonionella labradorica*, *Islandiella helenae*, *Cassidulina reniforme*, *Elphidium excavatum*, *Elphidium bartletti*) had brightly species-specifically colored cytoplasm implying they did not starve but had access to algal food. Transmission electron microscopy revealed that all specimens had well-developed mitochondria, Golgi apparatus and endoplasmic reticulum, therefore being metabolically active throughout the winter season. Moreover, some foraminiferans possessed intact

chloroplasts within cytoplasm. These results strongly indicate that high Arctic benthic foraminifera are not dormant during the polar night, and they access algal diet.

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#### TIM17 FAMILY PROTEIN IN THE MITOSOMES OF *GIARDIA INTESTINALIS*

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Protein transport across the mitochondrial membranes is mediated by the TIM, TOM and SAM complexes. These complexes are common to all supergroups of eukaryotes suggesting that they were already present in the last eukaryotic common ancestor. However, mitochondria-related organelles of *Giardia intestinalis* known as mitosomes were thought to lack both the SAM (a beta barrel assembly complex in the outer membrane) and the TIM (the translocase of the inner membranes) their membranes. The question was, how proteins pass the inner mitochondrial membrane. Here, we present the identification of Tim17 family protein in giardia mitosomes, which represents a core channel forming subunit of the TIM complex. The bioinformatic identification of this highly diverged subunit was only possible by including newly obtained orthologous metamonad sequences in the sequence profiles. We demonstrate that giardia Tim17 is specifically targeted to mitosomes, where it interacts with other proteins involved in the protein transport and the iron-sulfur cluster assembly.

#### HETEROTROPHIC NANOFLAGELLATES IN THE PLANKTON OF LAPTEV SEA

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The results of the species composition, abundance and biomass of heterotrophic nanoflagellates (HNF) were obtained for the first time in the Laptev Sea in August-September 2014-2015. An attempt was made to assess the role of HNF in grazing productions of bacterioplankton ( $P_B$ ) and virioplankton ( $P_V$ ). 24 species and forms of HNF 9 taxa and groups of uncertain systematic position were found. Kinetoplastea Honigberg, 1963 were characterized by the highest species diversity. The HNF community dominated by bacteriophages (eighteen species), also found four omnivorous and two carnivorous species.  $N_{HNF}$  ranged from 108-651 cells/ml, the biomass — 0.3-3.5 mg C/m<sup>3</sup>.  $N_{HNF}$  was three orders of magnitude lower than  $N_B$ , and  $B_{HNF}$  was 1.6-63.3% (mean 24.4±4.0%) biomass of bacterioplankton. Between  $N_{HNF}$  and  $N_B$  were detected high positive correlation ( $R=0.63$ ,  $p=0.05$ ). The virus particles (diameter of 200-531 nm) potentially can be edible objects for HNF. However, the ratio of the number of large viruses at  $N_{HNF}$  was low - 42-417. HNF could consume a significant amount of virus attached to the walls and inside bacteria. The number of bacteria with attached viruses was 8.7-26.5%  $N_B$ . On bacteria it was up to 11 virus particles. The number of bacteriophage-viruses in infected cells reached 111 particle/cell. Guided by literature data on clearance rates of water by Arctic HNF (Sherr et al., 1997), calculated that in the water column of the Laptev sea the HNF community consumed 1.1-30.4% (average 8.3%) daily  $P_B$  and 1.3-77.2% (average 14.9%) the daily  $P_V$ . The research supported by the RFBR grant 14-04-00130.

#### THE STATUS OF THE SPECIES *BALANTIDIUM ELONGATUM* FROM THE GUT OF EUROPEAN COMMON FROG

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The representatives of the genus *Balantidium* Claparède et Lachmann, 1858 have several common morphological features: sacciform or

slightly elongated body completely covered with cilia forming dense longitudinal rows. In the anterior part of the body, there is an opening of vestibulum. Such features are clearly visible under light micro-scope. Because balantidia have very few other morphological characters suitable for taxonomy, their species identity is usually determined using morphometric data. Comparative analysis of several described *Balantidium* spp. indicated that many of these specific names might be synonyms. All balantidia inhabiting the intestine of mammals and birds were recognized to belong to a single species — *Balantidium coli*, recently moved to the genus *Balantioides* Alexeieff, 1931. We supposed that some species from amphibians may be synonyms (balantidia are polymorphic) and some of them may belong to different genera. For solving this issue, we used modern electron microscopic techniques and molecular phylogenetic analysis. We studied the morphology of *Balantidium entozoon* and *B. elongatum* from the gut of European common frog *Rana temporaria* Linnaeus, 1758 using light and electron microscopy. The distinguishing features of *B. elongatum* are the long thin fusiform body and relatively short vestibulum. However, other traits (number of kinetes and the distance between them) are indistinguishable between two species. Their intracellular structures also showed no differences. The sequences of 18S rRNA gene of both species were identical to each other and to that of *B. entozoon* from the GenBank. Thus *B. elongatum* must be considered as a younger synonym of *B. entozoon*.

#### BRIDGING THE GAP BETWEEN TRADITIONAL TAXONOMY AND TRANSCRIPTOMICS IN ARCELLINIDS (AMOEBOZOA)

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Arcellinid testate amoebae are a diverse group of terrestrial and freshwater microbial Eukaryotes. Described by naturalists since the eighteenth century, Arcellinids are frequently used in biomonitoring and paleo-ecological reconstruction of past climates. They are supposed to include the oldest undisputed eukaryotic fossils, which vests them with a particular importance in the study of basal nodes of eukaryotic tree. However, difficulties in culturing these organisms as well as in the application of molecular biology protocols have limited the study of their diversity and evolutionary history. Our aim in this study was to investigate arcellinid (Amoebozoa, Tubulinea) diversity, and to reconstruct a solid and precise phylogeny combining comprehensive taxon sampling, multiple genes and detailed morphological characterisation. We obtained transcriptomes for twenty-one testate amoeba species, for most of which no molecular data was yet available. Our phylogenetic reconstruction of Arcellinids is based on the combination of morphological data and molecular data on about 350 genes and 100 000 amino acid positions.

#### HETEROTROPHIC FLAGELLATES IN THE WATER COLUMN AND BOTTOM SEDIMENTS IN THE RYBINSK RESERVOIR

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A total of 56 heterotrophic flagellates (HNF) from 15 large taxa were identified in water and bottom sediments of the large floodplain mesoeutrophic Rybinsk Reservoir during the summer period. Among them 35 species were detected in plankton and 45 species were found in benthos. Twenty-four species of HNF were common for these two habitats that constituted 44% of their composition. Ten species were recorded only in plankton and 20 species were recorded only in benthos. The orders Chrysomonadida, Euglenida and Cercomonadida differed, to a greater extent, in the species composition in water and bottom sediments. Most of the species of heterotrophic flagellates which were found in the Rybinsk Reservoir were bacteriophages. Three predatory species were also found. The abundance and biomass of HNF in the water column averaged  $991 \pm 326$  cells/mL and  $41.4 \pm 14.1$  mg/m<sup>3</sup>, respectively in the water column and  $(236 \pm 61) \times 10^3$  cells/mL and  $10.7 \pm 4.0$  µg/mL, respectively in bottom sediments. The average

values of the abundance and biomass of benthic heterotrophic flagellates were 238 and 259 times, respectively higher than in plankton. The biomass of flagellates constituted on average 11.2% of the bacterial biomass in the water column and only 0.8% in bottom sediments. The estimation of the role of flagellates in the consumption of the bacterial production demonstrated that HNFs were an important factor which regulated the bacterioplankton development grazing on average 32.3% of its daily production whereas their effect on bacteriobenthos was insignificant as they consumed only 2.0% of its production. The moderate positive correlation ( $r = 0.33$ ) and ( $r = 0.52$ ), respectively was found between the abundance and biomass of bacteria both in plankton and benthos.

#### MINIONS OF GREAT CTHULHU AWAKENING – A NEW INSIGHT INTO THE DIVERSITY OF PARABASALID SYMBIOTS OF COCKROACHES

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Parabasalian symbiots of xylophagous cockroaches from families Cryptocercidae and Isoptera (termites) have been attracting the interest of biologists for several decades; they often possess large, complex, and visually attractive cells and are of great ecological significance. Phylogenetic analyses showed that the complex forms of parabasalians (=hypermastigotes) have arisen several times independently. On the other hand, only a little attention has been paid to endosymbionts of “normal” cockroaches, although these insects show a great diversity in morphology and lifestyles. We have examined the intestines of 250 cockroaches belonging to 100 species and 20 subfamilies (out of 33), established 50 stable cultures of trichomonads, sequenced their SSU rRNA gene, and studied the morphology of some of them. Approximately one half of the obtained trichomonads formed a considerably diversified clade that contained the recently discovered hypermastigote *Cthulhu* with approximately 20 flagella and trichomonad genera *Hexamastix* and *Cthylla* with 6 flagella. By contrast, our strains have cells equipped with three or four. Thus, *Cthulhu* and *Cthylla* are not orphans anymore, but are surrounded by a cloud of lesser trichomonads. Most of the remaining strains belong to the understudied genus *Hypotrichomonas*, where they represent several novel species.

LAND USE AND LITTER CHEMISTRY IMPACT MICROBIAL DECOMPOSER COMMUNITIES AND LITTER DECOMPOSITION IN TROPICAL LOWLANDS OF SUMATRA, INDONESIA

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We investigated how the conversion of rainforest into jungle rubber, intensive rubber and oil palm plantations affects the decomposer community and decomposition processes. Further, we investigated the role of litter chemistry in structuring the decomposer system. In total 192 litterbags were placed into the field: 3 litter types (forest, rubber, oil palm) in 4 land use systems, with 2 harvest times and 8 replicates, each. After 6 and 12 months litterbags were collected and litter mass loss was measured, testate amoebae and microbial communities were studied, and litter element concentrations were measured. The decomposition in converted systems was slower as compared to rainforest; and litter of oil palms and rubber decomposed faster than that of forest. A total of 58 species of testate amoebae (TA) colonized the litter. Species number and density of TA, as well as microbial biomass (MB) were high in rainforest and jungle rubber and decreased significantly in rubber and oil palm plantations. Further, TA density and MB was highest in rubber litter, whereas TA species number was at a similar level in rubber and forest litter. Overall, our data indicate negative effects of rainforest conversion on the structure and functioning of decomposer community and, thereby litter decomposition. Further, testate amoebae and microorganisms were interactively affected by litter element concentrations. The data suggest that element ratios of litter material as basal resource of the decomposer food web exhibit strong bottom-up control on the structure of decomposer communities and decomposition processes

RHIZOPOD ANALYSIS IN THE PEAT-LAKE SEDIMENTS OF THE MOUNTAIN MIRE "BEZRYBNOE" IN THE SOUTH OF MIDDLE SIBERIA

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The research is devoted to rhizopod analysis (analysis of the testate amoebae complexes) in peat-lake sediments. The aim is the reconstruction of hydrological conditions in the mire during its development in the late Holocene. The study site Bezrybnoe Mire lies in Yergaki Natural Park in the Western Sayan Mts, southern Middle Siberia (52°51' N, 93°21' E, 1300 m a.s.l.). The thickness of sediments is 6 m, of which the lower 2 m – gyttja, the upper 4 m - peat composed mainly of remnants of sphagnum moss. Age of peat at a depth of 4 m is 2,420 cal yr BP. Total 80 testate amoeba taxa was revealed. The dominant species were: *Amphitrema wrightianum*, *Centropyxis aculeata*, *C. gibba*, *Diffflugia globulus*, *Euglypha rotunda*, *Schoenbornia smithi*, *Trinema lineare*, *T. enchelys*. There are both oligotrophic and minerotrophic species. 22 species were met only in gyttja. Water table depth (WTD) reconstruction is made on the basis of species optima obtained in our study of modern oligotrophic and minerotrophic mire habitats in the south of Western Siberia using the transfer function (training set includes 115 samples). Decrease of WTD in the mire are observed 2350-2300, 1850-1800, 1650, 1450, 1350 1100-900, 400-0 cal yr BP and increase – in 2400, 2000, 1900, 1800-1700, 1600-1500, 1400, 1300, 800-500 cal yr BP. Minerotrophic testate amoebae species were prevailed in the studied sediments. However, there were also oligotrophic species. For the reconstruction of WTD during development of such mires the information is needed about optima of both oligotrophic and minerotrophic species. The work was supported by RFBR grant (16-34-60057).

BENTHIC CILIATES ON HERON ISLAND REEF, AUSTRALIA

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Field studies conducted at the Heron Island Research Station, located in the Capricorn region of the Great Barrier Reef, revealed complex protistan communities. Heron Reef, home to about two-thirds of the coral species found on the GBR, has experienced repeated bleaching episodes in the past decade. This study, with comparisons to similar observations in 2006, emphasizes the diversity of ciliates and diatoms found in reef sediments. Observations included the interactive role of the protistan component of a coral reef, in particular the dynamics of opportunistic and/or potentially



pathogenic forms associated with damaged corals undergoing disease conditions, such as Brown Band disease. These altered communities are characterized by rapid tissue breakdown in hard corals, coupled with high levels of dissolved organic matter. Samples were taken by direct capture, observed with phase contrast and epifluorescence microscopy, recorded by video and photomicrography, and fixed for later identification and genomic studies. The relative abundance of diatoms/flagellates/ciliates appears to show a higher proportion of photosynthetic species as compared to previous observations. Brown Band ciliates were not found on the corals, in the sediments, on algal surfaces, or in the plankton prior to bleaching in late January, posing the question of their location when not acting as opportunistic pathogens.

“*CANDIDATUS MEGAIRA*”: AN EMERGING GENUS OF BACTERIAL ENDOSYMBIONTS WIDESPREAD IN CILIATES AND OTHER PROTISTS

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“*Candidatus Megaira*” (Rickettsiaceae) is a recently described genus of obligate intracellular bacteria, which are phylogenetically closely related to the pathogen *Rickettsia*. Sequences representatives of genus “*Candidatus Megaira*” can be split in at least 3–4 species level sub-clades. These sequences have been found in association to evolutionary far related host lineages (e.g. unicellular and multicellular algae, corals and hydras). The type species of the genus, “*Candidatus Megaira polyxenophila*”, has been found in several ciliates as well as in other protists. In ciliates, “*Candidatus Megaira polyxenophila*” can infect different cell compartments (macronucleus and cytoplasm) according to the host species. Herein, we provide the characterization of a second “*Candidatus Megaira*” species inhabiting the cytoplasm of the green ciliate *Paramecium bursaria*. Within the same host cell, this novel “*Candidatus Megaira*” presents polymorphic features such as the association with endosymbiotic algae, or the possible

presence of flagellum at some stage of the life-cycle. Moreover, we performed several trans-infection experiments using different species of newly characterized “*Candidatus Megaira*” in order to understand how representatives of this genus can be spread in the aquatic environment and how they influence the host fitness within the same ciliate population. We also investigated the interaction between “*Candidatus Megaira*” and other symbiotic bacteria already inhabiting or invading the same host cell. All together, our findings highlight the necessity of a better understanding of the life-cycles of this widespread, but poorly investigated, endosymbiont.

THE GENOMIC STUDY OF AGAR PRODUCING RED ALGA *GRACILARIOPSIS CHORDA*

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Red algae (Rhodophyta) played an important role in the evolution of photosynthetic organisms as plastid donor(s) in chlorophyll-c containing algae including cryptophytes, haptophytes, stramenopiles, and alveolates. Most red algal species are important components in marine ecosystems, serving as a shelter for diverse marine organisms and contributing as primary producers. Many red algal species are heavily cultivating as food and industrial purposes such as agar and carrageenan production. Despite of these importances, only several red algal genomes have been reported among more than 7,000 species. From the major multicellular red algal group of the Florideophyceae, only one genome was fully sequenced to date. To fill the gap of our understanding on red algae, here we analyzed a new genome of the multicellular red algal species *Gracilariopsis chorda*, which is one of major cultivar as food and agar production in Korea. Using the long-read single molecule sequencing (PacBio) platform, we completed whole-genome of 89.6 Mbp (49% GC content, 1,098 contigs) that encodes a total of 9,992 predicted proteins. We discuss the interesting story of genome reduction/expansion and gene duplication in red algal genomes at this meeting.

AN ANCESTRAL BACTERIAL DIVISION SYSTEM IS WIDESPREAD IN EUKARYOTIC MITOCHONDRIA

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

#### DIVERSITY OF PHOTOSYNTHETIC PAULINELLA SPECIES INCLUDING A COMPARATIVE PLASTID GENOME ANALYSIS

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Thecate filose amoeba *Paulinella chromatophora* is a good model organism for understanding plastid organellenogenesis because its plastid was derived from a *Synechococcus*-*Cyanobium* type of alphacyanobacterium. Recent studies have shown species-level of divergence after the acquisition of the organelle; however, a full investigation has not been conducted for the photosynthetic species. We surveyed the biodiversity of this interesting alga using samples collected from around the world. Using four gene markers (18S rRNA, 16S rRNA, dnaK1, psaL), two distinct lineages with high genetic variation were identified, including one new species candidate (i.e., *P. microporus*). In addition, the chromatophore genome was fully sequenced from *P. microporus* strain KR01 and the recently reported marine *P. longichromatophora*. Comparative genomic analysis showed 0.17% of sequence divergence between the Korean strain KR01 and the Japanese strain FK01. Among 1,626 variable sites, the divergence was converged on noncoding regions at a rate seven times higher than for coding regions. The chromatophore genome of *P. longichromatophora*, when compared to other photosynthetic *Paulinella* species, showed a higher mutation rate. These results suggest that the diversification of the photosynthetic *Paulinella* species has occurred at a rapid rate and that the diversification is still ongoing.

#### ROLE OF PROTEIN DISULFIDE ISOMERASE (PDI) ON TOXOPLASMA-HOST INTERPLAY: IMMUNOLocalIZATION ASSAYS USING ANTI-HUMAN PDI MONOCLONAL ANTIBODIES (MABS) ON IN VITRO CULTURE SYSTEMS

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The main goal of this study is to address the functional

roles of PDI on *Toxoplasma gondii*-host interplay, in the context of acute/chronic infection, and evaluating their usefulness as drug-targets using anti-human PDI commercial MABs. The potential cross-reactions of PDI anti-human MABs with *T. gondii*, confirming their usefulness for immunolocalization of PDI not only in human fibroblasts (HFF) target cells, but also in *Toxoplasma* tachyzoites/cysts were verified. In order to validate this hypothesis a bioinformatics analysis was conducted in the *Toxoplasma* genome database to search for homologous regions: of the antigens' complete amino acid sequence used in the manufacturing processes of the MABs utilized in this study; and/or of their more predicted immunogenic regions in case of antigens with longer sequences. Confirmation was obtained by reverse search on human genome for the predicted *T. gondii* antigens/peptides sequences. Results from indirect immunolabeling assays using immunofluorescence techniques with anti-human PDI MABs: PDI, PDIA3(ErP57), PDIA6, Calnexin(CNX), glucose-regulated proteins/immunoglobulin heavy-chain binding protein (GRP78/BiP) and GRP94 suggest both cross-reaction with target cell-lines and *Toxoplasma*, being suitable for their profile identification and to evaluate PDI usefulness as a drug-target against *T. gondii* infection. Different patterns of immunolabeling were observed in *Toxoplasma* infected and non-infected HFF cells according to the MAB used. The functional confirmation of the characterized PDI involved in the host-pathogen interaction, in the presence of PDI inhibitors, is in progress. Further analyses will be performed by RNA silencing to get complementary evidence of these specific proteins in *Toxoplasma*-host interactions.

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#### ANALYSIS OF THREE SINGLE-CELL AMPLIFIED GENOMES OF THE CHOANOFLAGELLATE *MONOSIGA BREVICOLLIS* REVEALS SINGLE-CELL GENOMICS AS A LIMITED APPROACH FOR EVOLUTIONARY PURPOSES

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Environmental metabarcoding data is providing a new view of the real protist diversity. This new diversity often occupies key phylogenetic positions

which can lead to important insights in the evolution of different eukaryotic lineages. This is the case of the Opisthokonta, in which the identification of new unicellular lineages close to animals has the potential to improve our understanding of the transition towards animal multicellularity. However, isolating these new organisms is a challenge. Instead, single-cell genomics (SCG) seems a promising approach to obtain directly the genomes from the environment. However, SCG technologies, which have mainly been employed in prokaryotes, are affected by important biases, specially during the whole genome amplification step. The potential of SCG for eukaryotes remains unclear. To test the potential of this technique for evolutionary studies in eukaryotes, we here analyze three environmental single amplified genomes (SAGs) from the choanoflagellate *Monosiga brevicollis*, whose genome is already sequenced. We show the genome recovery obtained from our SAGs was low (between 30–5%, around 3,684 out of 9,175 genes). Interestingly, the percentage of phylogenomic markers that our SAGs contains is good (almost 100% for some datasets). We also explored different techniques to improve the quality and the recovery of the assemblies obtained. We found that genome recovery increased up to 48% by pooling the data from the three different SAGs. Current SCG technologies seem a limited approach for addressing some questions but has the potential to provide enough data for phylogenomic analyses.

#### DIPLONEMIDS – NEW KIDS ON THE BLOCK

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Results from the Tara Oceans metabarcoding study revealed that a significant fraction of the plankton in the sunlit ocean is constituted by diplomonid flagellates, a poorly known group of heterotrophic excavate protists. In an analysis of V9 18S rDNA metabarcodes from planktonic communities collected in 123 globally distributed stations, diplomonids emerge as a highly abundant and extremely diverse group that lacks geographic structuring but displays strong vertical separation. Uniquely among planktonic eukaryotes, taxonomic identification of diplomonids is far from saturation. They are more abundant in polar regions and deeper waters, yet their biological function in the ocean – the largest ecosystem of our biosphere – remains unknown. We are attempting to isolate and culture new marine diplomonids in order to sequence their genomes, analyze their ultrastructure and establish protocols for their genetic manipulation. By this combined approach, we hope to shed more light on these „new kids on the block“.

THE SMALL SUBUNIT rRNA GENE SEQUENCE OF THE CHONOTRICH *CHILODOCHONA CARCINI* JANKOWSKI, 2007 CONFIRMS CHONOTRICHS AS A DYSTERIID-DERIVED CLADE (PHYLLOPHARYNGEA, CILIOPHORA)

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The chonotrichs are sessile ciliated protozoa ectosymbiotic on the body parts of a variety of crustaceans. They have long been considered a separate group because their sessile habit has resulted in the evolution of a very divergent body form and reproductive strategy compared to free-living ciliates. In the mid-20<sup>th</sup> Century, the free-living dysteriid cyrtophorian ciliates were proposed as a potential sister clade because the chonotrich bud or daughter cell showed similarities during division morphogenesis (i.e. ontogeny) to these free-living dysteriids. A single small subunit (SSU) rRNA gene sequence is available for the chonotrich *Isochona* sp. However, its authenticity has been questioned in the recent literature, and the placement of this sequence within the dysteriid clade has added to this controversy. In this report, the SSUrRNA gene sequence of the chonotrich *Chilodochona carcini* ectosymbiotic on the green crab *Carcinus maenas* is provided. Topology testing of the SSUrRNA gene phylogeny constructed by Bayesian Inference robustly supports the sister-group relationship of *Isochona* sp. and *C. carcini*, the monophyly of

these two chonotrichs, and the divergence of the chonotrich clade within the dysteriid clade.

MAPPING THE HIDDEN DIVERSITY OF FREE LIVING FRESH WATER CILIATES FROM DELHI REGION, INDIA

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India, with 2.4% of the world's area, has over 8% of the world's total biodiversity, making it one of the 12 mega diversity countries in the world. Despite this richness, the data concerning ciliate diversity from India is rather scarce. We at University of Delhi have been involved in the morphological and molecular taxonomy of ciliates from freshwater bodies namely from Okhla Bird Sanctuary, Sanjay Lake, Pond at Rajghat in Delhi region. A large number of species from different classes have been observed from these water samples namely from genus *Tetmemena*, *Stylonychia*, *Laurentiella*, *Euplotes*, *Aspidisca*, *Paraurostyla*, *Gastrostyla*, *Pseudourostyla*, *Urostyla*, *Oxytricha* and *Uroleptus* from class Spirotrichea; *Blepharisma*, *Spirostomum* and *Stentor* from class Heterotrichea; *Paramecium*, *Frontonia*, *Vorticella* and *Cyclidium* from class Oligohymenophorea; *Litonotus*, *Dileptus*, *Didinium* and *Lacrymaria* from class Litostomatea; *Colpoda* from class Colpodea; *Chilodonella* from class Phyllopharyngea; *Loxodes* from class Karyorelectia; and *Coleps* from class Prostomatea have been identified by live cell observations, protargol staining, silver staining and morphometric measurements. In the present study, we describe in detail few species from the subclass Stichotrichia and Hypotrichea. There is difficulty in understanding the phylogenetic relationship between different species of these group based solely on morphological markers. Therefore, we are trying to look into both morphological and multiple molecular markers like SSU- rRNA, ITS 1, ITS2, hsp70 and histone genes and we could depict morphological and molecular congruence in the species from subclass Stichotrichia and Hypotrichea.

GLOBAL SHIFTS IN GENE EXPRESSION PROFILES ACCOMPANIED WITH ENVIRONMENTAL CHANGES IN CNIDARIAN-DINOFLAGELLATE ENDOSYMBIOSIS

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Stable endosymbiotic relationship between cnidarian animal and dinoflagellate *Symbiodinium* spp. is a prerequisite for sustaining coral reef ecosystem. Recent studies have shown that elevated seawater temperature can cause collapse of endosymbiosis by expulsion of the symbiotic algae from cnidarians, which is known as ‘bleaching’, and subsequent mass mortality. However, the technical difficulty in maintaining and using corals as material in laboratory has hampered further understanding of the molecular biology of the cnidarian-dinoflagellate endosymbiosis. Here we show transcriptomic analyses using the symbiotic sea anemone *Exaiptasia pallida* (formerly *Aiptasia* spp.), an emerging model cnidarian, in multiple culture conditions. The genome-wide gene expression profiles were analyzed by mapping the RNAseq reads from symbiotic and experimental apo-symbiotic anemones onto the host and endosymbiont genome sequences. A large portion of the genes differentially expressed in response to light and elevated temperature in the symbiotic and apo-symbiotic anemones was not overlapped and, in a number of cases, different members in a single gene family were activated between the symbiotic and apo-symbiotic anemones. These suggest that the anemones use distinct gene sets to respond to environmental changes depending on the symbiosis states with *Symbiodinium*. From the endosymbiont side, several photosynthesis-related genes associated with the photosystem II core assembly were detected to be down-regulated under the heat condition in hospite, which potentially affect the photodamage-induced stress responses. Overall, our data suggest that the endosymbiosis with *Symbiodinium* substantially affect the host’s transcriptional profiles, potentially leading to the altered ecological contribution to the environment.

#### CHLOROPLAST DIVISION OF *TETRASELMIS* SP. IN THE FOOD VACUOLE OF MIXOTROPHIC ALGAE *RAPAZA VIRIDIS*

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*Rapaza viridis* is a euglenoid that always possesses chloroplasts in cells without exception. *R. viridis* requires not only light but also a specific strain of green algae (*Tetraselmis* sp.) as its prey for their survival/growth, hence apparently being of obligate mixotrophy. Nonetheless, the degradative process of chloroplasts of the prey was barely observed. Furthermore, only trace amount of 132,173-cyclophorbide enols (CPEs), the non-phototoxic catabolites of chlorophylls, was produced along with the predation, indicating that only a small proportion of chlorophylls was actually discarded from the ingested chloroplasts. We thus investigated cell dynamics regarding ingested chloroplasts in *R. viridis* to understand the fate of the chloroplasts and chlorophylls contained therein. Unlike other phycophagic euglenoids, decrement of chlorophyll fluorescence from the ingested chloroplasts was rarely observed, and any evidence for digestive degradation of the chloroplasts was not recognized. Furthermore, the chloroplastic eye-spots of *Tetraselmis* sp. became dispersed several hours after the ingestion and eventually disappeared. In the next hours, interestingly, the ingested chloroplasts became subdivided into more than several pieces with various sizes. Within a day, the unique, rough appearance of chloroplasts of *Tetraselmis* sp. in the DIC image became insignificant and changed into a rather smooth appearance that is no more distinguishable from those originally possessed by *R. viridis*. Afterward, the number of “chloroplasts” per cell began to decrease, suggesting progress of *R. viridis* cell divisions at this stage. We discuss the mechanism for the observed chloroplast division and the origin of “chloroplasts” of *R. viridis*.

#### HETEROGENEITY IN NUTRIENT UPTAKE BY INDIVIDUAL DINOFLAGELLATE CELLS REVEALED USING NanoSIMS

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Dinoflagellates are one of the most successful groups of marine protists. Many photosynthetic dinoflagellates can utilize not only inorganic, but also organic compounds as nutrients, which is advantageous in eutrophied coastal zones. Therefore, nutritional physiology of these organisms receives much scientific attention. Usually it is studied by bulk approaches; however, the use of

modern single-cell tools significantly advances our knowledge in this field. We used stable isotope tracers, isotope ratio mass spectrometry and nanoscale secondary ion mass spectrometry (NanoSIMS) to investigate concurrent uptake of nitrate and urea by dinoflagellates *Prorocentrum minimum* at the population and single-cell levels. Although bulk and averaged single-cell measurements revealed similar relationships between the urea and nitrate uptake, NanoSIMS disclosed significant heterogeneity in nutritional activity of individual cells. Dinoflagellates readily consumed urea even if this nutrient was new to the cells, but some cells took it up at a 9-folds higher rate than others. Furthermore, sudden urea input resulted in overall 30–40% suppression of the nitrate uptake. However, a closer look with NanoSIMS showed that in 30% of cells nitrate consumption was completely inhibited, whereas in other cells it might be not suppressed. We conclude that even sporadic inputs of urea to coastal ecosystems are likely to have a prominent effect on *P. minimum* populations. Physiological heterogeneity among individual dinoflagellate cells can represent a so far overlooked strategy towards ecological success. Funded by the Russian Science Foundation, project 16-14-10116 (to OM, SS) and Federal Ministry of Education and Research, grant BMBF 03F0626A (to AV, MV).

#### TRENDS IN ENDOSYMBIOTIC GENE TRANSFER ON PLASTID METABOLIC PATHWAYS IN DINOFLAGELLATES WITH NON-CANONICAL PLASTIDS

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The major photosynthetic dinoflagellates possess red algal-derived plastids, but some minor lineages established non-canonical plastids derived from phylogenetically diverse eukaryotic algae. Dinoflagellates *Karlodinium veneficum* and *Lepidodinium chlorophorum*, which bear non-canonical plastids derived from haptophyte- and green algal endosymbionts, respectively, and their nuclear genomes contain genes encoding plastid proteins that are likely transferred from the genomes of the endosymbiont algae (endosymbiotic gene transfer, EGT). Although EGT is generally considered to be an essential step in transforming an endosymbiotic alga into a plastid, it has yet to be fully understood to what extent metabolic functions in the *Karlodinium* and *Lepidodinium* plastids rely

on endosymbiotically acquired proteins. We here surveyed nucleus-encoded plastid proteins in *Karlodinium* and *Lepidodinium*, and investigated the origins of the proteins involved in two metabolic pathways localized in the plastid. Chlorophyll a biosynthetic pathway in the two species appeared to be reorganized in different ways. The pathway in *Karlodinium* was found to be occupied by proteins acquired from the haptophyte endosymbiont, while ‘laterally derived’ proteins, which were acquired from diverse eukaryotes rather than the green algal endosymbiont, comprise the pathway in *Lepidodinium*. In contrast, the majority of proteins which were detected in isoprene biosynthetic pathway in *Karlodinium* or *Lepidodinium* were derived from host dinoflagellate, suggesting that switch from the canonical to non-canonical plastids barely triggered reorganization of this particular pathway in the two species. In this talk, we discuss biological reasons for the marked difference in the impact of gene transfer between the two metabolic pathways in *Karlodinium* and *Lepidodinium*.

#### LAGENOPHRYS PATINA (CILIOPHORA: PERITRICHIA: LAGENOPHRYIDAE) ATTACHED TO TWO MEXICAN POPULATIONS OF *HYALELLA AZTECA* (CRUSTACEA: AMPHIPODA): STATISTICAL APPROACH TO PROBE SITE PATTERN PREFERENCE ON HOST

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Members of genus *Lagenophrys* are obligate symbiotic peritrichs of crustaceans, however the pattern of distribution on corporal surface varies according to involved species. To demonstrate with statistical tests the preference for an amphipod body region or appendage to attach, two *Hyalella azteca* populations were collected in two Mexican lakes, Xochimilco Lake and Cuitzeo Lake, and density and prevalence of *Lagenophrys patina* on host surface were calculated. We used a contingency table and



correspondance analysis to identify a microhabitat preference. Except for the head, pleopods and telson, lagenophryid ciliates were attached to 2-9 amphipod body regions, and greatest prevalence was found on coxae, followed by pereopods. For Cuitzeo Lake individuals the total *L. patina* loricae abundance was from 617-1165, being the coxae with the highest values (626), and for Xochimilco Lake *L. patina* showed a highly significative preference for coxae for their attachment ( $X^2=1752.01$ ,  $P<0.0001$ ); we also obtained the same highly significant result for Cuitzeo Lake symbiotic system (with  $X^2=1317.8$ ,  $P < 0.0001$ ). These results could be explained by the shape and function of appendages of amphipods, providing the water flow derived by host movements and swimming, carrying food and oxygen for symbiotic ciliate.

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#### COMPARATIVE MORPHOMETRY OF *LEIDYANA* SP. (EUGREGARINORIDA) AND ITS FIRST RECORD IN *CONOCEPHALUS ICTUS* (ORTHOPTERA: ENSIFERA: TETTIGONIIDAE) FROM MEXICO

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*Leidyana* is a genus of apicomplexan eugregarine inhabiting the digestive tract of insects, mainly lepidopterans and orthopterans. Its classification is mainly based on the shape of their epimerite, protomerite, deutomerite and oocyst, and also on their gametocyst dehiscence. Nevertheless, morphometrical data of the species belonging to this genus have been also considered in order to establish reliable descriptions but is not a main criterion for its identification. In the present work we compared the morphometric attributes of *Leidyana* sp. a parasite of *Conocephalus ictus* collected in Tlanchinol, Hidalgo, Mexico, with

two species that also infect orthopterans belonging to the same suborder (Ensifera) but different families and infraorder (Gryllidea) and belonging to the Old World. These species of *Leidyana* also possess epimerites resembling the ones found of *Leidyana* sp. The three species measurements comparison was made using a one-way ANOVA and a correspondence analysis (n=20 each sample). We found significative differences in the epimerite length and width, considered as an important character for the eugregarines taxonomy to genus and species level identification. Additionally we show the first record of a species of genus *Leidyana* parasitizing the gut lumen of an orthopteran of the infraorder Tettigoniidea, family Tettigonidae, as well for Mexico. Key words: eugregarines, Mexico, *Leidyana*.

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#### SOME NOTES ON THE MORPHOLOGY, GEOGRAPHIC DISTRIBUTION AND 18S rRNA GENE SEQUENCE OF A FRESHWATER OXYTRICHID CILIATE (HYPOTRICHIA: OXYTRICHIDAE) FROM MEXICO

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Species of genus *Oxytricha* are characterized by 18 frontal-ventral-transverse cirri, one right and one left row of marginal cirri, undulating membranes and dorsal kineties usually in *Oxytricha*-pattern, and also the presence of caudal cirri. We collected some samples from a Mexican freshwater lake and we isolated *Oxytricha granulifera* (Foissner & Adam, 1981). Our individuals presented six dorsal kineties compared with the Austrian population (with five dorsal kineties) collected in a terrestrial environment. All available records of *O. granulifera* were plotted and obtained a broad geographic distribution. Considering the 18S rRNA gene sequence, our species grouped with other *O. granulifera* strains and populations available in GenBank. Due to the number of dorsal kineties we propose that Mexican population could be considered like a subspecies of *O. granulifera*.

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#### DIVERSITY OF THECAMOEBID AMOEBAE (AMOEBOTAXA: DISCOSEA: THECAMOEBIDAE)

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Amoebae of the family Thecamoebidae are widely distributed in the different environments: salt and fresh water and soil. These protists are relatively easy to isolate and cultivate. They are relatively easy determining to genus, or even to species, thus representing a convenient object for many kinds of studies. The frequency of occurrence of species is very different: there are rather common ones like *Thecamoeba quadrilineata*, *T. similis* and *T. orbis* as well as numerous species known from few findings or never re-isolated since initial description. Many of these species were studied only at the light-microscopic level and require investigation with modern methods, including electron microscopy and molecular studies (this especially concerns the genus *Thecamoeba* and genera of unclear systematic position like *Pseudothecamoebea* and *Thecochaos*). Our studies show that “hotspot” of *Thecamoeba* diversity is terrestrial habitats – soil, grass, dry leaves and surface of trees. During our studies we isolated 20 strains of *Thecamoeba*; some were identified as known species (*Thecamoeba aesculea*, *T. similis*, *T. quadrilineata* and *T. terricola*) and 6 strains, which represent new species of the genus *Thecamoeba*. We have found two new strains of amoebae belonging to the genus *Sappinia*. In contrast, amoebae of the genus *Stenamoeba* were never found in terrestrial samples. Our data shows that species diversity of thecamoebid amoebae remains considerably underexplored.

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#### ESTABLISHING *CRYPTOSPORIDIUM* AS A MODEL FOR STUDYING THE BIOLOGY AND EVOLUTION OF APICOMPLEXANS AND UNIQUE ORGANELLES

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*Cryptosporidium* spp. display a number of unusual traits, distinct from other apicomplexans; notably a smaller genome and the absence of an essential organelle, the apicoplast. There are also intra-species differences, such as a reduced mitochondrion in *C. parvum* and *C. hominis*, in size and functions. Studying these organisms holds great potential for our understanding of how the apicomplexans diverged. Studies of *Cryptosporidium* are inhibited by a limited selection of *in-vitro* culturing systems, with short lifespans and production volumes. Using a novel cell line for propagation we have seen a significant increase in parasite production volume as well as a longer lifespan, which have enabled the development of many new techniques for studying *Cryptosporidium* and its cell biology and biochemistry. Amongst these new techniques is the development of NMR and Mass spectrometry protocols, which have been optimised to examine the metabolic flux of the parasite, as well as investigating the role of the mitosome. We have also acquired many electron microscopy (EM) images of the parasite during its life cycle, identifying details of structures at levels previously unseen in the literature. Furthermore, using Immuno-EM we have shown the first experimental evidence of the function of the mitosome in *C. parvum*, with the localisation of iron sulphur cluster biosynthesis proteins.

#### DIVERSITY AND TEMPORAL DYNAMICS OF CRYPTOMYCOTA AND APHELIDA, TWO OVERLOOKED GROUPS OF PARASITES IN FRESHWATER ECOSYSTEMS

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Basal-branching fungi, especially chytrids, are important heterotrophic members of aquatic microbial food chains, especially in freshwater. More recently, two additional groups very distantly related to fungi, Cryptomycota (or Rozellomycota) and Aphelida, have also been found to be ubiquitous members of freshwater microbial communities. Together with the Microsporidia, they form the recently described superphylum Opisthosporidia. All known opisthosporidian species are parasites of very diverse eukaryotic hosts. Remarkably, both cryptomycetes and aphelids are able to feed by phagotrophy, a chief difference with microsporidia

and true fungi, all of them osmotrophic. Despite the ubiquity of cryptomycetes and aphelids in diverse environments, little is known about their abundance and temporal dynamics. We have carried out a 2-year monthly survey of eukaryotic plankton diversity in five contrasted freshwater ecosystems (one brook, one small lake, and three shallow ponds) using massive 18S rRNA gene amplicon sequencing to compare cryptomycetes and aphelids with the much better-known chytrids. OTU analysis reveal that cryptomycetes and aphelids are less diverse than chytrids (556, 313, and 1274 OTUs, respectively) although in some moments cryptomycete+aphelid combined OTU number can exceed that of chytrids. Cryptomycetes show stable low numbers of sequences (<1% of total sequences) all along the year, in contrast with aphelids, which exhibit a more heterogeneous dynamics with recurrent abundance peaks in early autumn (>4% of sequences), when they become even more abundant than chytrids. These results suggest that cryptomycetes and aphelids are important overlooked members of freshwater ecosystems that most likely control other eukaryotic populations through their parasitic activity.

#### APPLICATION OF RECOMBINANT ANTIGENS FOR THE SERODIAGNOSIS OF TOXOPLASMOSIS

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*Toxoplasma gondii* is an opportunistic apicomplexan protozoon that can cause devastating disease in immunosuppressed patients and congenital infection. The diagnosis of toxoplasmosis is usually done by observing the parasite in biological samples or by the detection of specific IgM and IgG against *T. gondii* antigens in the patient's serum. The improvement of toxoplasmosis diagnostic techniques and the differentiation between the infection stages can be achieved using recombinant antigen. This study aims to use micronemal protein MIC3 (elicits a strong specific host immune response) recombinant antigens in the serodiagnosis of toxoplasmosis. *Toxoplasma* RNA was isolated using the Tri-Reagent method and a PCR was performed using primers for MIC3 nucleotide sequence. The bands corresponding in size to the recombinant plasmid were purified and cloned into the cloning vector pLATE 28 and in the expression vector

pLATE 31. An expression study was performed in different *E. coli* BL21 (DE3) strains: Star, XJB, RIPL and PlyS. These strains were transformed with the recombinant vector pLATE 31 in order to obtain clones. Three random clones were therefore selected and induced with IPTG. The result of the induction was observed on a SDS-PAGE electrophoresis. The recombinant protein was purified by high-affinity chromatography with immobilized nickel ions and subsequently analyzed by ELISA, SDS-PAGE electrophoresis and quantified by Nanodrop 1000. Preliminary results show that the best *E. coli* strains for expression are BL21 (DE3) RIPL and BL21 (DE3) PlyS, based on SDS-PAGE analysis. The optimization of the ELISA assay is in progress. Acknowledgments: Supported partially by FCT ref:VIH/SAU/0019/2011.

#### THE RAPUNZEL TINTINNID – REDESCRIPTION OF *TINTINNOPSIS SUBACUTA* JÖRGENSEN, 1899 (ALVEOLATA, CILIOPHORA, SPIROTRICHA)

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Tintinnids contribute distinctly to the microbial biomass in the marine plankton. Since the species have specific requirements concerning physico-chemical conditions and food items, reliable identification is indispensable for assessing their role in the food web. About one thousand extant tintinnid species are known, whose descriptions are exclusively based on the features of their loricae (houses); merely in about 30 species, cell characteristics have been studied. Since lorica shape and size are affected by environmental conditions and might show a polymorphism in the cell cycle, the tintinnid classification is artificial. Investigations of the cell, especially of the ciliary pattern and nuclear apparatus (generative micronuclei and somatic macronucleus nodules) are, however, supposed to provide features for a natural classification; these characters are revealed by protargol (silver proteinate) staining. *Tintinnopsis subacuta* was collected from surface waters of the Indiana River at the Atlantic coast of Florida (USA) and stained with protargol. Cell and lorica morphology were investigated under a compound microscope at up to 1250× magnification. The lorica is 55–119 µm, on average 79 µm long and consists of a cylindrical collar about 34 µm across and a subspherical bowl about 45 µm wide. The lorica wall has agglutinated mainly mineral particles. The contracted cell



measures  $30 \times 28 \mu\text{m}$  and is attached to the bottom of the lorica by a contractile peduncle. The somatic ciliary pattern is of the most complex type, i.e., it comprises a ventral, dorsal, and posterior kinety as well as a right, left, and lateral ciliary field. The ventral kinety has associated an extraordinary ciliary tuft of cell length that extends outside the lorica posteriorly, resembling the golden hair let down from the tower by Rapunzel; *T. subacuta* is unique in this respect. The right and left ciliary fields are composed of about 11 ciliary rows each, the lateral field consists of invariably 15 rows. While the majority of tintinnids have only two macronucleus nodules, *T. subacuta* has 4–34, on average 14 nodules. Financially supported by FWF Project P28790.

#### PROTISTAN VERSUS CYANOBACTERIAL PICOPHYTOPLANKTON PRODUCTION AND GRAZING MORTALITY IN SEVASTOPOL BAY AND ADJACENT WATERS (THE BLACK SEA)

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Seasonal dynamics of abundance, specific growth rate, daily production and grazing mortality of the major picophytoplankton components, eukaryotic protists and prokaryotic cyanobacteria, were studied at three stations in Sevastopol bay and adjacent waters (the Black Sea) in 2014 by flow cytometry and dilution method. In the shallow coastal waters, protistan picophytoplankton (PP) dominated ( $64 \pm 23$  (SD) %,  $n=26$ ) the community in terms of abundance (annual average of  $16.3 \pm 12.4 \times 10^3$  cells  $\text{ml}^{-1}$ ), with the latter increasing along the nutrient and pollution gradient from the coastal waters outside the bay ( $7.3 \pm 5.4 \times 10^3$  cells  $\text{ml}^{-1}$ ) to the eastern corner of the bay ( $28.7 \pm 11.4 \times 10^3$  cells  $\text{ml}^{-1}$ ). PP demonstrated significantly lower specific growth rates ( $0.20 \pm 0.19 \text{ d}^{-1}$ ) and significantly higher daily grazing mortality ( $4.0 \pm 5.8 \mu\text{g C l}^{-1} \text{ d}^{-1}$ ) than cyanobacterial picophytoplankton ( $0.70 \pm 0.46 \text{ d}^{-1}$  and  $1.1 \pm 1.1 \mu\text{g C l}^{-1} \text{ d}^{-1}$ , respectively) while the protistan and cyanobacterial daily productions did not differ significantly (paired t-test,  $p > 0.05$ ,  $n=26$ ). Matter flows through both the community components were comparable to or even exceeded their biomass stocks that indicated high biomass turnover rates. Thus, the protistan component has been shown to play a major role in the community functioning in the Black Sea coastal waters.

#### THE SPECIALIZATION OF THE PROTO-MITOCHONDRION AS A RESPIRATORY ORGANELLE

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The ancestor of mitochondria was an alpha-proteobacterium whose exact phylogenetic and phenotypic nature remains obscure. Therefore, the proximate selective force that drove the initial endosymbiosis is unknown, even though the ultimate selective advantage was undoubtedly greater efficiency in energy production through aerobic respiration. The specialization of the proto-mitochondrion as the respiratory organelle of eukaryotes required the host to exert increased control over the biogenesis of the newly evolving organelle. Among the several adaptations that transformed the ancestral endosymbiont into a respiratory organelle, two evolutionary innovations were of major importance. The first major innovation was the evolution of mitochondrial cristae to make respiratory sub-compartments. Cristae likely evolved from precursor structures in alpha-proteobacteria. Later molecular innovations further modified cristae to improve their respiratory function. This required the expansion of MICOS (Mitochondrial contact site and Cristae Organizing System) and the evolution of the capability of the ATP synthase complex to form multimers. The second major innovation was the evolution of the ability of the host to control the overall morphology, positioning and distribution of mitochondria within the cell. These adaptations optimized bioenergetic output in response to host needs. This was made possible by the origin of mitochondrial fusion, as well as the establishment of interactions between mitochondria and diverse endomembranes and the cytoskeleton. I discuss a detailed evolutionary scenario for the evolution of these two major adaptations in the context of the co-evolutionary integration of mitochondria and their host.

#### THE ULTRASTRUCTURE OF AMOEBOID FLAGELLATES *AMASTIGOMONAS* (CERCOZOA, RHISARIA)

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The cytoskeleton of three amoeboid flagellates *Amastigomonas* spp. dwelling in freshwater (one

strain) and marine waters (two strains) has been considered. The morphology of these strains is relatively similar. The anterior flagellum lies inside the hollow proboscis. The posterior flagellum goes along the ventral groove. Two heterodynamic flagella are smooth and have not been covered by any structures. The transitional zone of the flagella do not contain additional elements and are of the usual structure. The microtubule band and anterior rootlet are inserted from the kinetosome of the anterior flagellum, the microtubule right and left rootlets and single rootlet are inserted from the kinetosome of the posterior flagellum. The kinerosomes are located at obtuse angle or antiparallel and connected by the three fibrils and cross-striated structure. The rhizoplast has not been found. The thickened cell coverings consist of plasmalemma and epiplasm. The margins of the coverings form the folds, the ventral groove goes between them and is bounded only by the plasmalemma. The vesicular nucleus and Golgi apparatus are of the usual structure. The mitochondria contain tubular cristae. The pseudopodia inserting from ventral groove serve to capture bacteria. Front cytoplasmic outgrowth have been found for the first time. The resemblance and differences of given species with other apusomonads have been shown. This study was supported by the Russian Foundation for Basic Research (grant nos. 14-04-00500, 14-04-00554, 15-29-02518).

#### THE ULTRASTRUCTURE OF AMOEBOID FLAGELLATE *THAUMATOMONAS COLONIENSIS* WYLEZICH ET AL. 2007 (CERCOZOA, RHIZARIA)

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The ultrathin structure of amoeboid flagellate *Th. coloniensis* has been considered. The cell is surrounded by somatic scales which forming on the surface of the mitochondria. The heterodynamic flagella emerge from the small flagellar pocket. Both flagella are covered by the cone-shaped scales and thin twisted mastigonemes. The kinetosomes lie parallel to each other. The transitional zone of the flagella contains the thin-walled cylinder. The transversal plate of the flagella is located above cell surface. The flagellar root system consists of 3 microtubular bands and fibrillar rhizoplast. The vesicular nucleus and Golgi apparatus are of the usual structure. The mitochondria contain the tubular cristae. The extrusive organelles (kinetocysts) which contain the amorphous material and capsule have been found in cytoplasm. The capsule

consists of the muff and cylinder. Osmiophilic bodies of various shapes contain crystalloid inclusions. The pseudopodia capturing the bacteria are inserted ventrally. The groove is armored by the two longitudinal groups of closely situated microtubules. Microbodies and symbiotic bacteria have not been observed. *Th. coloniensis* differs from other *Thaumatomonas* species by the presence of osmiophilic bodies and absence of microbodies. This study was supported by the Russian Foundation for Basic Research (grant nos. 14-04-00500, 14-04-00554, 15-29-02518).

#### PLANKTONIC CILIATES OF THE SHEKSNA RESERVOIR

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The species composition, abundance, biomass and distribution of planktonic ciliates across the Sheksna Reservoir consisting of three parts have been studied. Fifteen species of ciliates, belonging to four classes: Spirotrichea - 6, Litostomatea - 4, Prostomatea - 4, Oligohymenophorea - 1 were recorded during the observation period in the pelagic zone. The maximal number of species (11) was registered in Beloye Lake, minimal one (6) - in Kovzhinsk part. The following species: *Tintinnidium fluviatile*, *Codonella cratera*, *Limnostrombidium viride*, *L. pelagica* and *Rimostrombidium velox* were dominants in the most part of sampling points. *Paradileptus conicus*, *Monodinium balbiani*, *Enchelis pupa* and *Prorodon ovum* were registered less frequently and in small quantities, and has been recorded for the first time on this site. The maximal average abundance ( $2502 \times 10^3$  ind./m<sup>3</sup>) and biomass (141 mg/m<sup>3</sup>) were registered in Beloye Lake. The maximal density ( $2750-4150 \times 10^3$  ind./m<sup>3</sup> and 156-352 mg/m<sup>3</sup>) observed in shallow waters of the western coast, in sampling points Mandoma, Kustovo, Kium-Mandoma and Belozersk. Lower density ( $1150-1250 \times 10^3$  ind./m<sup>3</sup> and 62-90 mg/m<sup>3</sup>) observed in sampling points near the center of the lake, and Sudovoy Khod station. The average values of abundance and biomass of Sheksna Reservoir accounted 1875000 ind./m<sup>3</sup> and 123 mg/m<sup>3</sup>, respectively. The trophic status of the Sheksna Reservoir during the study period can be described as mesosaprobic.

#### PUF PROTEINS IN *GIARDIA INTESTINALIS*

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*Giardia intestinalis*, an anaerobic protozoan parasite, contains highly compact genome with extremely short untranslated regions (UTRs). The regulation of gene expression during giardia cell- and life-cycle has been poorly studied and only a handful of RNA binding proteins have been characterized so far. PUF proteins bind 3' UTRs of cognate mRNAs, by which they regulate their stability, translation and localization. These eukaryotic proteins are evolutionarily conserved from protists to metazoans. We have identified five PUF genes in the genome of *G. intestinalis* and have initiated studies towards the characterization of PUFs in giardia biology.

EXPLORING CELL TYPE DIFFERENTIATION IN THE FILASTEREAN *CAPSASPORA OWCZARZAKI* BY SINGLE-CELL RNA-SEQ  
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The origin of multicellular animals from their unicellular ancestors is one of the most important evolutionary transitions in life's history. However, the specific cellular and genetic changes that led to this transition remain unknown. Phylogenomic analyses have shown that animals are closely related to three unicellular lineages: choanoflagellates, filastereans and ichthyosporeans, altogether forming the Holozoa clade. Recent phylogenomic studies have shown that those premetazoan taxa already had a complex repertoire of genes important for multicellularity, some of them previously thought to be exclusive of animals. Different versions of "simple multicellularity" are found among the unicellular relatives of Metazoa. There is the clonal development of colonial choanoflagellates, the aggregative behavior of *Capsaspora owczarzaki*, and the coenocytic development of ichthyosporeans. Those colonies and aggregates are assumed to be without cell differentiation. However, there is no molecular data proving that all cells within those colonies or aggregates or coenocytes are identical. Here we show microscopic evidence for the coexis-

tence of different cell types in *C. owczarzaki* aggregates. We also show our advances in developing single-cell transcriptomics methodology in these organism to molecularly characterize cell types. The possibility of analyzing differential gene expression at the single-cell level between diverse cell types of unicellular holozoans will allow us to better understand the molecular mechanisms underlying programs of cell differentiation in the origin of animals. The aggregates of *C. owczarzaki* offer us an ideal model in which to test this, and provide a better framework to understand the origin of the different metazoan cell types.

PHYLOGENY AND ECOLOGICAL IMPORTANCE OF PHAEODARIANS (CERCOZOA, RHIZARIA)

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Phaeodarians are a group of marine protists belonging to the phylum Cercozoa, composing Rhizaria (SAR). These unicellular siliceous zooplankton occasionally become abundant in the ocean, however their ecological importance and phylogeny are still wrapped in mystery. Plankton were sampled from several depths at ca. 40 stations in the Northern hemisphere during 2011–2015. Zooplankton were sorted and identified in order to clarify the species composition of each sample. Some phaeodarians were cultured to observe their behavior. The 18S rDNA sequences of phaeodarians were determined by single-cell PCR method. Two undescribed phaeodarians were found in the deep waters in the Sea of Japan, and one of the species was abundant through the year, occupying ca. 22% of the total zooplankton biomass on average. The abundance of phaeodarians was also seen in the East China Sea, where two species occupied 10.2–13.9% of the zooplankton biomass, suggesting that this group is an important component of the zooplankton community and the material cycle in the ocean. The cell division of phaeodarians was observed during the culture experiment. The species morphologically identified as phaeodarians formed a single clade together with other cercozoans in the phylogenetic tree, suggesting that almost all phaeodarians belong to Cercozoa and that Phaeodaria is a monophyletic



group. The branching pattern within the phaeodarian clade did not correspond to the families and the orders of the current classification system, and the system needs to be reconsidered.

#### CYANOBACTERIAL GENES IN THE NUCLEAR GENOME OF A DIATOM BEARING N<sub>2</sub>-FIXING CYANOBACTERIAL ENDOSYMBIONTS: POTENTIAL FACTORS INVOLVED IN THE HOST-ENDOSYMBIONT PARTNERSHIP

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The evolution of mitochondria and plastids from bacterial endosymbionts were key events in the evolution of eukaryotes. While the ancient nature of these organelles preclude understanding the transition from a bacterium to an organelle (organellogenesis), the study of eukaryotic cells with recently evolved obligate endosymbiotic bacteria has the potential to provide important insights into the early events in the organellogenesis. Diatoms belonging to the family Rhopalodiaceae and their N<sub>2</sub>-fixing cyanobacterial endosymbionts (spheroid bodies) are emerging as a useful model system in this regard. The experimental data accumulated to date suggest that the endosymbiont has been already integrated into the host cell during the endosymbiotic relationship. Our previous study on the genome sequence of the endosymbiont in a rhopalodiacean diatom provided insight into its reductive evolution and the metabolic dependency on the diatom host. However, it has yet to be elucidated how the host control the endosymbionts. In this study, to tackle this question, we obtained both genome and transcriptomic data of a rhopalodiacean diatom, *Epithemia adnata*, as well as the genome data of its cyanobacterial endosymbiont. Phylogenetic analyses showed that the nuclear genome encodes protein-coding genes of cyanobacterial origin, which are not seen in other diatom genomes. Some of these ‘cyanobacterial genes’ likely encode enzymes involved in the metabolism of peptidoglycan wall, which is a feature exclusively associated with the endosymbiont in the *E. adnata* cell. We will overview the cyanobacterial genes found in the diatom genome, and discuss their possible contributions to the host-endosymbiont partnership.

#### PHYLOGENOMIC INSIGHTS ON THE EVOLUTION OF METCHNIKOVELLIDS

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Metchnikovellids constitute a group of hyperparasites that infect gregarines living in the gut of polychaetes and other marine invertebrates. Despite they were described in the late 19th century, they are poorly known and their phylogenetic affinities have remained elusive for a long time. Morphological studies suggested an evolutionary relationship with Microsporidia, a group of highly derived intracellular parasites known for its extreme metabolic and genomic simplification, including e.g. loss of the mitochondrion. Microsporidia together with Rozellida (Cryptomycota) and Aphelida form a monophyletic holomycotan clade, the superphylum Opisthosporidia. The first molecular phylogenetic analyses based on SSU rRNA and beta-tubulin genes of *Metchnikovella incurvata*, a parasite of the gregarine *Polyrhabdina* sp. from the gut of the polychaete *Pygospio elegans*, supported a close evolutionary relationship with microsporidia. However, unraveling the phylogenetic position of these organisms is difficult due to their high evolutionary rate. To improve the phylogenetic signal and ascertain the phylogenetic position of metchnikovellids, we applied a single-cell genomics approach to individual gregarine cells infected with *M. incurvata*. We generated genome data by multiple displacement amplification followed by direct HiSeq 2500 Illumina sequencing. After assembly, we mined the genome dataset in search of conserved genes. Preliminary phylogenomic analyses of 31 conserved genes confirm the phylogenetic placement of metchnikovellids at the base of Microsporidia and after the divergence of *Mitosporidium daphniae*, a microsporidia-like mitochondrion-bearing parasite. Further exploration of metchnikovellid genomes would allow determining the genes and traits involved in the evolution of extreme parasitism. Supported by RFBR 15-04-08870 and ERC 322669.

#### EPIGENETIC INCOMPATIBILITY OF *PARAMECIUM TETRAURELIA* STRAINS

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Two epigenetic phenomena occur in crosses of *Paramecium tetraurelia* strains 32 and 51. Strain 32 is deficient for an IES present in one of the mating type genes, mtB, of strain 51. Internal eliminated sequences are excised from the developing macronuclear genome by a fascinating mechanism of genomic subtraction mediated by scanRNAs. However, if an IES is present in genome of one partner but absent in genome of another, then F1 hybrids deriving from the latter parent are unable to excise such IES from developing somatic genome: they can't produce a certain scanRNA. Moreover, F2 progeny of such cell will inherit this IES retained in macronucleus. IES inside a gene disrupts its function, thus reminding hybrid dysgenesis known for *Drosophila*. Indeed, in 25% of crosses we observed loss of mtB function in F2 progeny derived from parent 32. We also found unexpectedly that in 20% of crosses IES in mtB gene was retained in macronucleus of F2 progeny derived from parent 51, which normally produces scanRNAs and excises this IES. Analogous phenomenon was reported in cross of d12 and d48 deletion mutants of *P. tetraurelia* restoring functional gene of surface antigen A. We suggest that its mechanism may be connected with hemizygosity state of the deleted locus in F1 hybrids of such crosses, leading somehow to deviation of such sequence excision despite scanRNAs for it are present. These epigenetic effects may contribute into speciation in ciliates, as occasional hemizygosity may lead to lethality of interstrain hybrids. Supported by RFBR 16-04-01710.

#### RECONSTRUCTION OF CELLULAR SHAPE DEFORMATION THROUGH CONTRACTION OF CORTEX ACTOMYOSIN

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Giant free-living amoebae, *Amoeba proteus*, actively deform cellular shape during the locomotion. The deformation is induced by contraction of cortical actin and myosin (actomyosin). In the process, since actomyosin is connected to the cell membrane and transmit the generated force to deform the membrane. Although the contractile properties of

actomyosin networks have been reported, actual contributions to the membrane deformation are still unclear because of the cellular complexities. Here, in order to simplify the complex system, we attempted to reconstitute a simple model system, in which lipid monolayer was deformed by actomyosin. In living cells, the connection between actomyosin and lipid layer is achieved by various types of proteins. To simply accomplish the actin-membrane connection *in vitro*, we adapted positively-charged lipid DOTAP (1,2-dioleoyl-3-trimethylammonium-propane), expecting the electrostatic adhesion between negatively-charged actin and DOTAP. We extracted actomyosin from *A. proteus* and enclosed actomyosin fraction within a spherical space surrounded by a DOTAP monolayer. As a result, active deformation of the lipid monolayer was yielded. From analyses of the static and dynamic properties of the deformation, we found that the depth and width of the deformation were dependent on the curvature radius of the sphere. The observed curvature dependence is explained by the theoretical description including elasticity and contractility of the cortex. Our results provide a fundamental insight into the cellular membrane deformation induced by the actomyosin cortex during amoeboid locomotion. For more details, see Nishigami et al. (Sci. Rep. 6, 19864, 2016) and Ito, Nishigami et al. (Phys. Rev. E 92, 062711, 2015).

#### NUCLEAR DIVISION PROCESS IN TESTATE AMOEBA *PAULINELLA CHROMATOPHORA*

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*Paulinella chromatophora* is a euglyphid testate amoeba (Rhizaria, Cercozoa) living in a shell composed of ~50 rectangular siliceous scales. In this species, the complex shell construction process appears to be integrated under the cell cycle regulation, since the cell division does not proceed without the completion of shell construction. Before cell division, scales produced inside of mother cell are secreted out from the cell and assembled into a new shell by a specialized thick pseudopodium. Following the completion of shell construction, one of daughter cells moves into the new shell. Despite that knowledge, it is still unknown how the cell division process proceeds in response to the shell construction. In this study, we focused on how the nucleus divides along with shell construction process in *P. chromatophora*. In an intermediate stage of shell construction, the nucleus in the maternal cell was in prophase. In this phase, the nucleolus, which

is prominent in interphase, was disappeared and chromosomes were scattered in the nucleus. The newly formed shell was almost or fully constructed when the nuclear division reaches metaphase. In this phase, the spindle body was formed and the chromosomes were arranged at the equatorial plane randomly. At the time of completion of shell construction, the nucleus was observed to be in anaphase, and chromosomes were separated into anterior and posterior side of the nucleus. After the migration of a daughter cell into new shell, the nucleus with densely condensed chromosomes was observed to locate at posterior end of each daughter cell.

#### MITOSOMES IN *ENTAMOEBA HISTOLYTICA*: DIFFERENTIATION, METABOLITE TRANSPORT, AND FISSION

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Hydrogenosomes and mitosomes are mitochondrion-related organelles (MROs) in anaerobic/microaerophilic eukaryotes with highly reduced and divergent functions. *Entamoeba* possesses a highly divergent MRO known as the mitosome. The biological functions and their origin of *Entamoeba* mitosomes have been a longstanding enigma in the evolution of mitochondria. We previously demonstrated that sulfate activation, which is not generally compartmentalized to mitochondria, is a major function of *E. histolytica* mitosomes. We recently purified and identified cholesteryl sulfate (CS) as a final sulfate activation metabolite. We further identified the gene encoding the cholesteryl sulfotransferase responsible for synthesis of CS. Supplementation of CS to the culture increased the number of cysts, while, conversely, chlorate, a selective inhibitor of the first enzyme in the sulfate activation pathway, inhibited cyst formation. These results indicate that CS plays an important role in differentiation, an essential process for transmission of *Entamoeba* between hosts. Furthermore, *Mastigamoeba balamuthi*, an anaerobic, free-living amoebozoan species, also has the sulfate activation pathway in MROs, but does not possess the capacity for CS production. Hence, we proposed that a unique function of MROs in *Entamoeba* contributes to adaptation of its parasitic life cycle. Understanding of metabolite trafficking across the two mitochondrial membranes is important to understand metabolic functions of mitosomes. We recently discovered a novel mitochondrial  $\beta$ -barrel outer membrane protein of 30 kDa (MBOMP30) and several novel membrane-spanning

proteins from a list of the mitosome proteome. We experimentally confirmed their localization and integration to mitosome membranes by Percoll-gradient fractionation, carbonate fractionation, immunofluorescence assay, and immunoelectron microscopy. These new class of mitochondrial membrane proteins including MBOMP30 likely play unique and indispensable roles in *Entamoeba* mitosomes. We also found that two dynamin-related proteins, DrpA and DrpB, are involved in mitosome fission. Expression of a mutant form or gene silencing of these Drps caused abnormal morphology of mitoses and growth defect, suggesting that mitosome fission is mediated in part by these Drps.

#### MORPHOLOGY, PHYLOGENY, AND TRANSCRIPTOME DATA OF A NEW ANAEROBIC *METOPUS* SPECIES (CILIOPHORA, ARMOPHORIDA) FROM YANTAI, CHINA

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A new anaerobic *Metopus* species was discovered in a soil sample from fruit garden in Yantai, China, and investigated using morphological, morphometrical, and molecular methods. The morphology was studied using *in vivo* observation and protargol impregnation. The main features of the new *Metopus* species include: (i) size *in vivo* 75-105 × 35-55  $\mu$ m; (ii) body shape ellipsoidal to pyriform; (iii) nuclear apparatus invariably in preoral dome, macronucleus reniform, micronucleus globular to ellipsoidal attached to macronucleus; (iv) cytoplasm studied with lipid droplets especially in preoral dome; (v) five perizonal and 18-21 somatic ciliary rows of which three extend onto preoral dome (dome kineties); (vi) three to five distinctly elongated caudal cilia; and (vii) adoral zone composed of 21-29 membranelles and distinctly shorter than perizonal ciliary stripe (45% vs. 61% of body length on average). Moreover, this species contains numerous, conspicuous hydrogenosomes, anaerobically-functioning mitochondrial-related organelles, as an adaptation for the anaerobic lifestyle. SSU rRNA and mRNA were obtained using a single cell transcriptome protocol, and were sequenced with both Sanger and MiSeq Illumina technology. The obtained data were used for phylogenomic analyses and analyzing basic metabolic processes of this anaerobic ciliate, including searching for genes of putative anaerobic-adapting functions.

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SINGLE CELL RNA SEQUENCING, AN EFFECTIVE APPROACH FOR ANALYZING THE GENOME CONTENT AND EVOLUTION OF NON-CULTIVATABLE MICROBIAL EUKARYOTES

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Technical advances in culture-independent techniques have significantly contributed to the discovery of novel microbial lineages. Both metagenomics and single cell genomics have been shown to be useful tools for studying the genomes of prokaryotes. However, microbial eukaryotes generally have a complex genome structure, which leads to that these techniques tend to perform poorly. If it would be possible to apply single cell RNA sequencing, issues related to data assembly can potentially be avoided. With a method adapted from Smart-seq2, where template switching is used to amplify cDNA, transcriptomes have been generated for several single protist cells with close to full coverage of the coding potential. In Smart-seq2, transcriptomes can be generated in a 384 well format, which gives the potential for protist transcriptomes to be generated with high throughput. In transcriptomics data, the highly expressed house keeping genes are among the most likely genes to have high coverage and full length. Those genes are also suitable for constructing phylogenies that aim to resolve deep branches in the eukaryotic tree of life. Therefore, RNA sequencing of many cells in parallel has the potential to effectively generate sequence data for novel or poorly studied protist lineages, and to increase our understanding of their biology and evolution.

ULTRASTRUCTURAL AND TRANSCRIPTOMIC STUDIES OF KLEPTOCHLOROPLASTIDIC DINOFLAGELLATE *NUSUTTODINIUM AERUGINOSUM* (DINOPHYCEAE)

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Unarmored dinoflagellates *Nusuttodinium* spp. possess kleptochloroplasts which are ingested from cryptomonads and retained in the host cell for certain periods. Our previous studies revealed that *N. poecilochroum* digests cryptomonad nucleus

and never enlarges chloroplast. By contrast, *N. aeruginosum* enlarges single chloroplast throughout the cell and divides nucleomorph, retaining a cryptomonad nucleus. These differences are able to be interpreted as different evolutionary stages toward acquisition of 'true chloroplast' within the same lineage. It is, therefore, clear that these dinoflagellates are interesting materials to investigate evolutionary transitions toward establishment of endosymbiosis. To reveal fate of the cryptomonad organelles in *Nusuttodinium aeruginosum* after host cell divisions, we have further observed all daughter cells with LM and single-cell TEM methods. These observations showed that cryptomonad karyokinesis did not occur and that only one of the daughter cells inherited a cryptomonad nucleus. Among all daughter cells originating from a single cell through five generations, the cell that inherited the cryptomonad nucleus consistently possessed the largest kleptochloroplast. Therefore, this study suggests that the cryptomonad nucleus carries important information for the enlargement of the kleptochloroplast. These results suggesting cryptomonad nucleus remains transcriptionally-active in the host cell and we are examining changes in transcriptome of dinoflagellate nucleus, cryptomonad nucleus and nucleomorph during the course of transition in the kleptochloroplast development. In this presentation, methods and progresses of transcriptome analyses are discussed in addition to the results of morphological observation.

RETENTION OF BACTERIVORY IN THE DOMINANTLY PHOTOAUTOTROPHIC GREEN ALGA *CYMBOMONAS TETRAMITIFORMIS* IS INFLUENCED BY PHOSPHATE LIMITATION

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The lineage Chloroplastida (green algae and land plants) is defined by the presence of a primary plastid, which was acquired through the ingestion of a photosynthetic bacterium presumably during the early- or mid-Proterozoic eon. However, members of Chloroplastida are dominantly photoautotrophic and only a few members of the early-diverging class, Prasinophyceae, retain the ability to ingest bacteria. It is unclear why bacterivory is restricted to the prasinophytes. The prasinophyte *Cymbomonas tetramitiformis* was definitively confirmed through transmission electron microscopy to ingest bacteria into a large food vacuole. Recently,

this phagomixotroph’s genome was found to retain a unique combination of genes not present in obligate photoautotrophs or heterotrophs. Additional prasinophytes have been found to ingest fluorescently-tagged bacteria and synthetic particles. To investigate drivers of bacterivory in *Cymbomonas*, cultures of the alga were grown under limited N, P and light regimes and fed bacteria as a rescue source of nutrients. The *Cymbomonas* genome was also mined for metabolic genes related to nutrient uptake and assimilation. Surprisingly, bacteria only rescued *Cymbomonas* growth under phosphate-limited conditions, but not when nitrogen or light-limited. The genome contains genes related to phosphate metabolism that are not present in other Chloroplastida. A full GS-GOGAT pathway is present and no unique nitrogen-related genes were found. These results suggest that *Cymbomonas* retains the ability to extract phosphorous from prey, but relies on photoautotrophic pathways for nitrogen and carbon. This trait gives *Cymbomonas* a competitive advantage in P-limited cultures and may drive retention of bacterivory in this species.

#### SUPPLEMENTING SYMBIONTS: PATHWAY RESTORATION IN A LONG TIME PARASITE

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Apicomplexans are highly successful parasites, infecting every major metazoan lineage. The genus *Nephromyces* has recently been described as having a mutualistic relationship to its host *Molgula tunicates* (Saffo et al., 2010), making *Nephromyces* the only reported mutualistic apicomplexan genus. Apicomplexans have reduced genomes and have lost the ability to make many essential metabolites. These essential metabolites are instead scavenged from their host. Species of *Nephromyces* are known to have three different bacterial endosymbionts. Our data show that the bacterial endosymbionts encode a number of essential pathways lost in Apicomplexans. Here we describe insights from the transcriptome from *Nephromyces*, all three bacterial endosymbionts and the tunicate host. These data gives us a glimpse of the complex metabolic relationships and intertwined pathways of hosts and endosymbionts, with a particular focus on the biosynthesis of amino acids and vitamins.

#### TWO NEW NON-CANONICAL NUCLEAR GENETIC CODES FROM A RHIZARIAN AND A FORNICATE WITH UAG, BUT NOT UAA, AS A SENSE CODON

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The original presumption that all organisms use the same (standard) genetic code for translation of mRNA sequences into proteins has been challenged by discoveries of deviations of this universal language in both prokaryotes and eukaryotes. In eukaryotes the nuclear genetic code has proven to be much more conservative than that of mitochondria, and plastids; just a few its variants are known. Generally, we can sort them into 3 groups: (1) UGA serves as a sense codon; (2) UAA and UAG simultaneously serve as sense codons; (3) CUG encodes serine or alanine (rather than leucine). We analyzed transcriptomic data from two unrelated protists and found out that these organisms, as only eukaryotes known so far, use UAG as a sense codon in nuclear genetic code while retaining UAA as a termination codon. One of these organisms uses UAG as codon for leucine, similarly to a code variant described from certain mitochondria. The other one instead uses UAG to encode glutamine, resembling thus the non-canonical genetic code of several eukaryotic groups including many ciliates, hexamitin diplomonads, some oxymonads, and some ulvophytes; however, all these taxa have at the same time reassigned also the UAA codon. Phylogenetic analyses place the first organism into the rhizarian lineage Sainouroidea, whereas the second one represents an undescribed lineage of “*Carpediemonas*-like organisms” in Fornicata (Metamonada). Our findings thus once again show protists as an inexhaustible resource of peculiar departures from the “standard” biology.

#### AGAMOCOCCIDIANS: COCCIDIANS OR GREGARINES? NEW SPECIES AND NEW DATA ON THE PHYLOGENETIC POSITION OF THE GROUP

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Agamococcidians (Agamococcidiorida Levine, 1979) represent an enigmatic group of Apicomplexa. The life cycle of these parasites is characterised by presence of oocysts with sporocysts (similar to coccidian), sporozoites and trophozoites, and by absence of merogony and gamogony. This group combines two families Rhytidocystidae Levine, 1979, parasites of polychaetes, and Gemmocystidae Upton & Peters, 1986, parasites of stony corals. The phylogenetic position of these organisms is still unclear. Previous phylogenetic studies brought agamococcidians together with gregarines and cryptosporidians (Leander, Ramey, 2006; Rueckert, Leander, 2009; Kristmundsson et al., 2011; Cavalier-Smith, 2014). In contrast, morphological data (oocysts with sporocysts and nonmotile trophozoites located within host epithelial tissues) indicate a relationship of agamococcidians with coccidians. We isolated two putative new species of *Rhytidocystis* from polychaetes *Pectinaria hyperborea* and *Ophelia limacina* collected in the Keret Archipelago of the White Sea, Russia. The SSU rDNA sequences obtained from these new parasites clustered strongly with *Rhytidocystis cyamus* and *R. polygordiae* within the rhytidocystid clade. Phylogenies inferred from these sequences demonstrate a close relationship between rhytidocystids and marine coccidians. Interestingly, some coccidians closely related to rhytidocystids, such as *Margolisiella islandica* or *Aggregata* sp., have all three types of reproduction found among apicomplexans: sporogony, merogony and gamogony in their life cycles. Thus, our molecular data agree with known morphological data. We discuss the phylogenetic position and perspectives of further investigations of agamococcidians for more deep understanding of Apicomplexa evolution.

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**CAPSASPORA OW CZARZAKI AS A UNICELLULAR MODEL TO STUDY CO-OPTION OF THE ANCESTRAL INTEGRIN ADHESOME**  
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Adhesion systems and signaling networks are both essential in multicellular organisms. Some elements of the adhesion and signaling pathways of metazoans, such as proteins from the integrin adhesome are conserved in their closest unicellular relatives. This means these proteins already existed in the unicellular ancestor of metazoans and that they were co-opted for a multicellular lifestyle. To understand how the integrin adhesome was co-opted at the onset of Metazoa, we aim to unravel its function in a close unicellular relative of animals, the filasterean *Capsaspora owczarzaki*. This protist is the closest unicellular relative to metazoans that contains in its genome the basic core of proteins that constitutes the integrin adhesome. The expression of these proteins is upregulated during the aggregative stage in culture conditions. In order to understand its role, we are developing some molecular and genetic tools, such as immunostaining, transfection, and CRISPR/cas9. We will discuss preliminary data on the localization of several cytoskeletal and adhesion proteins of the integrin adhesome in *C. owczarzaki*, obtained by overexpression and by immunostaining with antibodies raised against our proteins of interest. We will also discuss the development of CRISPR system in this organism with the aim to develop a complete model system to analyze the origin of animals.

**ORAL PROTISTS: IMPORTANCE TO CANINE PERIODONTAL DISEASE**

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Periodontal disease is one of the most important health concerns for companion animals. Previous studies have demonstrated that at least half of all dogs will have some form of the disease within their lifetime which, without early intervention, can lead to painful periodontal ligament destruction, alveolar bone loss, and eventual loss of teeth. The recent focus of research into canine periodontitis has been the identification and characterisation of the bacterial communities present. However, other microorganisms are known to inhabit the oral cavity and could also influence the disease process. Our recent research has identified two oral protists that can inhabit the canine oral periodontium. *Trichomonas* sp. and *Entamoeba gingivalis* were detected using PCR and next generation sequencing and had an overall prevalence of 56.52 % (52/92) and 4.34 % (4/92) respectively in UK dogs. Both were statistically associated to animals with periodontal disease indicating their potential involvement in the periodontal disease process. Further work has led to the development of a quantitative PCR assay to measure protist abundance. The qPCR assay has been utilised on plaque samples, collected over a 60 week period from individual teeth of miniature schnauzer dogs (n = 52), to investigate longitudinal changes in abundance of both *Trichomonas* sp. and *E. gingivalis* as dogs progress from mild gingivitis to early stage periodontitis (<25 % attachment loss). These findings provide the first conclusive evidence for the presence of canine oral protozoa in dog plaque and suggest a possible role for protozoa in the periodontal disease process.

#### NAKED AMOEBAE OF UKRAINIAN POLISSYA FAUNA

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At present, the naked amoebae of the Kyiv, Zhytomyr and Volyn Polissya fauna are represented by 47 species, which belong to 5 classes, 11 orders, 15 families and 20 species.

According to our research the most distributed in the Ukrainian Polissya waters are: *Saccamoeba stagnicola* Page, 1974, *Korotnevella stella* Schaeffer, 1926, *Vexillifera* sp., *Vannella* (cf) *lata* Page, 1988, *Cochliopodium* sp.(1), *Mayorella cantabrigiensis* Page, 1983, *Thecamoeba striata* Penard, 1890, *Vahlkampfia* sp.(1), *Vahlkampfia* sp.(2). The such species as *Amoeba proteus* Leidy, 1878, *Polychaos* sp., *Saccamoeba wakulla* Bovee, 1972, *Rhizamoeba* sp. (2), *Cochliopodium* sp. (2), *Pellita digitata* (Greef, 1866) Smirnov et Kudryavtsev, 2004, *Mayorella*

*penardi* Page, 1972, *Thecamoeba sphaeronucleolus* Greef, 1891, *Penardia mutabilis* Cash, 1904 (have been registered by us in the Volyn Polissya waters), *Willaertia* sp. and *Acanthamoeba* sp. (have been registered by us in the Zhytomyr Polissya waters) are less distributed. Among the above listed species 3, which belong to genera *Vahlkampfia* Chatton et Lalung-Bonnaire, 1912 and *Acanthamoeba* Volkonsky, 1931 are parasitic. The 14 naked amoebae morphotypes are registered in the different water-bodies: polytactic, monotactic, flamellian, lens-like, striate, rugose, lingulate, lanceolate, fan-shaped, in the different waters mayorellian, dactylopodial, acanthopodial, branched and eruptive. The amoebae with polytactic and acanthopodial morphotypes might be mentioned as the least distributed.

#### EFFECT OF THE SPECTRUM OF AVAILABLE NITROGEN SOURCES ON *PROROCENTRUM MINIMUM* MORPHOLOGY AND PHYSIOLOGY

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Dinoflagellates are the prosperous group of aquatic eukaryotes. The ongoing eutrophication of coastal areas provides a competitive advantage to these organisms due to their ability to assimilate nitrogen from a variety of dissolved organic and inorganic sources. The analysis of genomic and transcriptomic databases revealed the presence of transporters and enzymes involved in uptake and assimilation of basic nitrogenous compounds present in seawater in dinoflagellates. In laboratory experiments with the culture of dinoflagellates *Prorocentrum minimum*, we investigated how the spectrum of available nitrogen sources influences morphological and physiological parameters of cells. We showed that addition of nitrate, ammonium, urea, glycine and their combinations to the culture growing on nitrate causes various physiological cell responses. The incorporation of H<sub>3</sub>-uridine by dinoflagellate cells revealed an increase in the RNA synthesis rate after the addition of supplementary nitrogen sources to the culture. Remarkably, the extent to which RNA synthesis was enhanced differed depending on the available nitrogen sources. For example, the largest increase in the level of RNA synthesis was achieved in response to addition of the ammonium/urea combination. The analysis of

the natural fluorescence of cellular photosynthetic pigments demonstrated that the observed increase in transcription was not directly linked to their concentration in cells. Moreover, this analysis revealed heterogeneity in the pigment fluorescence among distinct cells within the model culture. Funded in part by RFBR, project 15-29-02706.

#### THE HIGH COMPLEXITY AND DYNAMIC EVOLUTION OF THE RAS SUPERFAMILY OF GTPASES IN *NAEGLERIA*

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Ras superfamily GTPases constitute a vast group of proteins involved in many eukaryote-specific processes. The last eukaryotic common ancestor appears to have possessed at least several tens of Ras superfamily genes, but gene duplications and losses in different eukaryotic lineages have modified this ancestral set such that substantially different gene complements may be present in different eukaryote groups. One extreme are taxa harbouring an extensively expanded Ras superfamily complement, as is the case of a free-living amoeboflagellate *Naegleria gruberi* (Heterolobosea). Recently, genome sequences of three strains of *Naegleria fowleri*, a causative agent of primary amebic meningoencephalitis (PAM), became available for analysis. In order to assess the differences between the two species and the three strains, we identified and annotated the Ras superfamily genes in the newly sequenced *N. fowleri* genomes and reannotated the respective gene complement in the previously published *N. gruberi* genome. The sets of Ras superfamily genes turned out to differ substantially between the two species, as *N. gruberi* harbours over 350 genes, whereas *N. fowleri* exhibits a much less expanded set with “only” over 200 genes. In contrast, little, if any, differences were found for the three *N. fowleri* strains. Phylogenetic analyses revealed both species-specific duplications and losses as the factors responsible for the different gene numbers in the two species. The evolution of the Ras superfamily in the genus *Naegleria* is thus surprisingly dynamic and points to a hidden level of differentiation in cellular physiology of different *Naegleria* species.

#### EXPRESSION AND PURIFICATION OF A *PNEUMOCYSTIS JIROVECI* SYNTHETIC RECOMBINANT ANTIGEN AND APPLICATION IN THE DEVELOPMENT OF A SEROLOGICAL RAPID DIAGNOSTIC TEST

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*Pneumocystis pneumonia* (PcP) is an infectious disease caused by *Pneumocystis jirovecii*, an atypical fungus. PcP remains a major cause of respiratory illness among immunosuppressed patients. Current PcP diagnosis is based on the detection of *P. jirovecii* in respiratory specimens, obtained by invasive methods such as bronchoalveolar lavage, followed by cytochemical staining, immunofluorescent staining with monoclonal antibodies (IF/Mab) or PCR. Therefore, the possibility of an early diagnostic method allowing the use of biological specimens obtained non-invasively, is highly desirable. Rapid diagnostic tests (RDTs) using gold nanoparticles (AuNPs) allow a more sensitive, fast and cheap diagnosis, to be used in developing countries. The goal of this work is to develop an immunochromatographic RDT for the detection of *P. jirovecii* in non-invasive specimens like serum. In this test, spherical AuNPs are conjugated with a multi-epitope synthetic recombinant antigen (msr) which will allow the detection of circulating anti-*P. jirovecii* antibodies in sera. In order to obtain the highest amount of pure antigen, the expression vector *pLATE 31*, which contains the coding sequence for the MSG g antigen, was isolated and cloned in *E. coli* XJb (DE3). Extraction and purification through affinity chromatography with immobilized metallic ions, ELISA, SDS-PAGE and Western-Blot, were performed with the objective to obtain the maximum quantity of antigen and determine its purity. The antigen was then used to form bionanoconjugates with AuNPs, previously

functionalized with several types of ligands, which will be the core of the immunochromatographic RDT with potential for point-of-care diagnostics. Acknowledgments: Partially supported by Gilead GÉNESE-PGG/001/2014.

#### DIVERSITY OF PROTISTS IN SALINE AND BRACKISH CONTINENTAL WATER BODIES REVEALED BY HIGH-THROUGHPUT SEQUENCING

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Modern methods of high-throughput sequencing (NGS) are widely used for characterization of protists biodiversity in fresh and marine waters and often result in new data changing our knowledge about natural microbial communities. At present only protistian communities from marine biotops have been studied with NGS, whereas the data on continental saline water bodies are rare (Heidelberg et al., 2013; Triado-Margarit and Casamayor, 2013). The aim of this investigation was characterization of structure and biodiversity of protistian communities in saline and brackish water bodies of the South Urals (Russia) with 18S metagenomic sequencing. For this purpose water samples from saline and brackish lakes and a brackish river were filtered through membranes with diameter of pores 0.45 µm. Total DNA was isolated from the filters and DNA-libraries were made by PCR with universal primers for V4 region of the gene 18S. High-throughput sequencing was conducted with MiSeq (Illumina). The obtained reads were treated with complex of bioinformatic tools. In the report the first data on the biodiversity of eukaryotes in the deeply continental saline and brackish water bodies of the South Urals (Russia) will be presented.

The research was performed in the Center of Shared Scientific Equipment «Persistence of microorganisms» of ICIS UB RAS and was supported by RFBR (16-44-560234, 15-29-02749, 15-29-02518).

#### AN EARLY-BRANCHING CYANOBACTERIUM AT THE ORIGIN OF PRIMARY PHOTOSYNTHETIC EUKARYOTES

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Primary plastid-bearing eukaryotes evolved by the endosymbiosis of a cyanobacterium within a heterotrophic host. This gave rise to the supergroup called Archaeplastida, comprising Viridiplantae (green algae and land plants), Rhodophyta (red algae) and Glaucophyta. Although the monophyly of primary plastids has been extensively recovered, the present-day closest cyanobacterial lineage to the chloroplast ancestor is still debated. We performed phylogenetic analyses using two concatenated datasets containing 97 plastid-encoded proteins and the plastid 16S+23S rRNA cluster, and found in both phylogenetic reconstructions that the ancestor of primary plastids was an early-branching cyanobacterium related to *Gloeomargarita lithophora*, the first cultured member of a recently discovered freshwater cyanobacterial lineage widely present in stromatolites and thermophilic microbial mats. This discovery has implications for the environmental conditions in which the endosymbiosis took place.

#### THE SPECIAL CASE OF *HOLOSPORA CARYOPHILA*, BACTERIAL SYMBIONT OF CILIATES PARAMECIUM

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Infectious bacterium *Holospora caryophila*, described as symbiont of the macronucleus of *Paramecium bicaurelia*, is an unconventional *Holospora*. While other *Holospora* species are highly selective for the host, *H. caryophila* has been isolated from nature in several species of the *P. aurelia* complex, and in



*P. caudatum*. Infection by *H. caryophila* sometimes kills paramecia, but stable association may last for many years. These premises led to detailed investigation of 6 isolates of *H. caryophila*, 4 of them inhabiting the species of the *P. aurelia* complex, and 2 found in *P. caudatum*. Screening of more than 70 potential hosts – strains belonging to 10 morphological species of *Paramecium* – showed that *H. caryophila* can infect majority of species of the *P. aurelia* complex, *P. jenningsi*, *P. caudatum*, *P. multimicronucleatum*, and *P. putrinum*. Symbiosis could be formed only in certain combinations of the host and the symbiont, though some strains appeared to be universal recipients for all *H. caryophila* isolates studied. However, most of the checked strains were never infected. *P. caudatum* strains often died during infection by *H. caryophila* isolated from *P. aurelia* strains. Thus, the chance that symbiont can not develop in host or kills it is higher than possibility of successful infection, explaining why ciliates harboring symbiotic bacteria are rather rare in nature. Molecular phylogenetic analysis of 16S rDNA sequences proved that phylogenetic relationships of *H. caryophila* with other *Holospora* species are quite distant. Physiological and phylogenetic features support transfer of *H. caryophila* to the new genus *Preeria*.

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#### CHASING ION CHANNELS OF DINOFLAGELLATES

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Ion channels are transmembrane protein complexes permeable for ions and playing a crucial role in cell physiology. Ion channels of animals, plants and fungi have been intensively studied for many decades; however, noticeably less information is available concerning ion channels of other eukaryotes. This lack of knowledge hampers our understanding of both evolution of ion transport and physiology of protists. Dinoflagellates are the group of aquatic unicellular eukaryotes of high ecological relevance, but rather little is known about their physiology. At the same time, dinoflagellates are phylogenetically distant from animals, plants and fungi and thus are attractive objects to study evolution of ion channels. However, investigation of dinoflagellate ion channels is complicated by the lack of sufficient genomic data and obstacles in applying electrophysiological techniques to dinoflagellates due to their complex cell coverings. We analyzed publicly available transcriptomes of ten dinoflagellate species and found 12 ion channel

families, including four-domain voltage-gated ion channels (FDVGIC) that played a crucial role in evolution of exciting membranes of eukaryotes and nervous system in metazoans. We revealed a high degree of phylogenetic, structural and functional diversity in FDVGIC of dinoflagellates. We developed a new method to produce spheroplasts of armored dinoflagellates and for the first time obtained single-channel recordings of their ion channels. Our method allowed us to detect considerable diversity of cation channels in *Prorocentrum minimum* at the electrophysiological level: potassium-selective channels, inwardly rectifying cation channels, “fast” and “slow” cation channels, and nonselective cation channels. Funded by the Russian Science Foundation, project 16-14-10116.

#### PHYLOGENY OF PROTISTAN FOUR-DO-MAIN VOLTAGE-GATED ION CHANNELS

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Four-domain voltage-gated ion channels (FDVGIC) drive the initial phase of the action potential propagation in metazoans and many protists. Therefore, these channels are considered as major players in evolution of eukaryotic excitability and metazoan nervous system. In addition to cellular excitability, they are important for cellular motility, intracellular signaling and regulation of rhythmical activity. There are five well studied subfamilies of FDVGIC, and all of them are associated with the opisthokont lineage: voltage-gated and voltage-insensitive sodium channels ( $\text{Na}_v$  and NALCN, respectively), as well as voltage-gated calcium channels (LVA- $\text{Ca}_v$  and HVA- $\text{Ca}_v$ ) and voltage-insensitive calcium channels of fungi (Cch). Using publicly available genomic, transcriptomic and protein databases and blast search, we identified 277 members of FDVGIC family from different eukaryotic groups to reconstruct phylogeny of this ion channel family employing the maximal likelihood (ML) method and Bayesian analysis (BA). In this work, we demonstrated that most of the considered protist groups have their own subfamilies of FDVGIC that do not form clades with any known subfamily of FDVGIC (i.e.  $\text{Na}_v$ , NALCN, LVA- $\text{Ca}_v$ , HVA- $\text{Ca}_v$ , and Cch). Moreover, both ML and BA approaches showed that similar to metazoans some protist groups, such as alveolates and stramenopiles, possess high phylogenetic diversity of FDVGIC. Although obtained phylogenies are not fully resolved due to the limited data on ion channel sequences, the present study advances our understanding of the diversity and evolution of FDVGIC family. Funded

by the Russian Science Foundation, project 16-14-10116.

#### HETEROTROPHIC FLAGELLATES OF SPHAGNUM BOGS IN SOUTH PATAGONIA, CHILE

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Bogs are habitats of great environment-modifying value, in particular, play a major role in the formation of the hydrological regime of the surrounding territory. A considerable amount of data has been accumulated on the heterotrophic flagellates associated with wetland ecosystems of the Northern Hemisphere while the large wetlands of the south part of South America remain almost unexplored. Species composition and morphology of heterotrophic flagellates collected from six sphagnum bogs in Chilean part of South Patagonia and Tierra del Fuego in October–November 2015 are described. Fifty-eight species from 33 genera and 3 macrotaxa (Opisthokonta, SAR, Excavata) and heterotrophic flagellates ‘incertae sedis’ have been recorded. Most of the recorded species are bacterivorous, 7 species – are omnivorous, and 1 – is a predator, feeding on other flagellates. In two bogs of continental part of Chile we have recorded 38 species and forms (38 species in bog no. 1; 1 species in drained bog no. 2), and in four bogs in Tierra del Fuego – we have recorded 39 species and forms (15 species in bog no. 3; 8 species in no. 4; 13 species in no. 5; 16 species in no. 6). Among all aquatic habitats associated with bogs, the greatest number of species has been recorded in hollows (35 species); bogs streams (25); small boggy lakes (24); pool near trunks of *Nothofagus* and peat excavation with cyanobacterial mat at the bottom (5); only 1 species has been recorded in a drainage canal. All identified flagellates are known from different types of freshwater habitats of the Northern Hemisphere, and most of them (31 species and 10 not identified to species level taxa are known from the bogs of the Northern Hemisphere.

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#### ATTRIBUTABLE RISK OF *CAPILLARIA* SPECIES IN DOMESTIC PIGEONS (COLUMBIVIA DOMESTICA)

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Fecal samples were collected from 120 domestic pigeons to determine the Attributable risk of *Capillaria* spp. The *Capillaria* spp. was observed in 64 out of 120 (51%) pigeons (70 males and 50 females) under study. A total of 64 (39 males and 25 females) were naturally infected with *Capillaria* spp. with 51% and 50% in males and females respectively. Qualitative examinations include the direct microscopy and faecal floatation while quantitative examination includes McMaster technique (worms are calculated per gram of the faeces). Month wise Attributable risk showed that eggs of the worms were found to be abundant in the month of July (60% to 73%) because of high humidity in the month of July during the present study. Very high and very low temperature is not suitable for the proper development of the eggs. Qualitative and quantitative examination revealed that *Capillaria* spp. was more prevalent in males (51%) than females (50%) but overall there is no significant difference ( $p > 0.05$ ) in the male and female because both of individuals invest equal amount of energy in search of food and incubating the eggs. Different breeds of the pigeons gave different Attributable risk in different months during the whole study. Group of pigeons from different locations showed variable Attributable risk. Areas with high humidity were more suitable for the development of eggs that is the reason that higher Attributable risk was observed in shahdara (75%).

#### *NEBELA JIUHUENSIS* NOV. SP. (AMOEBOTROPHIC; ARCELLINIDA; HYALOSPHENIIDAE): A NEW MEMBER OF THE *NEBELA SACCIFERA* - *EQUICALCEUS* - *ANSATA* GROUP DESCRIBED FROM SPHAGNUM PEATLANDS IN SOUTH-CENTRAL CHINA

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Hyalospheniids are among the most common and conspicuous testate amoebae in high-latitude peatlands and forest humus. These testate amoebae were widely studied as bioindicators, and are increasingly used as models in microbial biogeography. However, data on their diversity and ecology are still very unevenly distributed geographically: notably, data are lacking for low latitude peatlands. We describe here a new species, *Nebela jiuhuensis*, from peatlands near the Middle Yangtze River reach of south central China with characteristic morphology. The test (shell) has hollow horn-like lateral extensions also found in *N. saccifera*, *N. equicalceus* (= *N. hippocrepis*) and *N. ansata*, three large species restricted mostly to *Sphagnum*-peatlands of Eastern North America. Mitochondrial cytochrome oxidase (COI) data confirm that *N. jiuhuensis* is closely related to the morphologically very similar North American species *N. saccifera* and more distantly to *N. ansata* within the *N. penardiana* group. These species are all found in wet mosses growing in poor fens. Earlier re-reports of morphologically similar specimens found in South Korea peatlands suggest that *N. jiuhuensis* may be distributed in comparable peatlands in Eastern Asia (China and Korea). The discovery of such a conspicuous new species in Chinese peatlands suggests that many new testate amoebae species are yet to be discovered, including potential regional endemics. Furthermore, human activities (e.g. drainage, agriculture, pollution) have reduced the known habitat of *N. jiuhuensis*, which can thus be considered as locally endangered. We therefore suggest that this very conspicuous microorganism with a probably limited geographical distribution and specific habitat requirement should be considered as a flagship species for microbial biogeography as well as local environmental conservation and management. Key words: Arcellinid testate amoebae; biodiversity conservation; biogeography; DNA barcoding; mtCOI.

EPIBIONTIC RELATIONSHIP IN AN ARTIFICIAL POND OF CHIAPAS (MEXICO): *PROCAMBARUS* (*AUSTROCAMBARUS*) SP. (CRUSTACEA: DECAPODA) AND *EPISTYLIS HENTSCHELI* (CILIOPHORA: PERITRICHIA)

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Several samples collected during years 2014 and 2015 contained epibenthonic fauna with the crayfish *Procambarus* (*Austrocambarus*) sp. Samplings were performed on the freshwater artificial pond Yaleon, located in Montebello (Chiapas, SE Mexico), using a 5 mm opening hand net. The environment was strongly polluted with organic matter (assimilated to a polysaprobic zone). The crayfish *Procambarus* (*Austrocambarus*) sp. harbored colonies of a peritrich ciliate identified as *Epistylis hentscheli* Kahl, 1935. This ciliate formed ramified colonies with a moderate number of zooids (up to 30). Stalks of the colony were dichotomously branched, and they presented peripheral fibres arranged longitudinally. The zooid was elongated; the form of body was similar to a bell (170–200 µm in length), with a C-shaped transversal macronucleus, and a spherical micronucleus near to the macronucleus. The peristomal lip was very thin. The buccal infraciliature had the general pattern of peritrichids and was composed by a haplokinety and a polykinety drawing a spiral with 1.5 rounds inside the buccal infundibulum. These epibionts were located exclusively on the pereopods. The goal of this contribution is to provide data about cell/colony structure of *E. hentscheli* and also its distribution on the crayfish. This is the first record of this epibiont peritrich ciliate on crustacea, being previously found on algae and fish. Acknowledgements: to Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México and CONACyT, for the support necessary for carry out this research. Additionally, we are indebted to Dr. Villalobos-Hiriart (Instituto de Biología, UNAM) for the crustacean identification and to Biol. Reyes-Santos (Facultad de Ciencias, UNAM) for their help with impregnation techniques. We also appreciate the technical support of the Department of Zoology (Universidad Complutense, UCM, España).

ORIGIN AND EVOLUTION OF *PARAMECIUM BURSARIA* / *CHLORELLA* SYMBIOTIC SYSTEM: WHAT CAN ONE SAY ABOUT THE HOST EVOLUTION STUDYING EVOLUTION OF SYMBIONT?

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**BACKGROUND.** *P. bursaria* belongs to one of the best studied Ciliate's genus *Paramecium*. This species diverged basically from other paramecia. They possess symbiotic *Chlorella* in the cytoplasm. These *Chlorella* were attributed to three *Chlorella* species – *Ch. vulgaris*, *Ch. variabilis* and *Micractinium reisseri* according to their phylogeny inferred from ITS1-5.8S-ITS2-5'LSU rDNA sequence and their sensitivity to specific viruses (PBCV). Morphospecies *P. bursaria* consist of 5 syngens – reproductively isolated intraspecies groups. Previously we have shown that syngens were represented by different branches on phylogenetic trees. The purpose of our investigation was to study phylogeny of *P. bursaria* and their symbiotic *Chlorella* using the same set of strains collected in different geographic regions. **RESULTS.** Gene RuBisCo have been used as molecular marker for *Chlorella* phylogeny. The tree consists of three separate branches. Diversity in each branch is negligible. Our data support strong correspondence between *P. bursaria* syngens and branch of *Chlorella*, while weak correlation between *Chlorella* type and its geographic location. **CONCLUSIONS.** According to our results we have concluded that successful symbiosis between *P. bursaria* ancestor and *Chlorella* has been established at least three times. The last time it had been happened before syngens R1 and R2 have diverged. We propose the strategy of evolution and geographic dispersion of this symbiotic system. Scientific research was performed at the Center for Culturing Collection of Microorganisms and Center for Molecular and Cell Technologies of Research park of St. Petersburg State University. Supported by RFBR grant 13-04-01714.

#### THE PLASTID GENOME OF *POLYTOMA UVELLA* IS THE LARGEST KNOWN AMONG NON-PHOTOSYNTHETIC ALGAE

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The polyphyletic assembly *Polytoma* and the species of the genus *Polytomella* represent different lineages within the Order Chlamydomonadales (Chlorophyta) that lost photosynthesis independently. The *Polytomella* clade is one of the two known groups of plastid-bearing eukaryotes that have lost completely the plastid genome (ptDNA)

after the loss of photosynthesis. Before the present report it was unknown if the absence of genome in the *Polytomella* plastids was a shared condition with species of the *Polytoma* genus. We present here the ptDNA of *Polytoma uvella* UTEX 964, which is the largest (circa 230 Kb) reported among non-photosynthetic algae. The ptDNA of *P. uvella* has lost all genes related with the photosynthetic function and its residual coding capacity is similar to ptDNAs from distantly related colorless chlorophytes, such as the pathogens *Prototheca* and *Helicosporidium*. The gene repertoire of the *P. uvella* ptDNA comprises mostly transcription and translation players and just few conserved proteins (e.g., FtsH-like, Ycf1-like and ClpP). Regardless of the substantial gene loss, the *P. uvella* ptDNA shows evidence of expansion due to the accumulation of intergenic short repeated sequences (average length of 2.9 Kb). It is plausible that recombination-based DNA repair mechanisms are responsible of the genomic expansion of the *P. uvella* ptDNA.

#### SELECTION AND CHARACTERIZATION OF SINGLE CHAIN VARIABLE FRAGMENT (SCFV) ANTIBODIES AGAINST *PNEUMOCYSTIS JIROVECI* FROM PHAGE DISPLAY LIBRARIES

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*Pneumocystis pneumonia* (PcP) is an infectious disease caused by *Pneumocystis jirovecii*, an atypical fungus. PcP remains a major cause of respiratory illness among immunosuppressed patients. Current PcP diagnosis is based on the detection of *P. jirovecii* in respiratory specimens, obtained by invasive methods such as bronchoalveolar lavage, followed by cytochemical staining, immunofluorescent staining with monoclonal antibodies (IF/Mab) or PCR. Therefore, the possibility of an early diagnostic method allowing the use of biological specimens obtained non-invasively, is highly desirable. Rapid diagnostic tests (RDTs) using gold nanoparticles (AuNPs) allow a more sensitive, fast and cheap diagnosis, to be used in developing countries. The goal of this work is to develop an immunochromatographic RDT for the detection of *P. jirovecii* in non-invasive specimens like serum. In this test, spherical AuNPs are conjugated with a multi-epitope synthetic recombinant antigen (msr) which will allow the detection of circulating anti-*P.*

*jirovecii* antibodies in sera. In order to obtain the highest amount of pure antigen, the expression vector pLATE 31, which contains the coding sequence for the MSG g antigen, was isolated and cloned in *E. coli* XJb (DE3). Extraction and purification through affinity chromatography with immobilized metallic ions, ELISA, SDS-PAGE and Western-Blot, were performed with the objective to obtain the maximum quantity of antigen and determine its purity. The antigen was then used to form bionanoconjugates with AuNPs, previously functionalized with several types of ligands, which will be the core of the immunochromatographic RDT with potential for point-of-care diagnostics. Acknowledgments: Partially supported by Gilead GÉNESE-PGG/001/2014.

#### CHANGES IN CILIATES COMMUNITY ACROSS AN EUTROPHICATION GRADIENT IN A LARGE TEMPERATE ESTUARY

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The ciliate community composition in Long Island Sound, USA, was investigated using high throughput sequencing (Miseq, Illumina) of variable region 4 (V4) of the small subunit ribosomal gene (V4-SSU). Due to its proximity to heavily populated areas, the western Sound (WLIS) is eutrophic, and strong gradients have been observed down-estuary (east to west) in nutrients, chlorophyll, and plankton biomass. We sampled six stations on the long axis of the estuary, at two depths (surface and 6 m), in the summer of 2015. Analysis of ciliate V4-SSU sequences grouped into operational taxonomic units (OTUs) corresponded to the eutrophication gradient, with higher OTU-richness found in WLIS compared to the east. The majority of ciliates were classified into Choreotrichia and Oligotrichia, contributing together 65% to 91% of all ciliate OTUs. Litostomatea and Prostomatea contributed from 4 to over 30% of the OTUs. The relative proportion of Oligotrichia was higher near the surface and increased in the eastern, more pristine stations. The Choreotrichia showed the opposite pattern. The relative proportion of Oligotrichs and Choreotrichs across the eutrophication gradient in Long Island Sound was correlated with water transparency and it could be partially explained by variation in abundance of major phytoplankton groups in the western, central and eastern Long Island Sound.

#### TERRESTRIAL ALGA *KLEBSORMIDIUM* IN THE LIGHT OF THE HYPOTHESIS „EVERYTHING IS EVERYWHERE, BUT THE ENVIRONMENT SELECTS“

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Baas Becking's hypothesis "Everything is everywhere, but the environment selects" first mentioned in 1934 still contains three current controversial issues in protists: disunity in the description of diversity, distribution patterns, and the ecological preferences. We examined a very common, globally distributed terrestrial algal genus *Klebsormidium* and tested (i) whether there is any biogeographical pattern of this microorganism, (ii) if there is a comparable diversity in the polar and temperate regions, and (iii) a potential role of ecological speciation processes on diversification. Our analyses revealed the presence of two different distribution patterns which are supposed to characterize both macroorganisms and protists. We demonstrated an unlimited dispersal and intensive gene flow within one of the inferred lineages (superclade B). However, the majority of *Klebsormidium* clades showed rather a limited distribution. In addition, we detected a significant decrease of species richness towards the poles, i.e. the macroecological pattern typical for macroorganisms. Species within a single protist genus may thus exhibit highly contrasting distribution patterns, based on their dispersal capabilities, which are usually shaped by both intrinsic and extrinsic factors. In addition, we determined a distinct ecophysiological differentiation among distantly and closely related lineages, thereby corroborating our hypothesis that the sympatric speciation of terrestrial algae is driven by ecological divergence. We clearly showed that pH is a critical ecological factor that influences the diversity of autotrophic protists in terrestrial habitats.

#### LYING ON THE SURFACE: NEW DATA ON THE EPISYMBIONTS OF CILIATES

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Protists often form symbiotic associations with prokaryotes. Ciliates are especially well known for harbouring endosymbiotic and ectosymbiotic bacteria, the relationship between the partners ranging from mutualism to parasitism. Epibionts can be found both in anaerobic and in free living aerobic ciliates, however, so far they have never been reported in paramecia. Here we present morphological (DIC, AFM, CLSM, TEM) and molecular characterization of the bacterium fouling cells of *Paramecium primaurelia* strain isolated from nature in Cyprus. At the last stage of infection, epibionts formed a thick coat on the cortex of the ciliate causing complete loss of cilia, changes of the cell shape, dwarfing, and finally, the host death. Fluctuations of the ciliate population density of the infected strain registered throughout long term observations of the laboratory culture imply parasitoid nature of the epibiont. In preliminary FISH experiments, epibionts were easily revealed with the universal eubacterial probe Eub 338, however, they never showed positive signal when hybridized with probes specific for Alpha-, Beta-, Gamma- or Deltaproteobacteria. Molecular characterization was done following the full-cycle rRNA approach and association of the epibionts with the host was confirmed by FISH experiments with newly designed species-specific probes. The obtained 16S rDNA sequence showed a similarity of circa 82% with Alphaproteobacteria class, thus suggesting that epibionts are extremely unusual novel organisms. Phylogenetic analysis, which is under way, will enlighten the evolutionary position of this enigmatic organism, presumably, either a basal alphaproteobacterium or a fast-evolving line within the order Rickettsiales.

#### BIODIVERSITY OF BENTHIC DINOFLAGELLATES ALONG THE SOUTHERN COAST OF OMAN WITH EMPHASIS ON POTENTIALLY TOXIC SPECIES

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Benthic dinoflagellates have attracted considerable research attention during the last decades due to their morphological and taxonomic diversity, ecological significance in marine benthic ecosystems, and their capability to produce toxins and cause toxic blooms in the coastal areas. A pilot taxonomic survey of the benthic dinoflagellates was performed for the first time at the Arabian Sea coast along Dhofar Governorate of the Sultanate of Oman in the vicinity of Salalah City at five sampling sites on February 2014 and 2016. It has been shown that the bottom sediments and the surface of brown and red macroalgae along the southern Omani coast were inhabited by taxonomically diverse and abundant assemblages of sand-dwelling and epiphytic dinoflagellates. A total of 38 dinoflagellate species belonging to five orders were recorded in this study including Dinophysiales, Gymnodiniales, Gonyaulacales, Peridinales, and Prorocentrales. High diversity of the sand-dwelling dinoflagellates was supported mainly by gymnodinioid and peridinioid taxa. In contrast, members of *Coolia*, *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum* were among the most abundant and diverse epiphytic dinoflagellates. A number of known toxin producers were observed including the ichthyotoxic dinoflagellates of the genus *Amphidinium*, the okadaic acid producing *Prorocentrum* species, the cooliatxin producing *Coolia*, the palytoxin producer *Ostreopsis*, and the ciguatera-related *Gambierdiscus* species. The presence of known toxic dinoflagellate species may indicate a potential risk of toxicity in the marine environment of the southern Omani coast, and underscores the need for further studies on taxonomy, ecology and toxicology of benthic dinoflagellates in Oman.

#### DEVELOPMENT OF A MULTIPLE-DRUG SELECTION SYSTEM FOR DUAL-TRANSFORMATION OF THE OYSTER PARASITE *PERKINSUS MARINUS*

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Plastids in apicomplexan parasites are highly degenerated. The organelle is nevertheless essential for completion of the parasite life cycle. Interestingly, an oyster parasite *Perkinsus marinus*, which is sister to dinoflagellates and close to Apicomplexa, also has a DNA-lacking, extremely degenerated plastid. Functional analysis of the cryptic organelle is attracting and required to understand the relationship between the organelle degeneration and parasitism. The transgenic technique is a convincing approach for the analyses of proteins of interest and is practicable in *P. marinus*. However, each transfected cell must be isolated from untransfected cells by hand labor using a micromanipulator multiple times to obtain any transfected cell lines. This is because drug selection system has not been established. Here, we identified two drugs that are available for selection of transfected *P. marinus* cells. Firstly, we screened antibiotics shown utility in apicomplexan parasites and determined that blasticidin S, bleomycin and puromycin effectively inhibited the parasite growth. Then, their resistance genes were fused downstream of *gfp* or *mCherry* gene, and each construct was transfected to the parasite. After two months, the fluorescent signals were observed in almost all cells cultured with bleomycin or puromycin. Furthermore, dual transfected cells were selected by using the two drugs, which enables us to examine colocalization of plastid proteins. We believe that this system provides new opportunities for functional analyses of the plastids in the parasite.

#### A DRAFT GENOME OF THE ANAEROBIC FLAGELLATE *CARPEDIEMONAS MEMBRANIFERA*, A FREE-LIVING RELATIVE OF METAMONAD PARASITES

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*Carpediemonas membranifera* is a free living flagellated metamonad related to diplomonad parasites such as *Giardia intestinalis* and *Spironucleus*

*salmonicida*. We are interested in elucidating the evolutionary transitions to anaerobiosis and parasitism within metamonada, and sequenced the genome of *C. membranifera*. The genome assembly is 22.4 Mb long with 11328 predicted protein-coding genes, 41% of those have introns. Automatic annotation was carried out by searching against the Interpro, PFAM, Prosite, TIGR databases to identify domains, assign putative functions and predict metabolic pathways. Expert manual annotation is underway for genes encoding proteins functioning in DNA repair, mRNA degradation, mitochondrion-related organelles, cell surface or external cellular processes involved in host tissue adhesion, immune evasion, pathogenicity, nutrient acquisition, metabolite transport and environmental sensing, among others. We have completed analyses of the DNA repair pathways. Those can drive sexual/parasexual pathways, antigen diversification and copy number variation, and are of great importance for adaptive evolution. *C. membranifera* possesses a complete system for excision repair, and the double strand break repair machinery including 1) a homologous recombination pathway and 2) microhomology-mediated end joining and single-strand annealing. Also, it has several gene family expansions, as well as, a complete repertoire of cell cycle checkpoints and sex-related proteins. *G. intestinalis* and *S. salmonicida* have minimalistic and slightly different versions of the pathways found in *C. membranifera* suggesting that there have been some secondary losses and modifications in diplomonads as a result of their parasitic lifestyle.

#### THE GENOMIC COST OF BECOMING A RED ALGAL FREELOADER

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An abundance of genomic and transcriptomic data have been gathered over the past decade providing a wealth of knowledge about what it takes to be a successful parasite. Genomes of highly derived eukaryotic parasites have been sequenced including those from formerly photosynthetic lineages including apicomplexans. These data have revealed fascinating innovations that evolved over hundreds of millions of years, enabling parasites to infect and evade their hosts. Unlike highly derived lineages of eukaryotic parasites, red algae appear to be fertile ground for adopting a parasitic life strategy as seen by numerous recent and independent evolutions of parasitic taxa. Red algal parasites provide a great system to investigate the early stages of genome

evolution as an organism gives up autotrophy in favor of moving onto its relatives' couch and raiding their refrigerator. We sequenced draft transcriptomes for the parasitic red alga *Choreocolax polysiphoniae* and its free-living host *Vertebrata lanosa*. These data, in combination with genomic DNA sequence data for these taxa was compared with other published red algal genomes and transcriptomes to investigate the early consequences of transitioning from autotrophy to parasitism. *Choreocolax polysiphoniae* appears to have lost genes involved in plastid maintenance and photosynthetic processes. Investigation of these data and their implications for the evolution of parasitism remains ongoing.

#### MOLECULAR DIVERSITY AND PHYLOGENY OF OLIGOTRICHIA AND CHOREOTRICHIA (CILIOPHORA, SPIROTRICHEA)

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We revise and expand the molecular data on the subclasses Oligotrichia and Choreotrichia in terms of both genetic markers and taxonomic sampling. Two approaches are used, one based on sequences from morphologically-characterized specimens, and another one including also the unidentified environmental sequences available in NCBI GenBank. First, concatenated sequences of ribosomal DNA markers (18S rDNA, 28S rDNA and ITS regions) from seventeen families and four clades of the paraphyletic genus *Tintinnopsis* were analyzed, including two families newly studied by single cell sequencing and six genera newly sequenced for at least one marker. Second, all the 18S rDNA sequences available in GenBank for these subclasses were retrieved and curated as part of the EukRef initiative, resulting in almost 3,200 sequences and 1,000 OTUs (operational taxonomic units clustered at 99% similarity). The concatenated dataset allowed confirming or discovering novel phylogenetic relationships within Choreotrichia. The 18S rDNA dataset allowed rough comparisons of molecular diversity between the two subclasses. Because Choreotrichia has been easier to barcode (mainly for the loricate Tintinnida), analyses have not revealed novel diversity, but exposed known and new misidentifications and classification inconsistencies. In contrast, three clades within Oligotrichia included only environmental sequences, thus highlighting a high proportion of partially-characterized or novel diversity in this subclass. These analyses help solving classification conflicts and will provide a reference for studying the diversity and distribution

of these abundant and ecologically relevant ciliates in marine plankton.

#### TAXONOMIC COMPOSITION OF PROKARYOTES-ASSOCIATES OF HALOPHILIC PROTISTS

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As known, taxonomic structure of communities in hypersaline environments is simple. It includes prokaryotes, photosynthetic and heterotrophic protists, fungi, and crustaceans. Under extreme salinity the community is simplified up to only microorganisms, where phototrophic protists are the main producers of organic matter, and heterotrophic protists are the only consumers. Symbiotic associations with halophilic phototrophic protists are well studied for green alga *Dunaliella* and its prokaryotic associates, while heterotrophic protists and their role in forming of halophilic microbial community are studied poorly. Aim of the study was to estimate taxonomic composition of prokaryotes – associates of autotrophic and heterotrophic protists isolated from salt water bodies in Salt-Iletsk and Elton regions. The method of 16S metagenomic sequencing was used. 111 OTUs were found in association with heterotrophic flagellate *Pleurostomum salinum*. The genera of archaea *Natronomonas* sp., *Haloarcula* sp., *Haloplanus* sp., *Halorubrum* sp. and bacteria *Halovibrio* sp., uncultured bacterium (Bacteroidetes) were predominant. Heterotrophic flagellate *Tulamoeba bucina* had very similar taxonomic composition of prokaryotes. There were 129 OTUs including uncultured bacterium (Bacteroidetes), *Natronomonas* sp., *Halovibrio* sp., *Haloferax* sp., *Halorubrum* sp. as predominant genera. There were 32 and 41 OTUs in associations with *Pharyngomonas* sp. and Heterolobosea aff. *Euplaesiobystra* respectively. Bacteria *Idiomarina* sp., *Halomonas* sp., *Gracilimonas* sp. were predominant in both cultures. Phototrophic flagellate *Dunaliella parva* had 19 OTUs of prokaryotes – associates. Bacteria *Halovibrio* sp. and archaea *Halorubrum* sp. were the most abundant. A crucial factor influencing the taxonomic composition of prokaryotes in the associations with halophilic protists was level of medium mineralization. Taxonomic diversity was higher in associations with heterotrophic protists than in associations with autotrophs. The highest

species richness of prokaryotes – associates has been registered in the culture *Tulamoeba bucina*. The research was performed in the Center of Shared Scientific Equipment «Persistence of microorganisms» of ICIS UB RAS and was supported by RFBR (16-44-560316, 14-04-01796).

#### CHARACTERIZATION OF MONOCLONAL ANTIBODIES FOR CATHEPSIN B AND CATHEPSIN B-LIKE PROTEINS OF *NAEGLERIA FOWLERI*

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*Naegleria fowleri* causes a fatal primary amoebic meningoencephalitis (PAM) in human and experimental animals. Cathepsin B (NfCPB) and cathepsin B-Like (NfCPBL) gene in *N. fowleri*, are consists of 1,038bp of DNA (345 amino acid) and 939bp of DNA (313 amino acid), and molecular weights of recombinant proteins are 38.4 and 34 kDa, respectively. In the previous study, rNfCPB and rNfCPB-L refolding protein may play important roles in host tissue invasion, immune evasion and nutrient uptake. In this study, we produced anti-NfCPB and anti-NfCPBL monoclonal antibody (rNfCPB-McAb and rNfCPBL-McAb) using the cell fusion technique and observed the immunological characteristics. Seven hybridoma cells secreting rNfCPB-McAb (2D6, 2D11, 2A7, 2B7, 2E2, 2E9, 2C9) and three hybridoma cells secreting rNfCPB-McAb (1E5, 1C8, 1D9) were produced. Among them, 2C9 (a cell line producing rNfCPB-McAb) and 1C8 (a cell line producing rNfCPBL-McAb) which showed high antibody titre, respectively, were selected. 2C9 monoclonal antibody was reacted with *N. fowleri* whole lysate or rNfCPB fusion protein by western blotting, as which showed about 28 kDa and 38.4 kDa of band pattern, respectively. 1C9 monoclonal antibody was reacted with *N. fowleri* whole lysate or rNfCPBL fusion protein by western blotting, as which showed 24 kDa and 34 kDa of band pattern. 2C9 and 1C8 monoclonal antibodies were not reacted with another amoebic lysates such as *N. gruberi*, *Acanthamoeba castellanii*, *A. polyphaga* in western blot analysis. In the results of the immunocytochemistry analysis, NfCPB and NfCPB-L protein were mainly detected in cytoplasm and cell membrane, especially pseudopodia, of *N. fowleri* trophozoites under a confocal microscope.

These results suggested that monoclonal antibodies against rNfCPB and rNfCPBL may be useful for the further immunological study.

#### BENTHIC PROTISTS (CILIOPHORA, GROMIIDA, FORAMINIFERA) IN THE BLACK SEA: THEIR CONTRIBUTION TO THE BOTTOM COMMUNITIES

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Ciliophora, Gromiida and Foraminifera (hard-shell and soft-shell forms) are the significant components of the benthic communities of the Black Sea. These protozoans are of great role in the transfer of bacterial and algal production to the next trophic levels. They themselves are consumers of bottom bacteria, microalgae, pollen terrestrial vegetation and marine filamentous fungi; as predators, they might prey upon unicellular organisms. In addition, the representatives of above listed groups are an important food source for many bottom invertebrates in different habitats. The changes in taxa composition and abundance, and also the proportions of these protozoan representatives in the benthic communities along deep-water oxic/anoxic interface (75-300m) in the Istanbul Strait's (Bosporus) outlet area of the Black Sea and NW shelf of Crimea Peninsula were studied. In the oxic zone of the Black Sea, benthic Protozoa and Metazoa were studied in area near the Kerch Strait's on NE shelf of Crimean Peninsula. Our data allowed concluding about tolerance of many benthic protozoans to the hypoxic / anoxic conditions and sulfidic pollution of bottom sediments of the Black Sea and of their significant share in the deep-water benthic communities. Benthic Ciliophora, Gromiida and Foraminifera are numerous, specific and diverse in these conditions. The contribution of these protozoans in the meiobenthos communities had high values and extremely prone to variability. The spatial-bathymetrical distribution of these protists along with benthic multicellular organisms had uneven character in the Black Sea.

#### CHARACTERIZATION OF “*CANDIDATUS GORTZIA SHAHRAZADIS*”, A NOVEL ENDOSYMBIONT OF *PARAMECIUM MULTIMICRONUCLEATUM* FROM INDIA

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Ciliates are known since a long time to be potential hosts for bacteria. Some of the most investigated species belong to the so-called *Holospora*-like bacteria (HLB) group, which includes all known *Holospora* species and “*Candidatus Gortzia*” genus. HLB are obligate endosymbionts, inhabiting the macronucleus or the micronucleus of certain species of *Paramecium* and, less frequently, *Frontonia*. They are Gram-negative, non-motile *Alphaproteobacteria* with a distinctive life cycle involving two different morphotypes: the smaller and almost roundish reproductive (RF) form, and the much more elongated, rod-like infectious form (IF). In the present work we describe a novel species belonging to “*Ca. Gortzia*” genus, detected in *P. multimicronucleatum*, a ciliate in which HLB have never been reported before. This novel endosymbiont shows unusual features respect its relatives, such as deep variations in IF morphology and capability to infect occasionally host cytoplasm. It presents small, roundish RF (2.5–3.3 µm) and rod-shaped IF. Two morphotypes of IF were detected in different periods: a shorter IF at the beginning (6.9–10.7 µm), and a longer one (8.2–14.7 µm) with irregular distribution of periplasm after one year of cultivation. The latest were found together with a high number of transient forms (TF), which appeared long (6.7–15.5 µm), rod-shaped and not yet differentiated in IF at ultrastructural level. All forms show a constant diameter of 0.7–0.8 µm. No production of “connecting piece” during host cell division was observed. We propose for this novel HLB from India the name of “*Candidatus Gortzia shahrazadis*”.

#### INTERACTION OF DIFFERENT SYMBIOTIC BACTERIA IN THE CILIATE *PARAMECIUM*

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Multiple bacterial symbioses are rather common phenomenon in ciliates. Some intracellular bacteria, like “*Candidatus Megaira polyxenophila*”, apparently do not produce any effect on the host, and their mode of interaction with ciliate is unknown; some other, like *Holospora*, are highly infectious bacteria actively exploiting the host resources. However, little is known about interactions among endosymbiotic bacteria inhabiting the same host cell. The simultaneous infection of a ciliate with several bacteria has been observed but not studied in detail. Finally, the ability of a bacterium to enter and associate with the host cell already occupied by another symbiont remains unclear; indeed the possible competition for the same cell compartment between the “native” bacterium and an “invader” has never been investigated. We analyzed how the host cell reacts to multiple infections, and how different symbiotic bacteria interact inside the host. For this purpose, four strains of *Paramecium caudatum* harboring “*Candidatus Megaira polyxenophila*” in macronucleus were experimentally infected with representatives of several *Holospora* species. During our observations “*Candidatus Megaira polyxenophila*” prevented development of macronuclear *Holospora* species, thus showing the tendency to be the only occupier of the host macronucleus. On the contrary, “*Candidatus Megaira polyxenophila*” allowed the entrance of *Holospora undulata* in the host micronucleus, even in presence of other cytoplasmic endosymbionts. Results on reciprocal infections (paramecia harboring *Holospora* infected with “*Candidatus Megaira polyxenophila*”) also suggested that symbionts protect their cell compartment from invasions by other bacteria in order to ensure their persistence and spreading in the environment.

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#### CANINE BABESIOSIS IN MOSCOW REGION OF RUSSIA

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The blood smears of dogs were examined in Moscow region under light microscope in 2011–2014. If *Babesia* merozoites were detected the samples were examined with IFA-tests SNAP 4Dx against for *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., *Dirofilaria immitis*. The microfilaria were visualized in samples after concentration. Differential staining of acid phosphatases was used to distinguish *Dirofilaria microfilaria*. Anamnesis of infected dogs was analyzed. The ticks were removed

from dogs inside veterinary clinic or collected from vegetation in different ecosystems biotopes of Moscow region. During the survey 1045 ticks were collected (*Dermacentor reticulatus*, *Ixodes ricinus*, *I. persulcatus*). In the natural habitats of Moscow region 480 ticks were collected (*I. ricinus*, *I. persulcatus*, *D. reticulatus*). It is presumed that only *D. reticulatus* is a natural agent of babesiosis transmission in Moscow region. In total 283 cases of babesiosis of dogs were reported, with 8,0% of cases reported for dogs never leaving city limits. Remaining 92 % of dogs were transported from time to time to country side. In the majority of dogs the babesiosis is developing without severe consequences, and only in 2,5% of cases the acute renal failure was diagnosed. In 1,7% of dogs autoimmune hemolytic anemia was observed. About 0,7% of studied dogs were seropositive for *D. immitis* all other pathogens were absent in these animals. In 1,7% of cases the microfilaria of *D. repens* were detected and in 0,7% of cases the microfilaria of *D. immitis* were found.

#### N-ACETYL ORNITHINE DEACETYLASE IS A MOONLIGHTING PROTEIN AND IS INVOLVED IN THE ADAPTATION OF *ENTAMOEBIA HISTOLYTICA* TO NITROSATIVE STRESS

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*Entamoeba histolytica* is a causative protozoan parasite of amebiasis, a disease which is characterized by acute inflammation of the colon. Adaptation of the parasite to toxic levels of nitric oxide (NO) that are produced by phagocytes may be essential for the establishment of chronic amebiasis and the parasite's survival in its host. In order to obtain insight into the mechanism of *E. histolytica*'s adaptation to NO, *E. histolytica* trophozoites were progressively adapted to increasing amounts of the NO donor drug, S-nitrosoglutathione (GSNO) up to a concentration of 110  $\mu$ M. These NO-adapted trophozoites (NAT) were more resistant to an acute exposure of 350  $\mu$ M GSNO) and activated macrophages and were better at invading porcine colon explants than wild-type trophozoites. The transcriptome of NATs was investigated by RNA sequencing (RNA-seq) and the results of the analysis revealed the existence of a weak overlap

with the transcriptome of acute nitrosative-stressed trophozoites (TEANS). N-acetyl ornithine deacetylase (NAOD) was among the 208 genes that were up-regulated in NAT. NAOD catalyzes the deacylation of N-acetyl-L-ornithine to yield ornithine and acetate. Overexpression of NAOD resulted in significant overproduction of putrescine and in a better adaptation to NS. Surprisingly, overexpression of a catalytically inactive NAOD (mNAOD) resulted in a better adaptation to NS but did not result in putrescine overexpression. These results suggest that NAOD has moonlighting activity. The binding of NAOD or mNAOD to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) prevents the formation of putatively toxic high molecular weight GAPDH species and the absence of these species may underlie the parasite's ability to survive NS.

#### DISTRIBUTION AND CRYOCONSERVATION OF PROTISTS IN THE ARCTIC SOILS

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Previous investigations have shown that resting cysts of soil protists can survive in permafrost for thousands of years at subzero temperatures. However, our knowledge about mechanisms of long-term cryobiosis remains incomplete. One of the uncertainties is the mechanism of cysts transition into the frozen deposits. The main goal of the study was to gauge the protists species abundance and diversity in the profiles of Cryosols - typical arctic soil, that are subjected to the different processes of cryogenic mass-exchange redistributed the fragments of the uppermost soil horizons with microorganisms inhabiting this material. Taxonomic analysis of ciliates and heterotrophic flagellates isolated from 21 soil samples revealed 40 species and forms of heterotrophic flagellates from 9 taxonomical groups and 32 species of ciliates from 7 taxonomical groups. Protists vertical distribution in soil profiles allows to select two groups of species: 1) species occur only in the modern uppermost soil horizons and 2) part of the protists' community (53% and 55% of ciliates and flagellates species respectively) that occurs in material of organic and organo-mineral soil horizons both a modern and buried into the middle and lowermost parts of

Cryosol profiles by different processes of cryogenic mass-exchange. According to our results about half of protists communities in Cryosols have adaptive and protective mechanisms enabling long-term cryptobiosis in the inhospitable conditions of the arctic soils and permafrost. Fragments of the uppermost soil horizons that were cryoturbated, buried by solifluction and accumulated in the upper layer of permafrost appear to be the ecological niche in profiles of Turbic Cryosols that can significantly sustain viability of protists.

#### GENOME ANNOTATION OF *ACRISIS KONA*

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Acrasids are single-celled amoebae that can undergo aggregative multicellularity in response to adverse environmental conditions, similar to the well-studied dictyostelid social amoebas. However, acrasids are unrelated to dictyostelids (supergroup Amoebozoa), being instead the only multicellular lineage in the eukaryotic supergroup Excavata. This makes *Acrasis* an interesting model system to study parallel evolution of social behavior in microbes as well as to explore the diversity of eukaryotes in general. We have sequenced the genome and transcriptome of *Acrasis kona* and are currently preparing transcriptomes from the four main stages of its life cycle. In initial work, we assembled the complete *A. kona* mitochondrial genome (mtDNA) and find that it is missing 14 protein genes present in the mtDNA of its closest sequenced relative, *Naegleria gruberii*. We further identified 11 of these protein genes in *A. kona* nuclear DNA and find that they carry mitochondrial important signals (transit peptides, Fu et al. 2014). We are now using RNAseq data and the *N. gruberii* genome in an annotation pipeline to create a fully annotated *A. kona* nuclear assembly. The results will be used to investigate parallel evolution of simple multicellularity, early steps in the evolution of eukaryotes and to aid in resolution of the eukaryote tree of life by breaking up some of the longer deep branches.

#### CHEMOTAXIS RESPONSE OF PHYTOPLANKTON TO CILIATES

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Marine environment at the micro scale level is

heterogeneous in terms of nutrient distribution and many microbial species can actively exploit these nutrient patches. Exudates of microbes act as nutrient hotspots and also provide chemical cues to their prey or predators. In the present study we examined the chemotactic response of starved and/or non-starved *Dunaliella salina* to the exudates of ciliate grazers (*Euplotes vannus*, *Euplotes* sp., and *Diophrys oligothrix*) by using two approaches; 1. Co-culturing of two species under different illumination conditions to evaluate the growth, ingestion and behavioural response of the phytoplankton and/or ciliates and 2. Using microfluidics and image analyses to evaluate the response of *D. salina* to the exudates of ciliates and related nutrient media. *D. salina* showed different active swimming and an ‘attack-like’ behaviour towards ciliates under different illumination conditions, despite the high ciliate grazing rates on them. When exposed to the patches of ciliate exudates, f/2 growth medium, yeast extraction and ammonium solution, both starved and non-starved *D. salina* showed chemotactic accumulation on/around the exudate and nutrient patches. In both cases positive chemotaxis indices were found towards ciliate exudates suggesting that *D. salina* could actively uptake nutrients released by its ciliate grazers. This specific behavioural response however, could be costly to *D. salina*. We suggest that this behaviour could also serve as a defence mechanism thereby increasing the benefits for *D. salina*.

#### PELAGIC PROTISTS FEEDING ON PICO-CYANOBACTERIA AND THEIR CHLOROPHYLL CATABOLISMS

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Pico-phytoplanktons dominate the primary production in pelagic oligotrophic oceans. Because these settings cover roughly half areas of the Earth surface, thus relatively dilute inhabitations of pico-phytoplanktons actually represent a considerable proportion of the photosynthetic primary production on the Earth. Nonetheless, ecological/bioenergetic networks extended from these microbial phototrophs have been poorly understood due to insufficient knowledge on the prey-predator relationship, where the minute cells should evade grazing by filter-feeding zooplanktons. We have thus investigated on pelagic protists that potentially prey on picophytoplanktons based on



microscopic observations and chemical detections of chlorophylls catabolites. Pelagic water samples from the northwestern Pacific were subdivided into hundred milliliter-scale flasks with additions of trace medium components and/or separately cultured picocyanobacteria as preys and incubated under dim light at 5 to 10 °C. In cultures with additions of *Acaryochloris marina*, a cyanobacterium ( $\varphi = 2\text{--}3\ \mu\text{m}$ ; nearly “pico”-scale) that produces chlorophyll *d* (Chl-*d*) instead of chlorophyll *a* (Chl-*a*) and,  $^{13}\text{C}$ ,  $^{17}\text{C}$ -cyclophosphoribide *denol* (a “CPE” derived from Chl-*d*; cPPB-*dE*) was detected from the cultures after a few day of incubation. CPEs such as Chl-*a*-derived cPPB-*aE* have been reported as non-phototoxic catabolites of chlorophylls and known to be produced by many varieties of protists. Because Chl-*d* was not detected from any of those samples without addition of *A. marina* that is allochthonous to the pelagic settings, the occurrence of cPPB-*dE* in these experiments suggests presence of phycophagic protists that potentially feed on picophytoplanktons. Some flagellates and amoeba were actually observed to have ingested cells of *A. marina* from microscopic measurements.

#### CHARACTERIZATION OF STRAIN SRT308; A NEW HETEROTROPHIC FLAGELLATE BASAL TO EUGLENOZOA

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We isolated a new heterotrophic flagellate, strain SRT308 from marine sediment sample collected in Republic of Palau on October 2013. The flagellate is round or oval shape with two long subequal flagella and shows unique rotating motion by beating the both flagella synchronously. Since the morphological combination of the flagellate is unique, the flagellate is apparently a novel lineage of eukaryotes. In molecular phylogenetic analysis using small subunit ribosomal RNA gene sequences, the flagellate shows no strong affinity with major eukaryotic lineages. Large scale phylogenetic analysis using 153 protein-coding genes placed the flagellate at the base of Euglenozoa with strong statistical support, suggesting that the flagellate is a previously undescribed member of the Discoba clade. Consistent with the position inferred from the phylogenomic analysis, the flagellate was found to

share morphological characteristics, namely discoid mitochondrial cristae and parallel basal bodies, with euglenozoans. Furthermore, the flagellate has a euglenozoan-like tripartite flagellar root system, albeit the ventral root splits into two bands, which is similar to the R2 of other typical excavates. On the other hand, the flagellate lacks some euglenozoan features, such as pellicle, paraxial rod, non-tubular mastigonemes, or feeding apparatus. Based on these morphological and ultrastructural features, the early character evolution of Euglenozoa, as well as that of Discoba as a whole, will be discussed.

#### VIALABLE AMOEBOID PROTISTS FROM THE ARCTIC PERMAFROST

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Viable amoeboid protists were isolated from the Arctic Late Pleistocene and Holocene permafrost buried soils and sediments. A total of 36 strains of protists of the phylum Amoebozoa have been obtained. Most isolates belong to the genera *Acanthamoeba* and *Flamella*. We also isolated *Vannella*, *Cochliopodium*, *Acramoeba*, *Phalansterium* genera. Many of them belong to new species. Two species belonging to the genus *Flamella* have been described. They were named *Flamella pleistocenica* n. sp. and *Flamella beringiana* n. sp. Two new species of giant *Acanthamoeba* viruses, *Pithovirus sibericum* and *Mollivirus sibericum*, were isolated from buried soil of the age 34 000 years, and described. These are the first representatives of the two new families of *Acanthamoeba* giant viruses. We isolated amoebas from the permafrost of the age up to 50,000 years. The age of viable organisms enclosed in the samples corresponds to the time of the last freezing of the strata. Mechanisms allowing amoebas to survive such a long cryptobiosis are poorly understood. All amoeba species from permafrost are cyst-forming. We studied the composition of the water extract of trophozoites, unmaturing, and mature cysts of *Acanthamoeba castellanii* recovered from permafrost. It was demonstrated that the accumulation of osmolytes such as trehalose, glycerol, tyrosine phosphate, alanine, choline, and  $\alpha$ -Glycerophosphocholine occurs during encystation. These substances are known to serve as cell protectants during desiccation and freezing. This study directly shows for the first time that amoeba cysts can be conserved not only for years and decades but for many thousand years and then recover, contributing to the formation of an active microbial community.

**PARATRYPANOSOMA, THE MOST BASAL BRANCH AND ANOTHER MODEL SPECIES AMONG TRYPANOSOMATIDS**

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Dozens of trypanosomatids (Trypanosomatida, Kinetoplastea, Euglenozoa) have been studied from the genomics perspective, however there is a strong bias towards species pathogenic for mammals, while numerous monoxenous (single-host) parasites of insects have been neglected. This year we have published the first high-quality genome of a monoxenous insect trypanosomatid, *Leptomonas pyrrocoris*. Here we present a comprehensive study of another probably monoxenous species, *Paratrypanosoma confusum*, a cosmopolitan parasite of *Culex mosquitoes*. This species forms the most basal branch of the trypanosomatid clade. *Paratrypanosoma* produces three distinct forms in axenic culture: swimming promastigotes, immobile amastigotes, and sessile cells, firmly attached to the surface. Using scanning and transmission electron microscopy, still and video light microscopy, and fluorescent antibodies we have fully characterized the ultrastructure and behavior of the unique sessile morphotype. Formation of transient sessile stage is stimulated by basic pH and by bipterin, and sessile cells are attached to the surface with the help of an extracellular ‘glue’ resistant to all enzymatic treatments. We have performed differential RNAseq analysis of the promastigote and sessile cells, and sequenced the genome of *P. confusum* using Illumina MiSeq reads of 250 nt (a paired-end and a mate pair library). A draft genome assembly has been produced with the following statistics: scaffold N50 of 458 kbp, 189 scaffolds, 31.6 Mbp genome size, ~8,800 genes. Using the *Paratrypanosoma* genome as an outgroup, we have illuminated patterns of gene family gains and losses in an important genus *Trypanosoma* and in trypanosomatids as a whole.

**POTENTIALLY TOXIC MARINE DINOFLAGELLATES: CELL AND MOLECULAR ADAPTATIONS TO STRESS**

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Potentially toxic marine planktonic dinoflagellates represent an ecologically important group of single-cell eukaryotes which show pronounced physiological adaptability to various types of environmental stress. These protists respond to harsh conditions by activation of their defensive mechanisms, which can differ from those of large multicellular forms. The dinoflagellates *Prorocentrum minimum* (or *P. cordatum*) in the Baltic Sea have recently become good model objects in the environmental and cell biology studies of unicellular eukaryotes. The goal of this paper is to demonstrate what cell and molecular mechanisms underpin the fast and effective adaptations of these protists in the brackishwater habitats. We focused on cell metabolism, ion channel research, and on the effects of different levels of salinity and elevated temperature on cell mortality, cell cycle pattern, RNA synthesis, and DNA replication in *P. minimum*. It appears that *P. minimum* can utilize urea as a source of organic nitrogen but also as a source of carbon. Moreover, urea inhibits assimilation of nitrate (the “classic” source of nitrogen) and can be the preferable substrate in the coastal brackish waters. Dinoflagellates show elevated biosynthetic activity and low cell-death level at critical salinity 5-8 ‰. We infer that this potentially toxic, bloom forming dinoflagellate species displays great colonizing ability across new ecosystems likely due to high physiological plasticity and pronounced adaptation potential at different levels of biological organization, from molecules and cells to populations. Funded by the Russian Science Foundation, project 16-14-10116.

**GENOMIC ANALYSIS OF NEPHROMYCES SHEDS INTO AN ENIGMATIC SYMBIOTIC SYSTEM BETWEEN A TUNICATE AND A DIVERGENT APICOMPLEXAN**

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Nephromyces is a divergent apicomplexan that lives as an endosymbiont in the renal sac of molluscid tunicates. The nature of Nephromyces as an apicomplexan remained enigmatic for long

time, mostly due to: (i) its peculiar habitat, (ii) the presence of cytoplasmic bacterial endosymbionts, (iii) a complex life-cycle with unusual morphologies, and (iv) a presumably mutualistic association with its animal host. Moreover, several lines of evidence suggest that the renal sac of an individual tunicate host harbors a complex community of diverse *Nephromyces* lineages: the multiple-infection hypothesis. We decided to further investigate this hypothesis and elucidate the phylogenetic position of *Nephromyces* within Apicomplexa by using deep sequencing of renal sacs in order to better understand the developmental dynamics of this symbiosis. Our initial analyses revealed the presence of eleven apicoplast genomes in one single renal sac. Surprisingly, there was considerable sequence divergence among the apicoplast genomes, although their gene content and order was highly conserved. We then used the phylogenetic information contained within 27 apicoplast proteins to infer *Nephromyces*' phylogenetic placement within Apicomplexa. These preliminary results support the idea that molgulid renal sacs are complex ecosystems inhabited by a diverse community of different *Nephromyces* lineages. In addition, we clarified the phylogenetic position of *Nephromyces* as sister to 'core' apicomplexans (Hematozoa+Coccidia), and highlighted the significant intra-clade divergence among these apicoplasts. Future efforts will focus on elucidating the metabolic contribution of each partner to the dynamics of this complex symbiotic system.

#### PHYLOGENY, DIVERSITY AND EVOLUTION OF AMOEBOZOA: NEW FINDINGS AND NEW PROBLEMS

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Amoebozoa is a major group of protists that unifies naked and testate lobose amoebae, varioseans, slime molds and pelobionts. The diversity and systematics of some groups, like slime molds and testate amoebae, possessing stable morphological characters - fruiting bodies or tests - was relatively well studied, also some evolutionary traits derived from morphology were further disproved by molecular phylogeny. In contrast, naked amoeboid protists with no constant shape remain relatively poorly known, molecular phylogeny nearly entirely rearranged earlier views on their evolutionary relationships. SSU-based phylogeny shows that naked amoebae belong to three different amoebozoan lineages and the most of organisms formerly known

as protostelids are dispersed among different groups. The basic finding was the split of naked amoebae in two lineages, named Tubulinea and Discosea and the fact that testate lobose amoebae form just a single branch within Tubulinea. The basal branching of Amoebozoa could not be properly resolved with SSU trees. Multigene data help to clarify many essential questions, e.g. they show that Discosea does not appear to be a monophyletic group. However, with the increment of taxon sampling multigene trees seem to experience the same problems and artifacts as SSU trees did. Recent studies of extreme and exotic habitats revealed a number of new species, especially among the smallest amoebozoans, some were found to be key taxa for rooting Amoebozoan tree. Genbank contains numerous unnamed sequences belonging to new amoebozoan lineages. This indicates that environmental diversity of Amoebozoa is still in much unexplored. Supported with RSF grant 14-14-00474.

#### TRANSCRIPTOME ANALYSIS OF *NAEGLERIA FOWLERI* CYSTS AND TROPHOZOTIES BY NEXT-GENERATION SEQUENCING

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*Naegleria fowleri*, ubiquitous pathogenic amoeba causing the fatal primary amoebic meningoencephalitis (PAM) in experimental animal and humans, is predominantly living in the ponds, lakes, rivers and swimming pools. *N. fowleri* trophozoites are encysted under unfavorable conditions such as cold temperature, starvation and desiccation. However, the information in differential expression genes between cysts and trophozoites of *N. fowleri* is very limited. In this study, RNA-sequencing libraries from *N. fowleri* cysts and trophozoites were investigated by Next-Generation Sequencing (NGS) analysis. In the NGS database, the assembly procedure resulted in mean full length of 11, 254 nucleotides in total 42,220 transcript contigs and 37.21 % of C+G contents. RNA sequencing indicated that upregulated 143 genes in cysts showed 2 folds expression in comparison with trophozoites and 163 genes were downregulated. These genes were found to participate in Kyoto Encyclopedia



of Genes and Genomes (KEGG) pathway. KEGG pathway included metabolisms(131), cellular processes(43), environmental Information processing(22), genetic information processing(66) and organismal systems(20). On the other hands, by analysis of 10,713 sequences via the gene ontology database, their annotations included biological processes(1,069) which were cellular process(228), metabolic process(214) and single organisms process(193), molecular functions(415) containing catalytic activity(195) and binding(186) and cellular components (923) possessing cells(240) and cell parts(225). Increased differential expression transcriptome levels in *N. fowleri* cysts compared to trophozoites were mainly categorized as serine/threonine protease, kinase, and lipid metabolisms related protein. Finally, this study may provide new insights into the environmental resistant genes or pathogenic related genes in *N. fowleri* survival and infectivity. Keywords: *Naegleria fowleri*, cyst, trophozoite, Next-Generation Sequencing, transcriptome.

#### AMOEBICIDAL ACTIVITY OF CHLORINE DIOXIDE AGAINST PATHOGENIC *ACANTHAMOEBA CASTELLANII*, *A. POLYPHAGA* AND *NAGELERIA FOWLERI*

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Pathogenic free-living amoebae, *Acanthamoeba* spp. and *Naegleria fowleri*, are widely distributed in soil and water. Several species of *Acanthamoeba* and *N. fowleri* can cause serious human infections such as granulomatous amoebic encephalitis (GAE) or primary amoebic meningoencephalitis (PAM) which almost results in death. Recently, there has been an increased incidence of *Acanthamoeba keratitis* (AK), particularly in contact lens wearers. Chlorine dioxide (ClO<sub>2</sub>), yellow-green gas is a powerful disinfectant which is 2.5 and 500,000 times more effective than chlorine-based disinfectants and alcohol, respectively. In this study, we examined the amoebicidal effect of ClO<sub>2</sub> gas against *A. castellanii*, *A. polyphaga* and *N. fowleri* trophozoites or cysts. ClO<sub>2</sub> gas was induced from “Puristic” that it was a ready-to-use product using a tubing stick without necessitating the ClO<sub>2</sub> gas generation on site.

Amoebic trophozoites or cysts were exposed to ClO<sub>2</sub> gas (0.064ppmv/min) for 12- 48 hr. Amoebae maintained for 12 - 48 hr without exposure to ClO<sub>2</sub> gas were used for the control groups. By microscopic examination, amoebic viability was assessed, and then growth rates were estimated. The results showed that the viability of amoebae was effectively inhibited by treatment with ClO<sub>2</sub> gas, as which their viability were assessed by re-cultivation with each flesh medium. The growth rates of *A. castellanii* and *A. polyphaga* trophozoites which were exposed to ClO<sub>2</sub> gas for 24 hr were decreased by 50 and 60 % respectively. Whereas, the growth rate of *N. fowleri* exposed with ClO<sub>2</sub> gas for 24 hr was completely inhibited. Furthermore, the actin mRNA levels of amoebae checked by RT-PCR were significantly reduced by treatment of ClO<sub>2</sub> gas. Similarly, the growth rate of *A. castellanii* and *A. polyphaga* cysts exposed with ClO<sub>2</sub> gas for 12 hr was significantly inhibited. Taken together, it is suggested that ClO<sub>2</sub> gas has amoebicidal effect on *A. castellanii*, *A. polyphaga* and *N. fowleri*. Therefore, the chlorine dioxide is proposed as an effective agent for the prevention and control of *Acanthamoeba* and *N. fowleri* infections. Keywords: *Acanthamoeba castellanii*, *A. polyphaga*, *Naegleria fowleri*, chlorine dioxide, amoebicidal effect.

#### MOLECULAR DEFENCE MECHANISMS IN SPIROTRICH CILIATES TO PREVENT HEAVY METAL TOXICITY

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Heavy metal pollution of water is of major concern these days especially in developing countries like India. Anthropogenic activity elevates the concentration of such elements, mainly in aquatic systems. About 85 percent of the water pollution is caused by domestic and industrial sources. A wide range of contaminants are continuously introduced into the water and among these contaminants, heavy metals due to their toxicity, accumulation and non-degradable nature, constitute one of the most dangerous groups. Prolonged exposure to heavy metals such as cadmium, copper, lead and zinc disturb normal biological function and evoke cellular stress response. Due to stress condition, apart from the release of antioxidant enzymes, other proteins like Heat Shock Proteins (HSPs) and Metallothioneins (Mts) are the major ones that get induced and these proteins trigger changes at the

gene transcription level. In the present investigation, we studied the acute toxicity of heavy metal - cadmium and the transcriptional changes of hsp70, metallothionein and glutathione peroxidase gene in spirotrich ciliates. The effect of cadmium on the expression of these gene transcripts was investigated by real-time qPCR. The gene expression increased significantly in cadmium treated cells against the control cells indicating that these genes may be involved in cellular defence mechanisms by elevating their gene expression against heavy metal-induced stress.

#### EVALUATION OF ACUTE TOXICITY OF HEAVY METALS AND THE ACTIVITY OF ANTIOXIDANT ENZYMES IN FRESHWATER CILIATES

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Heavy metal concentration in the environment is increasing world-wide due to anthropogenic activities and India is no exception to this. Metals are biologically important though increase in the threshold concentration of heavy metal in the environment may interfere with the metabolic activity of organisms. As studies on the cellular level could detect stress reactions earlier in time compared to common physiological methods, and since ciliates offer higher sensitivity towards heavy metals as compared to other microorganisms mainly due to lack of cell wall, ciliates were used as model organism in this study. In the present investigation, the toxicity of heavy metals, viz., Cadmium and Copper was assessed in freshwater ciliates; *Tetmemena* sp. and *Euplotes* sp. It was noted that growth rate exhibited inverse relationship with the heavy metal concentration. Mortality rate and various doses of heavy metals (control, LC<sub>30</sub>, LC<sub>50</sub> & LC<sub>70</sub>) were determined in the ciliates. Generation of reactive oxygen species (ROS) was determined in these ciliates by using specific fluorescent dye in the presence of heavy metals. Increase in ROS production indicated that heavy metal stress induced ROS in living cells. Since it is known that antioxidant enzymes get activated to detoxify ROS, activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined in ciliates at various doses of heavy metals. The levels of SOD and GPx were found to be significantly higher in the presence of heavy metals. This study indicates that antioxidant enzymes can be used as an effective

biomarker to evaluate cytotoxic effect of heavy metals.

#### GENOMIC AND TRANSCRIPTOMIC SURVEY OF OSMOTROPHIC EUGLENID *RHABDOMONAS COSTATA*

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Euglenids represent a diverse group of protists with diverse modes of feeding including heterotrophy and photosynthesis, e. g. in *Euglena gracilis*. Until today, there is not available any complete genome sequence of euglenids and this probably represents the biggest gap in the sampling of eukaryotic genomes. We are running genomic and transcriptomic projects on a primary osmotroph *Rhabdomonas costata*. To avoid bacterial contamination, the DNA sample was prepared from cells picked using laser microdissection and amplified by GenomePlex®. Almost 1GB of reads was generated in four runs on Illumina MiSeq platform. The current assembly is too fragmented to be used for genome annotation, nevertheless the comparison of transcriptomic and genomic data allow us to estimate gene density and the features of introns. Annotation of the mitochondrial backbone metabolism provides the first data on *Rhabdomonas* mitochondrion.

#### GENE TRANSFER ACCOMPANYING THE SECONDARY ENDOSYMBIOSIS OF EUGLENID PLASTID

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Autotrophic euglenids (Euglenophyta) form a monophyletic group with secondary green plastids, which were most probably acquired by their common ancestor. However, the acquisition of the plastid earlier in the evolution of euglenids (plastid-early hypothesis) cannot be ruled out. The process of organelle acquisition is accompanied by

the transfer of genes from the endosymbiont to host (EGT), the presence of such genes indicates past endosymbiosis. To test the plastid-early hypothesis and to learn more about the contribution of EGT to euglenid genome, we have analyzed transcriptomes of 5 euglenids (2 osmotrophic, 3 autotrophic) using a pipeline, which enabled us to select genes related to algae. The contribution of algal genes in autotrophic euglenids (around 2 % of genes) is higher than in primary osmotrophs (around 0.07 %) supporting the plastid-late hypothesis. Surprisingly, we observed a high number of genes related to other algal groups than green algae.

CHARACTERIZATION OF A NOVEL *PARAMECIUM* ENDOSYMBIONT AND A CRITICAL REVISION OF “BASAL RICKETTSIALES”  
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In the past ten years, the number of newly described endosymbionts within the bacterial order Rickettsiales has constantly grown, and 18 novel Rickettsiales genera inhabiting protists—such as ciliates or amoeba—have been described since 2006. Half of these new species belong to the recently proposed family “*Candidatus* Midichloriaceae”, whereas five novel bacterial symbionts were found belonging to Rickettsiaceae and six belonging to Holosporaceae and other Rickettsiales with uncertain position (“basal Rickettsiales”). In this work, we provide molecular and ultrastructural data on a novel bacterial endosymbiont inhabiting a *Paramecium biaurelia* isolate collected near Bloomington, Indiana (USA). We propose the name “*Candidatus* Jurandia parameciophila” in honor of Dr. Artur Jurand. The Gram-negative bacteria (1.5–2.0 × 0.35–0.38 μm in size) occupies the host cytoplasm and is devoid of flagella. For molecular characterization, the SSU rRNA gene was sequenced and used for taxonomic assignment and the design of species-specific oligonucleotide probes. Phylogenetic analyses indicate that the endosymbiont belongs to “basal Rickettsiales” (Alphaproteobacteria), clustering with the recently proposed family “*Candidatus* Paracaedibacteraceae”. We obtained tree topologies showing a strong separation of Rickettsiales into at least two groups; one represented by the families Rickettsiaceae, Anaplasmataceae, and “*Candidatus* Midichloriaceae”, the so-called RAM clade, and the other group represented by “basal Rickettsiales” including “*Candidatus* Jurandia parameciophila”. Therefore, and in accordance with other recent publications, we propose to limit the order Rickettsiales to the RAM clade and rise “basal Rickettsiales” to an independent order, Holosporales ord. nov., inside Alphaproteobacteria, presently comprising four clades at the family level.

‘ANAERAMOEBEA’ - NEW LINEAGE WITHIN METAMONADA  
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We have isolated and cultured nine strains of anaerobic amoebae (‘Anaeramoeba’) from marine coastal hypoxic sediments worldwide. ‘Anaeramoeba’ cells were fan-shaped, with extremely flattened pseudopodia and trailing uroidal filaments, and morphologically belonging to flammellian or flabellate morphotype. We distinguished six morphospecies that differed in a number of features including cell size and nuclear morphology. ‘Anaeramoeba’ species shared a unique combination of morphological features. All three strains examined by TEM possessed double membrane-bound organelles with no cristae, presumably hydrogenosomes, associated with prokaryotes (probably methanogens). Cytoplasm contained a large acentriolar centrosome, and no basal bodies were observed. Peculiar isokont flagellates with two or four flagella were rarely observed in two strains representing different species. Single- and also five-gene analyses showed that ‘Anaeramoeba’ clade was monophyletic, but were completely unable to assess its phylogenetic position. We analysed transcriptomic data of two ‘Anaeramoeba’ species. Phylogenomic analysis based on 160 protein-coding genes surprisingly showed that ‘Anaeramoeba’ instead of being a member of Amoebozoa, represents a novel deep lineage within Metamonada (Eukaryota: Excavata).



THE DRAFT GENOME OF *KIPFERIA BIALATA* REVEALS THAT THE GAIN OF FUNCTION CONTRIBUTES THE MASSIVE REDUCTIVE EVOLUTION IN METAMONADA

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Metamonada is a unicellular eukaryotic group known to consist of free-living and parasitic organisms. Almost all of metamonads have adapted to anaerobic or micro aerobic environments, and lost the several mitochondrial functions such as the oxidative phosphorylation. The biological pathways localized in those reduced mitochondria (so-called mitochondrion-related organelles or MROs) vary depending on the species. The nuclear genome of the model parasites such as *Girardia intestinalis* was also reduced in terms of the genome structure and the number of coding proteins, presumably throughout the adaptive evolution to the intracellular lifestyle. However, little is known how the genome reduction progressed in this parasite. Here, we present the draft genome sequence of the free-living *Kipferia bialata*, which is a phylogenetic relative of *G. intestinalis*, and compare it to the genomes of the model metamonad parasites, *G. intestinalis* and *Trichomonas vaginalis*. Our data show that 1) *K. bialata* possesses two substrate-level phosphorylation pathways—one is homologous to that in *G. intestinalis* and the other is to that in *T. vaginalis*, suggesting the once expansion of ATP synthesis pathways in the metamonad evolution to reach parasitic life style of *G. intestinalis*, and 2) no variant-specific surface protein (VSP), possibly an evasion mechanism of the host immunity in *G. intestinalis*, was detected from *K. bialata* genome, suggesting that the VSPs were acquired somehow on the line leading to *G. intestinalis* after the divergence of *K. bialata*. In sum, our results suggested that the gain of function/protein conversely contributed to the massive reductive evolution in metamonads.

AUTOTROPHIC PROTISTS OF THE LITTLE RIVER AND ITS TRIBUTARIES (ON THE EXAMPLE OF THE ZIVIL, THE REPUBLIC OF CHUVASHIA)

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Small river is a complex natural object. Natural and human-induced change affect the health of its components. In the summer of 2013 was a study of

the algae flora in plankton of river Zivil and 10 of its tributaries. In the composition of autotrophic plankton were recorded 105 species of protists. They accounted for 25% of the total species richness of the algae flora. In the Zivil was met 77 species of protists, in the rivers – 67. However, the average number of species in one sample in the tributaries was 6,7, and in the Zivil – 2,75. The qualitative composition of protists of Zivil and its tributaries are similar. The coefficient of species similarity of Sorenson calculated for the Zivil and its tributaries - 53%. Abundance and biomass of the protists was significantly higher in the Zivil, compared to the tributaries. The contribution of protists to the formation of the total abundance and biomass of phytoplankton was also higher in the river Zivil. Thus, the average specific number of species of protists in the little river is lower than in its tributaries. This is probably due to a much more varied conditions. However, in the river compared to the tributaries, the indices of quantitative development of higher protists. Great is the contribution of protist in the formation of the total abundance and biomass of phytoplankton in the Zivil, compared to the tributaries.

CHANGE COMMUNITY OF AUTOTROPHIC PROTISTS FROM THE SOURCE TO THE MOUTH OF A SMALL RIVER (ON THE EXAMPLE OF THE ZIVIL, THE REPUBLIC OF CHUVASHIA)

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Zivil river – inland waterway of the republic, the right tributary of the Volga. The study of the biota of the river began in 1913. In the summer of 2013 conducted a comprehensive study of the reservoir from the headwaters to the mouth. In the composition of algae flora was discovered 77 species of protists from divisions Chryzophyta, Cryptophyta, Dinophyta, Euglenophyta and Raphidophyta. This is 25% of the total species richness of the algae flora. The river experiences significant agricultural load, so the maximum diversity differed the division Euglenophyta. The specific number of species of protists varied from 3 to 28. Maximum species richness was registered in the region of the mouth of the Warm river, experiencing significant development pressure. In most cases, the increase in species richness of protists after the confluence of the river tributaries. From its source to the mouth of the river is the growth of the species richness of protists. The population and biomass of protists also increase

from the headwaters to the mouth. The maximum development of the protists received at the mouth of Warm Creek. Here the number of protists in 50, and biomass 25 times higher than the average. Thus, the protists of a small river with significant agricultural load, have high species diversity. The trends of increasing species richness, abundance and biomass of protists from the source to the mouth of the watercourse.

#### THE PROTISTAN SPECIES MAXIMUM CONCEPT AND ITS IMPLICATIONS TO INVASIVE BIOLOGY

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The paper discusses possible implications of the novel protistan species maximum concept for the predictive modelling of ecosystem resilience and vulnerability to alien species invasions. Recent development of the protistan species maximum concept for the challenging zone of critical salinity 5-8‰ (horohalinicum), where macrozoobenthos experience destructive osmotic stress (Telesh et al., 2011), is presented. This concept arose from the discovery that not all brackish waters are poor in plankton species, which was exemplified by the Baltic Sea data. The concept has gradually evolved to the understanding that in the environment with sharp salinity fluctuations community regulations and life strategies of small unicellular planktonic organisms differ substantially from those of large multicellular bottom-dwellers. Special efforts were undertaken to define the major organismal traits and environmental factors responsible for this new peculiar biodiversity pattern. Phytoplankton cell size, seasonality in development, and water salinity were tested as key characteristics. A long-term (1972-2006) phytoplankton dataset from the Baltic Sea (“the sea of invaders”) was analyzed by means of correlation analysis, non-metric multi-dimensional scaling, and rarefaction analysis. Results prove statistically that algal cell size minimum underpins the protistan species maximum in the horohalinicum. Seasonality in phytoplankton development promotes the shift in community composition towards dominance of the small-sized species in the critical salinity regions. The protistan species maximum in the horohalinicum is largely backed up by the significant between-sample variation in species richness in the highly changeable brackish-water environment. The work was funded in part by the Russian Foundation for Basic Research, project 15-29-02706.

#### PROFILING THE PROTOZOAN TAXONOMIC AND FUNCTIONAL DIVERSITY OF AN ANTARCTIC DRY VALLEY

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The McMurdo Dry Valleys of Victoria Land Antarctica are some of the harshest terrestrial habitat on earth. At 78°S and 163°E, the average temperature of the region is <-20°C, precipitation is <10 cm yr<sup>-1</sup> and the growing period during the austral summer lasts only a few months when the landscape is warmed enough for glacier-fed streams to flow and some permafrost to thaw. Life is almost entirely microbial in these valleys and the landscape is dominated by extremely dry soils that are highly saline, oligotrophic, basic in pH and subject to frequent freeze-thaw cycles. This system is a great outdoor laboratory as the extreme conditions have reduced complexity such that intricate interactions between soil taxa can be unraveled. Our research aims to investigate protozoan diversity in these valleys in order to understand fundamental aspects of protozoan ecology that can be applied broadly. However, our understanding of the taxonomic diversity, distribution and functional roles of these organisms in these valleys is still lacking. To begin to address this, a variety of soils were sampled from two valley systems and metagenome and transcriptome datasets were constructed using next generation sequencing. Our results so far suggest that there is greater taxonomic and functional diversity than was previously thought present, with relatively high representation from many major ciliate and cercozoan clades. Future sequencing efforts will undoubtedly shed more light on distribution and richness of individual OTUs.

#### MANY PREVIOUSLY UNCERTAIN AMOEBOZOANS FIND A HOME WITHIN THE CENTRAMOEVIDA

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The Centramoebida is a group of amoebozoans with flattened trophic cells that display finely pointed subpseudopodia and a prominent lamellate microtubular organizing center (MTOC). This group of amoebozoans has received considerable attention from the broader scientific community due to the pathogenic nature and ecological importance of some of its members. Here we increase the known diversity of the Centramoebida by demonstrating through multi and single gene phylogenetics along with light and electron microscopy that the formally incertae sedis amoebozoans "*Protostelium*" *pyriformis*, "*Protostelium*" *arachisporum*, and *Stereomyxa ramosa* belong within the Centramoebida. We also describe *Vacuolamoeba acanthaformis* n.g. n.sp. a new centramoebid isolated from soil in Tibet. Our phylogenetic analyses recover a highly supported Centramoebida that includes highly supported clades comprised of "*P.*" *arachisporum*, *Stereomyxa ramosa* and *Vacuolamoeba acanthaformis* respectively. We also show "*P.*" *pyriformis* is robustly nested within the genus *Acanthamoeba* making it the first reported protosteloid member of the genus. As a result of these analyses, we formally transfer "*Protostelium*" *arachisporum* into the genus *Luapelameoba* and "*Protostelium*" *pyriformis* into the genus *Acanthamoeba* establishing the combinations *Luapeleamoeba arachisporum* n. comb. and *Acanthamoeba pyriformis* n. comb. Our results greatly increase the known diversity of this medically and ecologically important group, and further demonstrate the widespread nature of sporocarpic fruiting across the tree of Amoebozoa.

#### THE EVOLUTIONARY IMPORTANCE OF PREDATORY FLAGELLATES: NEW DEEP BRANCHES ON THE EUKARYOTIC TREE OF LIFE

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The origin and early radiation of major protist supergroups are linked to understanding key transitions in the evolution of eukaryotes: endosymbiosis, genome evolution, and morphological novelty. This understanding is often limited by the absence of

reliable comparators; organisms with ancestral or intermediate features that represent sister groups of well-known taxa. Recent studies on the diversity of heterotrophic protists and environmental sequence data have indicated that many such "sister" lineages exist but remain insufficiently known primarily because they are not available in stable cultures. Among heterotrophs, predatory protists are of particular interest because their diversity may be the least understood of all protists: they are comparatively rare in natural samples and often difficult to maintain in the laboratory. However, their ability to feed on protozoa and microalgae is relevant to understanding the role of phagotrophy in the origin of mitochondria and plastids, and many represent deep-branching evolutionary lineages that could help us illuminate the origin of major protist supergroups and resolve their relationships. Here we report the establishment of multiple cultures of rare, free-living predatory flagellates, which occupy basal or intermediate evolutionary positions within major eukaryotic supergroups (Alveolata, Stramenopiles, Rhizaria, Opisthokonta, Kinetoplastida), or represent independent deep-branching eukaryotic lineages. Molecular and morphological data from these isolates reveals ancestral and unique morphological and ultrastructural features, slowly evolving nuclear genes, and large, slow-evolving mitochondrial gene sets.

This study was supported by the Russian Science Foundation (grant no. 14-14-00515).

#### DEVELOPMENT OF THE INTEGRATED INFORMATION WEB-SYSTEM ON A BIODIVERSITY OF PROTISTS "PROTIST.RU"

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Protists are the most heterogeneous at cellular and molecular levels group of eukaryotes. They play an important role in ecosystem functioning as a part of microbial communities and have a high potential for human practical activity as a resource for the development of biotechnologies. Inventory, systematization, and integration of the data on the diversity of protists are of primary importance. We have made the technical platform and started the development of web-system, which allows



integrating the information on morphological, molecular, and ecological features of different protist species. The content of the web-system will include morphological descriptions and differential diagnoses of the species, photos of living cells and whole mounts, electron-microscopic illustrations of coverings and cell ultrastructure, movies of live cells, drawings, sequences of phylogenetically important genes, molecular phylogenetic trees, databases on geographic and biotopic distribution of the species, protistological literature. Now we focus on four groups of free-living protists: heterotrophic flagellates, testate amoebae, ciliates, centroheliozoans. The work on interactive illustrative keys for some taxonomic groups was started. Registered on the website users can upload and edit content, create personal profiles, and topics on the forum. We invite all protistologists to join our team. The integrated web-system is available for observing on the Internet at <http://protist.ru/>. This study was supported by the Russian Foundation for Basic Research (grant 15-29-02518).

#### HIDDEN DIVERSITY OF FILOSE TESTATE AMOEBAE IN THE MIDDLE WATERSHED OF THE DANUBE

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Diversity of filose testate amoebae in the middle watershed of the Danube has been searched for decades, but still features with new detections. *Corythionella golemanskyi* is first time detected in Europe in satisfactory number of individuals for correct identification. *Cyphoderia calceolus* and *Cyphoderia myosurus* specimens found during the last twenty years make possible to distinguish between these two similar and rare taxa. Reviewing all the rare filose testaceans found in the middle watershed of River Danube from 1962 and considering their sampled microhabitats, we conclude that these species are generally adapted to low organic material content and high oxygen concentration. Testate amoeba species lists from Hungary are compared throughout five decades, highlighting habitat requirements for rare filoseans. We argue, that water flow has an important role in formation of riverine testate amoeba communities: Space clearing floods open new microhabitats for colonization from local refugia. Microhabitats like sandy riparian zone remaining free from intensive organic load are occasionally supplied with additional fresh ground water, and can maintain favourable environmental conditions for filoseans for a long time, while prevent

development of diverse lobosean assemblages. Striking similarity between assemblages of Penard in the profundal zone of deep Swiss lakes and the middle watershed of the Danube are attributed to the above environmental factors. It is still debated, why majority of the presented species have not been reported out of Europe. Finally, a concise methodological proposal is presented to the detection of these inconspicuous testate amoeba species. Funding: Hungarian Scientific Research Fund (T49632).

#### RARE *CORYTHIONELLA* AND *CYPHODERIA* SPECIES IN THE HUNGARIAN SECTION OF THE DANUBE

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Sandy sediment and seston were investigated in several sites north from Budapest along the Danube riparian zone in order to find rare filose testate amoebae. Finally, ca. 20 kilometers north from the capital *Corythionella golemanskyi* tests were detected in 2015, enough for morphometric analysis. A repeated sampling four months later resulted specimens of *Cyphoderia calceolus* with enormous form variations. Shell structure details and morphometry are given for both species. Characteristics of the latter are compared with those of *Cyphoderia myosurus*, detected in 1996–97 sampling of the Danube in the Szigetköz area. As Penard (1908) has stated, *Cyphoderia myosurus* has elongated shell plates. *Cyphoderia calceolus*, however, comprises non-overlapping plates, often kidney shaped, without a regular circular outline. Morphometry of *Corythionella golemanskyi* shells leads us to conclude that the formerly found active *Corythionella* specimens (sampled in Szigetköz Danube, 1996) belong to another species. The very first *Corythionella* specimens ever found in Hungary were detected as early as 1996 at the same site, but being not yet described, they were preserved as weird *Cyphoderia laevis* specimens. These data suggest that although *Corythionella* is present in the Danube, its shy appearance prevents it from recognition during analysis of turbid sediment samples. Funding: Hungarian Scientific Research Fund (T49632).

*PARVULARIA* GEN. NOV. REPRESENTS A FILOSE AMOEBEA RELATED TO NUCLEARIIDS, THE EARLIEST-BRANCHING LINEAGE OF HOLOMYCOTA (OPISTHOKONTA)  
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Opisthokonts, one of the largest eukaryotic supergroups, is divided into two major clades: the Holozoa, encompassing animals and their unicellular relatives, and the Holomycota, including fungi and their unicellular relatives. The earliest-branching lineage in Holomycota, which has received many names (e.g. Nucleariidae, Cristidiscoidia, Discicristata), remains poorly studied. This group of phagotrophic filose amoebae bears contrasting features in comparison with those of their close relatives, the parasitic Opisthosporidia and the osmotrophic fungi. It originally contained a single genus, *Nuclearia*, which includes naked amoebae of ca. 40 µm cell diameter that feed on filamentous cyanobacteria in freshwater environments. More recently, *Fonticula alba* was included as sister to *Nuclearia* spp. With much smaller cell size and bacterivore, this amoeba presents an aggregative multicellular fruiting body. Here we describe the new genus *Parvularia* nov. gen., a small filose amoeba formerly called *Nuclearia* sp. ATCC50694, and we compare its morphological features with those of the genera *Nuclearia* and *Fonticula*. We also review the whole nucleariid lineage at the onset of Holomycota, focusing on their diversity, ecology and evolutionary importance. SSU rRNA-based phylogenetic analyses including environmental sequences suggest that nucleariids are relatively scarce and thrive exclusively in freshwater systems. Based on existing transcriptomic data for *Parvularia* and novel data for a canonical *Nuclearia* strain, we carried out phylogenomic analyses to study the internal phylogeny of the whole group. Nucleariids occupy a key evolutionary position, such that mapping phenotypic traits on the phylogeny of Opisthokonts will help understanding important evolutionary transitions such multicellularity, parasitism or osmotrophy.

#### TESTATE AMOEBEA IN BAU SEN AND BAU TRANG LAKES, BINH THUAN PROVINCE, VIETNAM

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The species composition and distribution of testate amoebae in Bau Sen and Bau Trang lakes (Binh Thuan Province, Vietnam) were investigated. Fifty-three species, varieties and forms have been

identified from the water column and sediments. Twenty-seven species, varieties and forms from the genera *Arcella*, *Centropyxis*, *Cyclopyxis*, *Diffflugia* and *Pyxidicula* were found in Vietnam for the first time. The updated list of testate amoebae in Vietnam now includes 286 taxa. The genera *Arcella*, *Centropyxis* and *Diffflugia* are characterized by the highest frequency of occurrence. The species diversity of *Arcella*, *Centropyxis*, *Diffflugia* was the highest. *Centropyxis aculeata*, *Arcella discoidea*, *Diffflugia schurmanni*, *Netzelia oviformis* and *Diffflugia limnetica* were the most common species. Some taxa have shown restricted distribution, e.g. *Lesquereusia modesta* was found in Bau Trang lake with a high frequency of occurrence, but was not observed in Bau Sen lake. The number of observed species, varieties and forms in each lake is 34. The average number of the species per sample on the shores of the lakes subjected to human agricultural activity was significantly higher than in the samples from the shores of the lakes with less anthropogenic disturbance. The curves of “cumulative species number vs. sampling effort” are well fitted by equations  $S = 7.60N^{0.69}$  for Bau Sen lake and  $S = 12.52N^{0.46}$  for Bau Trang. The curves are unsaturated, which indicates that more intensive investigations of testate amoebae should be expected to reveal more species.

#### DIVERSITY OF THE GENUS *MONOCERCOMONOIDES*

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*Monocercomonoides* sp. is a genus of small flagellates belonging to the order Oxymonadida. They live as intestinal endosymbionts of insects, but some of them can be found also in the intestine of vertebrates. In this work we sequenced the SSU rRNA of 35 different strains of *Monocercomonoides* isolated from various insect and vertebrate hosts and from unused cesspit. We have performed phylogenetic analysis in order to understand the diversity of this genus. Our preliminary results indicate large variation among strains at the genetic level with some strains having a typical 1800 bp SSU and some having around 3000 bp SSU sequence. The strains formed two clades, potentially distinct genera, however the position of these clades in the oxymonad trees is unclear and we have so far not found any good diagnostic feature discriminating

between them. Morphological analysis of selected strains from the major clade, which we consider as the genus *Monocercomonoides*, failed to find suitable characters for species delineation and it is also very difficult to assign lineages to described species. Based on the morphology and host origin, we assume that the lineage containing Chinchilla isolate PA203, and isolates from guinea pig and *Chameleo cristatus* represent species *M. exilis*.

#### CILIOFAUNA OF NATURAL AND ARTIFICIAL ECOSYSTEMS OF KHABAROVSK TERRITORY

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Environmental degradation due to pollution by industrial, agricultural and municipal waste gave rise to serious problems, associated with the violation of the ecological balance in ecosystems and the biosphere. Ciliata - an important object in the model laboratory experiments, as they are used as an indicator of the degree of human impact on the environment. Sampling was carried out in small rivers of the Khabarovsk territory (rivers Krasnaya, Chernaya, Berezovaya) and aeration tanks in Khabarovsk (town Berezovii). During inventory ciliofauna has been identified 152 species of ciliates. The greatest number of species represented Oligohymenophorea class - 33 and Vorticellidae family - 12 species. 3 species of ciliates were eurybiontic (*Paramecium caudatum*, *Colpidium campylym*, *Uronema marinum*). Species ciliofauna similarity between small rivers and aeration tanks is 30% (according to Sorensen coefficient). Ciliata specific types of small rivers Khabarovsk - 14 (32% of the number of species recorded in small rivers), in aeration tanks - 32 (57%). Specific types belonged Armophorea classes - 3 kinds, Litostomatea - 3 kinds, Phyllopharyngea - 8 species, Oligohymenophorea - 13 species. Thus, faunal analysis of ciliofauna aquatic ecosystems Khabarovsk shows how the presence of similar species found in a variety of biological communities and the presence of specific representatives. In particular, this phenomenon is characteristic of biocenosis aeration.

#### OVERVIEW OF THE WHOLE SPECTRUM OF MITOCHONDRIAL DIVERSITY WITHIN THE CILIATE CLADE

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Mitochondria are important organelles in all eukaryotes and their protein composition and function(s) depend on the lifestyle of the organism harboring them. While being very well studied, their functional distribution amongst ciliates remains a dark box. The Ciliophora group is currently one of the most studied lineages of single-cell eukaryotes, mainly due to their abundance in diverse habitats. Despite this, investigations on the cell biology and the functional diversification of their organelles have been limited, with the exception of certain species, mainly *Paramecium*, *Tetrahymena* and *Nyctotherus*. These organisms have been shown to harbor either typical mitochondria or hydrogen-producing mitochondria, while their mitochondrial genome has an unusual codon usage. In this project, we used the recently acquired transcriptomic and genomic data to predict the functional diversity of mitochondria and related organelles within the group of ciliates. Our analyses demonstrate that members of the ciliates harbor distinctive classes of mitochondria, with a variety of functions and adaptations. Two species have been predicted to have remnant mitochondria (mitosomes) and one of them hydrogen-producing mitochondria (hydrogenosomes). Surprisingly, we are currently observing the whole spectrum of functional classification of mitochondria just within ciliates; Ciliophora is an exceptional model group to monitor in real time the functional adaptations of mitochondria.

#### TESTATE AMOEBA TRANSFER FUNCTION PERFORMANCE ALONG LOCALISED HYDROLOGICAL GRADIENTS

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Testate amoeba transfer functions are widely used for reconstruction of palaeo-hydrological regime in peatlands. However, the limitations of this approach have become apparent with increasing attention to validation and assessing sources of uncertainty. This paper investigates effects of peatland type and



sampling depth on the performance of a transfer function using an independent test-set from four Sphagnum-dominated sites in European Russia (Penza Region). We focus on transfer function performance along localised hydrological gradients, which is a useful analogue for predictive ability through time. The performance of the transfer function with the independent test-set was generally weaker than for the leave-one-out or bootstrap cross-validations. However, the transfer function was robust for the reconstruction of relative changes in water-table depth, provided the presence of good modern analogues and overlap in water-table depth ranges. When applied to subsurface samples, the performance of the transfer function was reduced due to selective decomposition, the presence of deep-dwelling taxa or vertical transfer of shells. Our results stress the importance of thorough testing of transfer functions, and highlight the role of taphonomic processes in determining results. Further studies of stratification, taxonomy and taphonomy of testate amoebae will be needed to improve the robustness of transfer function output. This research was funded by the Russian Scientific Fund (grant 14-14-00891) and grant of the President of Russian Federation (MD-7930.2016.4).

#### CELL COAT EVOLUTION AND PHYLOGENY OF DACTYLOPODID AMOEBAE (AMOEBOSOA, DISCOSEA)

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An amoebae order Dactylopodida was established on the base of the results of the molecular phylogenetic analysis. To date it includes genera *Vexillifera*, *Cunea*, *Pseudoparamoeba*, *Korotnevella*, *Paramoeba* and *Neoparamoeba*. All representatives of this order share the ability to form non-furcating finger-shaped subpseudopodia (dactylopodia), which considered as morphological synapomorphy for this group. Up to now all these genera were distinguished by morphological features: peculiarities of locomotive form, presence or absence of kinetoplastid endosymbiont (*Perkinsella amoebae*-like organism, or PLO) and structure of the cell coat. The details of the cell coat evolution in Dactylopodida are still unclear. Recent studies shown that situation with generic distinctions in this group actually is much more complex. One of the studied species formally fits the diagnosis of the genus *Korotnevella*, because it has scales and lacks PLO. At the same time its

18S rRNA gene sequence robustly groups with *Pseudoparamoeba pagei* (which lacks both PLO and scales) and never branches among those of *Korotnevella* spp. Thus the situation described above shows that to the moment the boundary between two genera *Pseudoparamoeba* and *Korotnevella* can be correctly drawn only on the basis of 18S rDNA sequence. Our data probably mean that actually the presence of scales is a primitive feature for a whole clade, which unifies genera *Pseudoparamoeba*, *Korotnevella*, *Paramoeba* and *Neoparamoeba*. Within this group scales retained in some species and lost in others.

#### ECOLOGICAL STUDY OF THRAUSTOCHYTRIDS (LABYRINTHULEA, STRAMENOPILES)

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Thraustochytrids have been recognized as important decomposers in the marine ecosystem. However, in many cases, this recognition is based on the short investigation of the biomass of whole thraustochytrids without distinguishing the species. In this study, we investigated the seasonal changes of biomass and species composition of thraustochytrids by continuous monitoring at river mouths and coastal areas in Japan for five years. Remarkable peaks of cell numbers of the thraustochytrids were observed in early summer and/or summer at the river mouths. This phenomenon was named "thraustochytrid spikes". Thraustochytrid biomass didn't correlate with chlorophyll a, but it seemed that the peaks occurred after the decrease in salinity. This phenomenon probably suggested that the thraustochytrid biomass is affected by the supply of the terrestrial organic matter from river water. Although thraustochytrid biomass was only 1.59% that of bacterial biomass, the fixed energy (as biomass) transferred directly from thraustochytrids to zooplankton was estimated to be 15.9% of that transferred from bacterioplankton via phagotrophic protists. Moreover, a clear seasonal succession of thraustochytrid species was repeatedly observed every year. However, species composition differs

between the river mouths and coastal areas. It is suggested that habitat segregation occurred among these species and was caused by the environmental factors and the differences of utilization of terrestrial or marine organic matter.

#### ANALYSES OF PHOTOSYNTHETIC OXIDATIVE STRESS RESPONSES IN HERBIVOROUS UNICELLULAR ORGANISMS

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Photosynthesis generates reactive oxygen species (ROS) which destroy biomolecules. It is known that photosynthetic organisms, such as plants and algae, have strategies to cope with the photosynthetic oxidative stress to perform photosynthesis safely. When unicellular predators, such as amoebae, feed on photosynthetic organisms under illumination, they are probably exposed to ROS that are generated by engulfed prey during digestion. The aim of my study is to examine whether the algal predators are really exposed to oxidative stress, and if so, to understand how they cope with the photosynthetic oxidative stress. To this end, I have isolated amoebae that feed on both photosynthetic and non-photosynthetic bacteria from marsh. From them, I chose three amoebae which were evolutionally distantly related. These amoebae were co-cultured with the cyanobacteria or *E. coli* under dark or light condition and I measured ROS generated by engulfed photosynthetic prey and examined transcriptome changes of amoeba cell. The results show that singlet oxygen, which is believed to be the main ROS produced by photosynthesis, is generated in amoeba by engulfed photosynthetic prey under illumination but not in dark condition or during predation of non-photosynthetic prey. On the other hand, higher level of hydrogen peroxide is produced under illumination than under dark condition regardless of the photosynthetic ability of prey. Even if they are evolutionally distantly related species, three species of amoebae exhibited similar pattern of transcriptome changes. Now, I am conducting some assays to investigate whether phenomena which are predicted based on transcriptome changes are really occur.

#### IRON SULFUR CLUSTER ASSEMBLY IN AMITOCHONDRIATE OXYMONAD *MONOCERCOMONOIDES*

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Oxymonads are a group of anaerobic or micro-aerophilic protists living in guts of insects and vertebrates. They are the only group of eukaryotes without mitochondrion, however in their closest free-living relative *Paratrimastix pyriformis* have been found organelles which are morphologically ntsimilar to hydrogenosomes. Concomitantly with the absence of mitochondrion, *Monocercomonoides* lacks classical mitochondrial ISC system for synthesis of Fe-S clusters. Instead, subunits of SUF system were found in genome and transcriptome of *Monocercomonoides*: SufB, SufC, SufS and SufU. All these proteins contain well conserved catalytic sites which are needed for their function in FeS cluster assembly. Heterologous localization of SufB and SufC in *Trichomonas vaginalis* expression system showed cytosolic localization. We have also found subunits of SUF system in transcriptomic data from *Paratrimastix pyriformis* and two other members of Preaxostyla – oxymonad strain NAU3 distantly related to *Monocercomonoides* and isolate MORAITICA, the deepest branching lineage of Preaxostyla available at the moment. Phylogenetic analyses of SUF subunits showed that all preaxostyla SUFs forms single clade, which is clearly distinct from clades of other eukaryotes – proving that common ancestor of all known Preaxostyla acquired SUF system by horizontal gene transfer independently from other eukaryotes. To prove that SUF subunits are indeed functionally active in *Monocercomonoides* we have performed several complementation experiments in *E. coli*. Preliminary experiments with complementation proved that SufB of *Monocercomonoides* can substitute SufB of *E. coli* in synthesis of Fe-S cluster and therefore SUF system is functionally active in Fe-S cluster assembly. Heterologous localization of SufB and SufC in *Trichomonas vaginalis* expression system showed cytosolic localization. Our results indicate that *Monocercomonoides* is the first known

organism, which assemble Fe-S clusters in the cytosol by concerted action of SUF and CIA pathways.

#### SYNCHRONIZED AND ER-DEPENDENT DYNAMICS OF MITOSOMES

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Mitosomes are the smallest evolutionary forms of mitochondria that evolved in eukaryotes adapted to anaerobic environments. While abandoning many attributes of the aerobic mitochondria such as the genome, respiration and the cristae, mitosomes have retained the double membrane and the bare bones of the pathways for the protein import and the synthesis of the iron-sulfur clusters. Here, we studied the dynamics of the mitosomes in the parasitic protist *Giardia intestinalis*, which belongs to one of five supergroups of eukaryotes known as Excavata. We found that mitosomes are extremely steady organelles during the interphase undergoing neither the fission nor the fusion during the interphase, thus being highly prone to become heterogeneous. Surprisingly, the mitosomal division is restricted to mitosis, when both central and peripheral organelles divide in a synchronized manner. The mitosomes also divide during the encystation of the parasite, thus preconfigure the cyst for the rapid excystation in a new host. Interestingly, the division involves the association of the mitosomes with the endoplasmic reticulum, a relationship typical for the mitochondria of Opisthokonta. While several such tethering mechanisms, which enable lipid transfer between the organelles, have been described for Opisthokonta, none of these have been shown to function in other eukaryotic supergroups including Excavata. However, we were able to show that lipid enzyme long chain acyl-CoA synthetase 4 is distributed to the mitosome-ER interface.

#### MORPHOLOGICAL AND MOLECULAR INVESTIGATION OF MARINE PARAMOEBI-DAE (AMOEBOSOA, DACTYLOPODIDA)

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We present a revision of marine dactylopodid amoebae containing an intracellular eukaryotic symbiont traditionally called ‘parasome’, and currently known as ‘*Perkinsela*-like organism’ (PLO) related to Kinetoplastida. This group traditionally consists of two genera: *Paramoeba* Schaudinn, 1896 and *Neoparamoeba* Page, 1987 which differ in their cell coat structure; the former being covered with scales, while the latter, with the thin, scaleless glycocalyx. The third PLO-containing genus, *Janickina* Chatton, 1953 has no clear taxonomic affinities yet, as no molecular data are available for its members. We present the results of investigation of the biodiversity and phylogenetic relationships within the genera *Paramoeba* and *Neoparamoeba* based on 15 marine and brackish water strains isolated from a broad range of habitats. The conclusions are based on morphological, ultrastructural and molecular evidence. The data obtained allow us to conclude that (1) Morpho-species of *Paramoeba* and *Neoparamoeba* show considerable levels of intragenomic and intraspecies variability based on the SSU rDNA and ITS region sequences; (2) A detailed study combining analysis of light-microscopic data, ultrastructure and molecular evidence is necessary in most of cases to discriminate species within this group; (3) The cell coats of *Paramoeba/Neoparamoeba* clade have evolved from the scale-bearing ancestral taxa through several independent scale losses in various lineages of this clade. We also present novel data that contribute to further understanding of the co-evolution of amoebae and their intracellular symbionts.

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#### CILIATES AS BIOINDICATORS OF MARINE WATER QUALITY

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Although protists, and especially ciliates, have long



been used as indicators of freshwater quality, they are rarely used in this capacity in marine waters. Here I will summarize the results of a series of studies carried out in Jiaozhou Bay, on the Yellow Sea coast of NE China, in which we investigate the relationships between ciliate communities, both planktonic and periphytic, and certain physico-chemical parameters that varied at different sites within the Bay. In each study, ciliates were identified and enumerated by direct microscopy, and data were analyzed using various statistical packages mainly within PRIMER. A main aim of this investigation was to develop protocols that maximize the efficiency of sampling and analyses of the ciliate communities. Our main findings were: (1) the 8-sampling events per year may be an optimal sampling strategy for planktonic ciliated protozoan seasonal research in marine ecosystems; (2) 90% of the periphytic community could be recovered on 10 microscope slide replicates immersed at one depth for 3 – 21 days; (3) multivariate (step-best-matching) analysis allows a subset of the most reliable indicator species to be identified without losing accuracy of water quality prediction; (4) applying taxonomic efficiency, i.e., identifying to the highest rank without losing critical bioindicator information, revealed that genus-level identification allows for accurate prediction of water quality; (5) efficiencies of sample analyses can be achieved by omitting ubiquitous groups, and using presence/absence of others rather than abundance data.

#### SPECIES CLASSIFICATION AND MATING IN FORAMINIFERA

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Many groups of foraminifera are characterized by the formation of elaborate shells, which provide detailed morphological features, useful for species classification. Since the majority of works focuses on their fossilized shells, a comprehensive morphotaxonomy has been established. Yet, genetic analyses revealed an even higher diversity on the molecular level, hidden within the traditional morphospecies. These cryptic species are marked by large genetic distances and differentiated distribution patterns, implying that cryptic species rather than morphospecies represent the level of species. As a consequence, today we are facing a conflict between the morphological species concept and the interpretation of genetic diversity. The

biological meaning of both is still unclear and the relationship between genetic divergence and the level of species or populations remains uncertain. In order to overcome this conflict, we try to combine aspects of morphological variability, genetic diversity and reproduction to achieve an integrative approach for species delimitation in foraminifera. To this end, we carry out breeding experiments on benthic foraminifera to observe the mating behavior between genetically divergent lineages to detect the level of divergence that corresponds to reproductive isolation. In addition, we plan to observe the mating behavior among genetically homogenous populations to examine the existence of different mating types within a population. The mating system largely influences the generation of genetic variation and contributes to the process of adaptation. Understanding its mechanisms in foraminifera is thus essential to understand the diversification and evolution of the group.

#### THE EVOLUTION OF MITOCHONDRIAL MEMBRANE CONTACT SITES

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It is commonly accepted that mitochondria evolved from an alpha-proteobacterial endosymbiont to become the major energy producing organelle of the eukaryote cell. Accounts describing the integration of the pre-mitochondrial symbiont into host cell processes often focus on this transfer of the control of energy production from the symbiont to the host. However, mitochondria are more than mere ATP generators and have several physical and functional links to various cell systems. One such link is manifested in the physical and functional link between mitochondria and the endomembrane system in the form of membrane contact sites (MCSs). These MCSs are important for non-vesicular lipid transport between apposed membranes. Recent progress has identified the protein complexes responsible for maintaining MCSs in *Saccharomyces cerevisiae*. A surprising number of functionally overlapping mitochondrial MCS tethering complexes have been described, but the extent to which MCS tethers are conserved between distant lineages appears to vary. Thus, while being functionally redundant in *S. cerevisiae*, MCSs appear to have a high degree of evolutionary plasticity in eukaryotes. Taken together, these data suggest that the last eukaryote common ancestor had a mitochondrion highly connected to diverse endomembranes, but over the course of eukaryote

divergence ancient MCS tethers were lost while others were gained in a lineage-specific manner.

#### UNRAVELLING THE DIVERSITY OF DIPLO- NEMIDS BY CULTURE-BASED TAXONO- MIC STUDY

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Diplonemids (Diplonemida) belong to the phylum Euglenozoa and they are unicellular flagellates that are free-living or parasitize algae and invertebrates. Although Diplonemida included for decades only the genera *Diplonema* and *Rhynchopus* yet, the genus *Hemistasia* was recently transferred to Diplonemida based on the phylogenetic analysis. *Hemistasia phaeocysticola* is a representative of a large environmental clade that remained to be identified in Diplonemida. Members of this environmental clade have attracted attention not only from taxonomic/phylogenetic perspective, but also due to highly unusual RNA processing in their mitochondria, which is reminiscent of trans-splicing and editing, so far reported only from them. Hence, the studies focusing on *H. phaeocysticola* and the other members of this environmental clade may expand our knowledge about the structural diversity and evolutionary history of unusual structural genes in this protist group. We have isolated new marine diplomemids and keep some of them in culture. In the present study, we report their morphology and phylogenetic positions in Diplonemida: some cultures branch within the clade that is composed of environmental sequences and *H. phaeocysticola*, while others are also shown to be novel lineages in the clade of *Diplonema* and *Rhynchopus*. Our findings significantly expand the known diversity of diplomemids and it is also suggested that the classification system under Diplonemida should be revised: the establishment of a novel family is possibly needed for the members of the environmental clade.

#### RED ALGAL PHYLOGENY AND EVOLUTION BASED ON MITOCHONDRIAL GENOME

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The Florideophyceae (5 subclasses and 31 orders) and Bangiophyceae (1 subclass and 1 order) distribute in both marine and freshwater habitats, and cover the most described red algal diversity, ca. 7,100 species (98% of Rhodophyta). The monophyly those two classes and relationships among subclasses were well-supported in many phylogenetic analyses, however, interordinal phylogeny remain largely unknown in the subclass Rhodymeniophycidae. In order to resolve ordinal relationships and evolution of the subclass, we have determined 11 mitochondrial genomes (mtDNA) from representative species and compared with published data. Concatenated phylogeny based on 37 mtDNA (6,345 amino acid data) of red algae showed stronger support among order level than previous studies. The mtDNA synteny comparison showed 47 events of gene evolution (indel and rearrangement) among Bangiophyceae, Hidenbrandiophycidae, Nemaliophycidae, Corallinophycidae, Ahnfeltiophycidae, and Rhodymeniophycidae. We demonstrate that all rhodymeniophycidan mtDNA have conserved architectures with little variation of contents. Based on the best phylogeny, we inferred the ancestral mtDNA content, evolution and reduction of protein coding genes in red algae.

#### NEW MITOCHONDRIAL GENOMES AND MOLECULAR MARKERS FOR THE COASTAL DIATOMS OF KOREA

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The Bacillariophyceae is the largest class of the stramenopiles (ca. 11,200 species), and one of the most important and abundant primary producers of the aquatic ecosystem. Diverse diatoms are occurred in inter-tidal mudflats of the west coast of Korea, the Yellow Sea. In spite of ecological importance of the phytoplankton community, there are a little number of molecular markers have been developed, such as nuclear encoded 18S rRNA (SSU) and plastid encoded *rbcL*. In present study, we determined two new mitochondrial genome (mtDNA) of common tube-dwelling diatoms, *Berkeleya fennica* TA424 (GenBank accession NC\_026126, 35,509 bp) and *Navicula ramosissima* TA439 (48,652 bp) isolated from the Taean mudflat (36°44'14.79"N 126°10'41.89"E), and one mtDNA

of pelagic diatom, *Skeletonema marinoi* JK029 (NC\_028615, 38,515 bp) isolated from an intertidal site of the Sihwaho (37°18'38.91"N 126°36'16.35"E). Complete mtDNA of each species shows typical bacillariophycean mitochondrial contents, including of 35–42 coding sequences (CDS), 2 ribosomal RNA (rRNA), and 25–26 transfer RNA (tRNA) genes. All CDS of mtDNA aligned with all available diatoms data, including *Phaeodactylum tricorutum* (NC\_016739), *Fragilaria acus* (NC\_013710), and *Thalassiosira pseudonana* (NC\_007405). Based on the saturation test and preliminary sequencing, we suggest mitochondrial markers for diatoms survey on the west coast of Korea.

#### HORIZONTAL TRANSFER OF A NOVEL SIX-GENE OPERON FROM A BACTERIUM INTO THE PLASTID GENOME OF EUSTIGMATOPHYTE ALGAE

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Genes in plastid genomes (plastomes) have been primarily inherited from the cyanobacterial plastid ancestor, but cases of genes acquired by horizontal gene transfer (HGT) have been occasionally reported from plastomes of different algal lineages. Here we report an interesting case of HGT-mediated gene acquisition revealed by sequencing plastomes of the eustigmatophyte algae *Monodopsis* sp. MarTras21 and *Vischeria* sp. CAUP 202. While the gene complement of the newly and previously sequenced eustigmatophyte plastomes proved to be highly conserved, those of *Monodopsis* sp. and *Vischeria* sp. harbour a cluster of six genes not reported from any plastid genome sequenced so far. All six genes have homologs in various bacteria, where they are usually organized in the same six-gene cluster, i.e. a putative operon. Phylogenetic analyses showed that the cluster from eustigmatophyte plastomes is nested among sequences from the order Cytophagales (phylum Bacteroidetes), with the cluster from *Sporocytophaga myxococcoides* constituting a robustly resolved sister group to the eustigmatophyte clade. Sequence analyses using different homology-detection tools failed to detect functionally characterized homologs of the protein

encoded by the first gene of the operon, whereas the remaining five proteins could be assigned only to broader enzyme superfamilies. Nevertheless, based on these analyses we speculate that the newly detected operon encodes enzymes of a pathway synthesizing a prenylated aromatic compound, possibly an antimicrobial or other protective substance. To our knowledge, this is the first report of an expansion of the metabolic capacity of a plastid mediated by HGT into the plastome.

#### SPECIES DIVERSITY AND MORPHOLOGY OF CENTROHELID HELIOZOANS OF SOME SITES IN EUROPEAN PART OF RUSSIA AND SOUTH-EAST UKRAINE

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The species composition and cytoskeleton morphology of centrohelid heliozoans from different water biotopes of European Russia and South-East Ukraine have been investigated. 21 species from 6 genera and 3 families have been found. Two forms (*Acanthocystis* sp. nov. 1. and *Acanthocystis* sp. nov. 2.) have appeared to be as new species. Following species *Acanthocystis turfacea*, *Heterophrys myriopoda*, *Polyplacocystis symmetrica*, *Polyplacocystis coerulea*, *Acanthocystis dentate* have been marked for South-East Ukraine for the first time. Also *Acanthocystis dentata*, *Raineriophrys fortesca*, *Raineriophrys kilianii*, *Pterocystis foliaea* and *Pterocystis pulchra* have been found for the Central Russia for the first time. *Pterocystis pulchra* has been revealed for heliozoan fauna of Russia firstly at all. *Heterophrys marina* has been found in fresh waters of Russia. *Polyplacocystis symmetrica* has been found in slightly salted biotopes. The morphological descriptions of all studied species have been given. New data on spicule structures of *Acanthocystis dentate* have been done. Most frequent species was *Acanthocystis pectinata*. This study was supported by the Russian Scientific Foundation (grant nos. 14-14-00515).

#### RUBISCO IN NON-PHOTOSYNTHETIC ALGA *EUGLENA LONGA*: DIVERGENT FEATURES, TRANSCRIPTOMIC ANALYSIS AND REGULATION OF COMPLEX FORMATION

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*Euglena longa*, a close relative of the photosynthetic model alga *Euglena gracilis*, possesses an enigmatic non-photosynthetic plastid. Its genome has retained a gene for the large subunit of the enzyme RuBisCO (*rbcL*). Here we provide new data illuminating the putative role of RuBisCO in *E. longa*. We demonstrated that the *E. longa* RBCL protein sequence is extremely divergent compared to its homologs from the photosynthetic relatives, suggesting a possible functional shift upon the loss of photosynthesis. Similarly to *E. gracilis*, *E. longa* harbors a nuclear gene encoding the small subunit of RuBisCO (RBCS) as a precursor polyprotein comprising multiple RBCS repeats, one of which is highly divergent. Both RBCL and the RBCS proteins are synthesized in *E. longa*, but their abundance is very low compared to *E. gracilis*. No RBCS monomers could be detected in *E. longa*, suggesting that processing of the precursor polyprotein is inefficient in this species. The abundance of RBCS is regulated post-transcriptionally. Indeed, blocking the cytoplasmic translation by cycloheximide has no immediate effect on the RBCS stability in photosynthetically grown *E. gracilis*, but in *E. longa*, the protein is rapidly degraded. *E. longa* appears to lack the chaperone RBCX essential for assembly of a conventional RuBisCO complex. Altogether, our results revealed signatures of evolutionary degradation of RuBisCO in *E. longa* and suggest that its role in this species may be rather unorthodox.

#### PROTIST COMMUNITIES IN WATER AND SEDIMENT OF A SEA CUCUMBER FARMING SYSTEM

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Little is known about the distinctive communities of uncultured protists within the aquaculture ecosystem. In this work, the planktonic and benthic protist communities in a sea cucumber farming system were simultaneously investigated on three sampling dates. Analyses of SSU rRNA gene libraries of six samples revealed 108 eukaryotic taxonomic units, among which 17.5% were rare. Stramenopiles and Alveolata are predominant groups in both sediment and water samples. Dinophyta, Rhizaria and parasitic Mesomycetozoa were only detected

in water samples. Parasitic Apicomplexa were found frequently from both water and sediment samples. Based on terminal-restriction fragment length polymorphisms, distinct succession and contrasting protist community structure was found among temporal samples and between planktonic and benthic habitats. Redundancy analysis indicated that the temperature, concentration of dissolved phosphate, and N:Si in surface water were the most significant abiotic variables shaping the planktonic communities. This study indicates that temperature and stoichiometric ratios play important roles in driving succession of protist communities, and parasitic protist could be early examined by molecular technologies for the farming pools.

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#### MAKE PERCISE IDENTIFICATION USING DNA TAXONOMY TECHNIQUES FOR CLOSELY RELATED *EUPLOTES* CONGENERS (PROTOZOA, CILIOPHORA) WITH HIGHLY PHENOTYPIC PLASTICITY

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Ciliated protists constitute astounding diversity and play crucial role in different biotopes yet still incompletely characterized microbes. DNA barcoding is promising to address this dilemma. This molecular technique based on COX 1 gene has become a routine method for animal organism identification and taxonomic clarification. This common region also can distinguish ciliates species, but has not displayed universality, and establishing a standardized DNA barcoding system for ciliated protists is still confronted with many difficulties and challenges. Here, we collected the species-rich taxon *Euplotes* from brackish and fresh waters. In the framework of traditional classification system, the *Euplotes* spp. with available morphological features can provide a useful template against which to test the accuracy of DNA-based taxonomy. Using >30 samples, we have assessed the most common COX 1 region and the alternative SSU-V4, SSU-V9, LSU-D1/D2, ITS1, ITS2 markers. And SSU-V9, LSU-D1/D2 and ITS1 could distinguish the most

closely related species and are proper markers for ciliates barcoding.

#### THE INTRICATE EVOLUTIONARY HISTORY OF RhoBTB PROTEINS

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The Rho family is one of the major subgroups of the huge superfamily of Ras-like GTPases. Rho proteins are known primarily as regulators of various pathways that are connected to the actin cytoskeleton (cell movement, polarization, morphogenesis etc.). These proteins usually contain only the Rho-type GTPase domain, but some of them possess also a tandem of two so-called BTB domains and are called RhoBTB proteins. The BTB domain is a protein-protein interaction domain and is often a part of proteins that are connected to protein ubiquitination through Cullin3-dependent E3 ligases. Proteins with the domain architecture characteristic for RhoBTB proteins have been reported only from metazoans and dictyosteliid slime moulds, but this distribution was deduced from a phylogenetically very limited survey. We have utilized the currently available wealth of genomic and transcriptomic data from diverse eukaryotes, including a wide coverage of protist taxa, and found out that RhoBTB proteins occur in many additional eukaryotic lineages. Although scattered, the phyletic pattern of RhoBTB genes is compatible with a hypothesis that a primordial RhoBTB gene was present already in the last eukaryotic common ancestor. Interestingly, RhoBTB proteins from some taxa (Amoebozoa, Apusomonadida, and Cryptomonadida) proved to possess a RING/U-box domain inserted into the first BTB domain. RING and U-box are related domains that constitute a class of E3 enzymes, so our findings further support the idea that RhoBTB3 proteins ancestrally served as components of ubiquitin-mediated regulation. These and other findings of our evolutionary analyses of RhoBTB proteins will be presented and discussed.

#### THE STATE OF ART IN THE TAXONOMY OF “HELIOZOA”

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“Heliozoa” is a former taxon of spherical axopodial protists. These organisms, being outwardly similar have revealed to be not necessarily related. Some of lineages are now placed into the proper “supergroups”, while many deviant forms still remain “orphans”. The most diverse group – the centrohelids – recently was placed in Haptista along with haptophytes and their internal taxonomy also was considerably revised showing interesting examples of parallel evolution. But in comparison to other groups with a developed taxonomy “Heliozoa” still seem to be undeservingly neglected. Many taxa of flagellates, ciliates and in a less degree of amoebae get much more attention, while heliozoan taxonomy suffer from the lack of skilled taxonomists. The great amount of their diversity is likely to be unknown. The heliozoans require closer attention, being ubiquitous in freshwater, marine (both planktonic and benthic) and soil environments, important consumers of bacteria, protists and even micrometazoans. But the taxonomic impediment currently prevents them from being incorporated into ecological and biogeographic studies. Many heliozoans, especially centrohelids, are easy to maintain in the culture, have a rich morphology and potentially can serve a good model for addressing many of fundamental questions of protistology, including a species problem, the endemism vs ubiquity dilemma and the evolution of cell coverings. Many of them can be indicators of water quality and should be included to biodiversity indexes. But first of all, their taxonomy need to be evaluated and the main proportion of species should be described on the modern level. Study support: RFBR grants 15-04-18101\_a, 15-29-02749-ofi\_m.

#### THE RELIABILITY OF PROTOZOANS AS INDICATORS OF AQUATIC ECOSYSTEM HEALTH

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Metapopulations of microbial organisms occur worldwide wherever their required habitats are realised. This is a consequence of ubiquitous dispersal driven by huge population sizes, and the consequently low probability of local extinction (Finlay, 2002). Microorganisms should be used in biomonitoring for several compelling reasons. (1) A cosmopolitan distribution facilitates comparisons of test results in geographically different regions. (2) Problems of scale are diminished. (3) Replicability

is as good as, or better than, tests with larger organisms. (4) Environmental realism is higher than in tests using larger organisms. (5) The number of test species is dramatically increased when using microorganisms, thus displaying natural variability much better than tests with a limited array of larger organisms, etc. (Cairns, 2005). Biodiversity of the whole aquatic ecosystem depends on the biodiversity of the microbial communities (Algae, Protozoa, Bacteria) at the basis of the trophic pyramid. The term 'indicator species' is usual in literature on bioindication of water quality, but we more tend to accept the term 'indicator communities', or 'model communities', that could be robust both for biological monitoring and ecotoxicology. Diversity and relative abundance of ciliates and other protozoans can be used as indicators of toxic pollution and acidification. Multivariate statistics were used to design "the scale of toxicity" across a gradient of toxicant stress and organic compounds. A new index of periphyton flagellates (IPF) as indicator of the trophic status of a water-body was developed (Zolotarev, 2007). For the foreseeable future, protozoans could be robust for ecosensors and ecorobots.

**NONOOGAMOUS SEXUAL REPRODUCTION IN THE POLAR CENTRIC DIATOM *ARDISSONEA CRYSTALLINA***

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At first glance, according to some morphological and habit features *Ardissona crystallina* (C. Agardh) Grunow should be regarded as belonging in pennate diatoms. However, molecular phylogeny analysis strongly suggests this species to be placed among centric diatoms. It is well known that centric diatoms reveal oogamous mode of sexual reproduction. Exceptions are cases of autogamy. We studied clonal cultures of *A. crystallina* and were able to initiate sexual reproduction in the mixtures of clones. Heterothallic sexual reproduction was shown to be nonoogamous. Gametes produced by male and female gametangia were more or less equal by size. Gamete behaviour and morphology were different. Male gametes had no flagella; at the same time, they were able to produce thin cytoplasmic projections similar to those of some araphid pennates (*Tabularia fasciculata*, *T. tabulata*, *Ulnaria ulna*, etc.). Female gametes were located in the maternal frustules and did not leave them until fertilization. Each male gamete moved in part amoeboidly to the place of singamy and penetrated in the gap between slightly opened valves of the maternal frustule. The described finding raises a question about possibility of independent evolution of isogamy in polar centric diatoms. This study was supported by the Russian Foundation for Basic Research (grant No 15-04-00237 A).