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## CHARACTERIZATION OF MICROSPORIDIAN METHIONINE AMINO PEPTIDASE TYPE 2 (METAP2): A THERAPEUTIC TARGET

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Microsporidia are parasites of all classes of vertebrates and most invertebrates. They have recently emerged as important infections in various immunosuppressed patient populations. Current therapies for microsporidiosis include benzimidazoles, which bind tubulin inhibiting microtubule assembly, and fumagilin, which binds and inhibit Methionine Aminopeptidase Type 2 (MetAP2). We have initiated a program to define the unique structural features of microsporidian MetAP2 using *Encephalitozoon cuniculi* (Ec) MetAP2. Despite extensive efforts using *E. coli*-based expression systems, no active/soluble material could be obtained. However, baculovirus-driven expression in Sf9 cells yielded multiple milligrams per liter of EcMetAP2. This material was enzymatically active and has been characterized. In addition, this material yielded structures of the native enzyme and the EcMetAP2-fumagilin complex at a 0.21 nm resolution. Residues contacting fumagillin that are not conserved between human MetAP2 and EcMetAP2 are D256 and H210, and these differences give clues as how to modify fumagillin in order to enhance its specificity for EcMetAP2. To further expedite and facilitate the discovery of safe and effective MetAP2 inhibitors, we have also engineered *Saccharomyces cerevisiae* to be dependent on EcMetAP2 for its growth, where EcMetAP2 is harbored on an episomal uracil selectable tetracycline regulated plasmid. By comparing yeast dependent on human and microsporidian MetAP2 in these assays we are able to screen for new compounds for the treatment of microsporidiosis. Finally, we have recently identified and cloned the *Enterocytozoon bieneusi* MetAP2 gene, allowing studies on this non-cultivatable human pathogen. Supported by NIH AI31788 and AI 069953.

## ABUNDANCE, DYNAMICS AND SPECIES SUCCESSION IN SOIL PROTISTS

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Soil protozoa are responsible for a significant portion of the bacterivory and cytotrophy. They also contribute to other trophic functional groups. According to empirical models, soil protozoa are an important rate regulating component of decomposition food webs. The activity of these protozoa varies with local abiotic conditions on the short term, and there are seasonal and succession patterns on the long term. We have shown that day to day abundance variations, in response to weather, fluctuate by up to one thousand times. In forest litter, we have shown there are successional patterns with seasons, and with plant litter chemistry. Over longer periods, we observed increase in species diversity over a 50 year chronosequence, in agriculture fields. These fields were transferred from tillage to no-till management, so the soil profile was undisturbed and allowed to develop. Although abundance fluctuations were governed by abiotic changes, longer term successional and diversity changes were correlated with changes in the profile due to organic matter accumulation. Similarly, post-mining sites under remediation showed similar trends of diversity changes with soil profile development. These studies are being extended at a forested site under conservation that offers several soil profile chronosequences. This study site allows comparison of soil protozoa community structure with plant species composition, along with different soil profiles and organic matter content, due to past land-use history, at the same climatic region. The aim is to

conduct sufficient studies to distinguish between the plant rhizosphere effect, the soil profile organic matter, the mineral chemistry, as they interplay and affect soil community structure changes on the long term.

**REDESCRIPTION OF *STENOSEMELLA NIVALIS* (CILIOPHORA, SPIROTRICHA, TINTINNINA) BASED ON LIVE OBSERVATION, PROTARGOL IMPREGNATION, AND SCANNING ELECTRON MICROSCOPY**

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Although *Stenosemella nivalis* (Meunier, 1910) Kofoid & Campbell, 1929 is widely distributed in coastal waters, its cell and lorica morphology were poorly known. The specimens were collected in the pelagial of the Irish Sea near the Isle of Man during spring. The investigations comprised live observation, protargol impregnation, and scanning electron microscopy. Lorica ~ 53 × 40 μm in size, amphoriform, incrustrated by quartz particles, except for hyaline collar with ~ 7 ellipsoidal windows; occasionally, with agglutinated second collar anterior to the hyaline one. Cell in extended state ~ 55 × 18 μm, attached to bottom of lorica by ~ 7 μm long stalk; in protargol preparations, specimens usually contracted and only 34 × 21 μm in size. Nuclear apparatus composed of two ellipsoidal macronuclear nodules and usually two globular micronuclei. Capsules (probably extrusive organelles) in striae (beaded strands) on the collar membranelles and tentaculoids (finger-like cytoplasmic processes) originating from the intermembranellar ridges. Somatic ciliary pattern is complex and comprises the right, left, and lateral ciliary fields, as well as the monokinetidal ventral, dikinetidal dorsal and dikinetidal posterior kineties. Oral apparatus is transversely orientated at the apical cell end. Adoral zone of membranelles is closed, composed of one buccal and ~ 18 collar membranelles. Endoral membrane probably is of monostichomonad-type. Oral primordium originates in the subsurface pouch posterior to lateral ciliary field. Study supported by the Austrian Science Foundation (FWF; project P17752-B06).

**CONJUGATION IN *HALTERIA GRANDINELLA*: THE MYSTERY OF ITS GENEALOGY PERSISTS**

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The conjugants are isomorphic and fuse partially to a homopolar pair. The partners become ventrally concave, obtain an interlocking arrangement, and one is shifted slightly posteriorly. The pair almost achieves the size and outline of the morphostatic specimen. Before the macronucleus fragments, it becomes surrounded by argyrophilic vesicles (probably autophagosomes). The micronucleus performs three typical maturation divisions. While the mates are still connected, the synkaryon divides twice: one derivative becomes a micronucleus, one - a macronuclear anlage, and two disintegrate. Likely, the somatic bristle rows are reduced from seven to four in each partner, i.e., the first becomes imperceptible on the ventral side during pair formation, the second and third - when the contact of the partners becomes more intimate. During the second maturation division, somatic anlagen become recognizable as in ordinary ontogenesis; their fate remains obscure. During prophase I, the partners become dimorphic: the collar membranelles of the more anteriorly located conjugant arrange around the pair's anterior end, forming a membranellar zone for both partners; its buccal membranelles as well as the collar and buccal membranelles of the more posteriorly located conjugant disappear. Simultaneously, an oral primordium originates on the ventral side of both partners, probably generating only the collar membranelles. *Halteria* resembles the choreotrichid ciliate *Pelagostrobilidium* in the interlocking arrangement of the partners, while the dimorphism and the shifting of the partners as well as the common membranellar zone are like those in the Stichotrichida. Since data on the Oligotrichida are not available, the apomorphic character states are unknown. Study supported by the Austrian Science Foun-dation (FWF; projects P17752-B06 and P19699-B17).

**DIVERSITY OF EPIBIONT CILIATES OF VARIOUS ORGANIC SUBSTRATES IN MEXICO**

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The total number of free living and symbiotic ciliates reported from Mexico is 952 species. The epibiont ciliates (106 species) represent 11.13% of the total species richness. Results are based on studies of ciliate diversity on different organic substrates during several years using optical and scanning electron microscopy. On the leaves of the angiosperms *Thalassia testudinum, Phyllospadix* sp. (marine), *Halodule beaudetti, Rhizophora mangle* roots (brackish) and freshwater plants, 77 species of epibiotic ciliates have been identified. On seven species of brackish algae, 47 ciliate species have been observed, and on filamentous freshwater algae we found seven species. The most studied invertebrate has been the freshwater crustacean *Cambarellus patzcuarensis* (a decapod), on which distribution of 28 species of epibiotic ciliates has been studied. On *Hyalella azteca* (freshwater amphipod) and other crustaceans, five species have been reported, and on *Penaeus* (brackish

decapod) only one species. Of the seven groups of epibiotic ciliates (apostomatids, folliculinids, licnophorid, peritrichs, prostomatids, stichotrich and suctorians), the peritrich ciliated were the most diverse group, represented by 62 species (58.5%). *Acineta tuberosa, Vorticella campanula* and *Platycola decumbens* are the three common species reported from angiosperms, algae and *Cambarellus patzcuarensis.*

**FAUNA OF FREE-LIVING CILIATES IN AZERBAIJAN: MODERN STUDIES AND PERSPECTIVES**

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At present, fauna of ciliates in Azerbaijan includes ca. 1300 species. It is known that 620 ciliate species are from the Caspian Sea, 780 species - from inland waters, and about 550 species - from soil samples from different places of Azerbaijan. A lot of species have been found in two or three different biocenoses. The analysis of the published data and our own experience showed that the ciliate fauna consists of three main groups: the first group of eurybiont cosmopolitan species includes about 25% of ciliates widely distributed worldwide. The second group, about 20%, is stenoterm (cold-resistant or heat-loving) species which are distributed in various regions with similar ecological conditions. The third and the greatest group (55% or more), are stenobiont ciliate species, some part of which might be endemic. On my opinion, for the full statistically significant investigation of ciliate fauna of any region of interest, not less than 15 years should be spent, because usually during the first years the researchers register ciliate species belonging only to the first and second group. The real estimation of ciliate fauna of any region (first of all, the soil species as inhabitants of the most heterogeneous environment) is possible only after long-term regular observations, when the number of rare stenobiont species increases from year to year. For example, despite of previous long-term research performed by Agamaliev, we found about 180 new ciliate species in the Caspian Sea.

**PILOT STUDY ON MICROSPORIDIAN INFECTION OF TERRESTRIAL VERTEBRATES IN AZERBAIJAN**

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Microsporidia is a group of obligatory intracellular parasitic protozoans of high practical significance. Infection of HIV-positive individuals with microsporidia, especially with the species of genera *Encephalitozoon* and *Enterocytozoon*, can radically complicate the process of the main disease, since microsporidia may cause opportunistic infections. To determine ability of wild rodents and domestic animals to serve as potential reservoirs of opportunistic Microsporidia in HIV-infected humans, we examined farm animals and rodents in semi-desert areas of Azerbaijan: in vicinity of Baku City megalopolis, and in Gobustan-Absheron and Davachi regions. Fecal samples from 144 rodents, 20 cows and buffaloes, 20 horses, 20 donkeys, 30 sheep and goats were collected. Samples were kept in 70% ethyl alcohol. Fixed fecal smears were analyzed under fluorescent microscope. Positive samples were double checked with PCR. Microsporidia-like objects were found in calcofluor-stained fecal smears of 22 *Meriones libycus* and 1 red fox (*Vulpes vulpes*). Fecal samples of *Microtus sociallis, Allactaga mayor, Allactaga elater* were free from any spores of Microsporidia. Additionally, we did not detect any spores in fecal samples from farm animals. PCR-based method failed to detect microsporidia in positive samples. Supported by US Collaborative Program, Post Doctoral Fellowship Ref. Nr 05-113-4445 INTAS foundation.

**CHANGES IN RAT HEPATOCYTE PLOIDY AND HYPETROPHY AFTER CRYPTOSPORIDIAL GASTROENTERITIS (*CRYPTOSPORIDIUM PARVUM*, SPOROZOA, APICOMPLEXA)**

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Using the laboratory model of mild, moderate, and acute intestinal cryptosporidiosis in suckling (10-14 days old) and weaned (18-22 days old) rats, we tested the extent of cryptosporidial impact on hepatocytes (where no cryptosporidian development occurs) at the peak of infection and after recovery from disease. Light and confocal microscopy and image analysis were applied for studying changes in cell morphology. Protein amount and cell ploidy levels were measured by means of absorption and fluorescent cytophotometry. Liver hypertrophy and obvious growth retardation were remarkable outcomes at the 4th day of cryptosporidian infection. Our results show that cryptosporidiosis is able to provoke a burst-like premature hepatocyte polyploidization and hypertrophy (in proportion to parasitic load). In 14 and 22 days old infected rats, the percentage of hepatocytes with multiplicated genomes, including 4n, 4nx2, 8n, and occasionally 16n cells, were substantially raised above control values and were similar to those in 1 to 2-3 month old uninfected rats respectively, i.e. rats after transition from fluid (milk) to solid food and liver parenchyma reorganization. At day 21 after cessation of diarrhea, the difference in hepatocyte ploidy still persisted, whereas the difference in cell protein content almost completely smoothed out. This is the first documented evidence of serious patho-

logical changes in hepatocytes of early postnatal rats experimentally infected with the intestinal pathogen *C. parvum*. The observed alterations in hepatocytes suggest that cryptosporidiosis modulate liver ploidy through yet unknown mechanisms, and to predict some presumably negative consequences of cryptosporidiosis in later life of infected rats. Supported by CRDF Project RUB2-002707-SP-05 and RFBR.

## PHYLOGENETIC RELATIONSHIPS WITHIN PENICULINE CILIATES AND THE PARAPHYLY OF *FRONTONIA*

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The peniculine ciliates *Frontonia* are usual members of the pelagic and benthic fauna in both freshwater and marine environments. The genus comprises about thirty species but the validity of some of them still has to be confirmed. Despite the high number of species, few molecular data are available for this genus. Here we present 18S rRNA molecular data of nine different, morphologically recognizable, *Frontonia* species. Five species were classified as *Frontonia leucas, Frontonia salmastra, Frontonia minuta, Frontonia atra* and *Frontonia fusca*. The identification, or de novo description, of the remaining species is still ongoing. The phylogenetic analysis based on 18S rRNA data showed that the genus *Frontonia* is paraphyletic, with the clade represented by genera *Apofrontonia* and *Paramecium* branching from within. In particular, *Frontonia* is split into three subgroups. One of these (Cluster 1) is associated with *Apofrontonia dohrni*, despite the significant morphological differences. Preliminary analyses suggest that the genus *Paramecium* associates to both *Apofrontonia dohrni* and *Frontonia* spp. Cluster 1. Despite the observed molecular differences, all the analyzed species display the typical gross-morphological features of *Frontonia*. According to these data, many distinctive traits of the genus *Frontonia* could be plesiomorphic characters of limited systematic value. A taxonomic revision of the genus will be necessary in the next future.

## THE *EPISTYLIS* SP. (CILIATA, PERITRICHA) AS EPIBIONTS OF CALANOID COPEPODS *ARCTODIAPTOMUS SALINUS* (DADAY) IN BRACKISH LAKES IN KHAKASIA

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We examined *Epistylis* sp. associated with calanoid copepods *Arctodiaptomus salinus* in brackish lakes in 2005-2007. The lakes Shira and Shunet are characterized by presence of hydrosulphuric layer and absence of fish. *A. salinus* is a dominating species of zooplankton (up to 100%). Till now, despite of high prevalence of peritrich epibionts of calanoid copepods, the ciliates have not been considered as a component of fauna of these lakes. Identification and a morphological characterization of peritrichs were performed by live observations in light microscope equipped with Lumenera INFINITY digital camera. Infestation density, load, mean number of zooids per colony on the host (adults, copepodites and nauplii of *A. salinus*) and morfometric characteristics of *Epistylis* sp. were registered. Zooids were 37.8±1.3 μm long and 24.8±1.0 μm wide. Colonies contained up to 15 bell-shaped zooids (in the lake Shira). Zooids in the lake Shunet were 39.1±1.6 μm long and 26.7±1.5 μm wide; number of zooids per colony was 2-15. Infestation rates were high. In the lake Shira, 18.4% of nauplii, 53.4% of copepodits and up to 66.7% of adults were infected in summer; 39.3% of copepodits, up to 34.2 % of adults were infected in winter. In the lake Shunet, 52.6% of nauplii, 82% of copepodits, and 80-87.5% of adults were infected in summer; in total, 98-100% of calanoid copepods were infected in winter. Peritrich epibions were localized on the cephalothorax, abdomen, swimming legs, antennae, or on the whole surface of the host.

## GENOME PLASTICITY IN THE DICYEMID MESOZOANS

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Dicyemid mesozoans are obligate parasites that inhabit the cephalopod renal appendage. They have a simple body construction: one large cylindrical axial cell contains intracellular stem cells (called axoblasts) from which embryos are derived, and is surrounded by some 30 ciliated peripheral cells. Previously, it was shown that numerous unique DNA sequences are first amplified and then eliminated during early somatic cell development, in the form of extrachromosomal circular DNAs, leading to genome reduction. Here, we demonstrate using *in situ* hybridization and incorporation of BrdU that other DNA elements, such as single copy genes and repetitive sequences, have very different fates. Single copy genes represented by b-tubulin are initially amplified, presumably via endoreduplication, but subsequently decrease in copy number through development, suggesting that a whole genome is initially amplified and then the amplified DNAs are simply diluted in successive cell divisions, with little DNA replication. In contrast, tandemly repetitive sequences are maintained even in terminally differentiated somatic cell

nuclei. Taking the increasing intensity of *in situ* hybridization after somatic cell differentiation, incorporation of BrdU, and a general correlation between nuclear content and cell size in consideration, those repetitive sequences must be selectively endoreplicated in the peripheral cell nucleus, concomitant with the increase of cell size. Similar genome plasticity is also observed in mitochondrial genome, where initial amplification of mtDNA and subsequent copy number reduction occur, accompanied by minicircle formation in somatic mitochondria. Biological significance of the different behaviors of these DNA elements is discussed as a unique adaptation to parasitism.

**MACROECOLOGICAL PATTERNS IN DIVERSITY OF MARINE BENTHIC CILIATES**

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The data on occurrence of over 1230 species of marine benthic ciliates compiled from more than 170 literature sources were used for studying the world-wide distribution and diversity of the group. Among 14 regions that were distinguished, the Northeastern Atlantic is the richest one (totally 608 species), then follow the Caspian, Baltic and Black seas (516, 456 and 434 species, respectively); Northwestern Atlantic, North Pacific and Antarctic regions have the lower-range richness (226, 218 and 94 species). Thirty one percent of species were found in one region only; the others, however, mainly had wide geographical distribution covering both hemispheres. There is no tendency to narrowing the occupancy ranges for species found at high latitudes. Comparison of faunistic composition reveals that species-level similarity between regions roughly corresponds to their geographical position. This correspondence, however, disappears when similarity is estimated at the levels of genera or higher taxa, though ciliofaunas of the Northwestern Atlantic, North Pacific and particularly Antarctic show noticeable specificity in their composition. Regional-scale diversity (RSD) depends neither on the total area, nor on the total coastline length, and does not show any latitudinal gradients. At the same time, it highly correlates with investigation effort (estimated as total time of studies carried out in the region). Besides, RSD correlates with average salinity (negatively, which is unusual!). Local-scale diversity (LSD, estimated as species richness at the selected well-studied sites) does not depend on RSD values, possibly indicating the high species-saturation level of local ciliate communities. Also, RSD values do not show any latitudinal trends. Moreover, region-averaged LSD values demonstrate striking resemblance (120-140 species per site). By contrast, the point diversity (estimated as average number of species per station for one sampling session) turns to be positively related with absolute latitude (i.e., samples taken at higher latitudes tend to bring more species). We suggest that marine benthic ciliates demonstrate many macroecological patterns that consistently contradict the regularities commonly obtained for macroorganisms. Thus, even if ciliates have biogeography, it is very specific and quite different from that of macrobiota.

**FACTORS INFLUENCING THE DIVERSITY AND DISTRIBUTION OF CERCOMONADS (CERCOZOA) AT DIFFERENT PHYLOGENETIC SCALES: UBIQUITOUS AND ECOLOGICALLY DIVERSE HETEROTROPHIC ZOOFLAGELLATES WHICH YET SHOW CLEAR PATTERNS OF COMMUNITY DIFFERENTIATION**

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We created and sequenced 18S rDNA libraries for *Cercomonas* and *Eocercomonas* from around the world, with particular emphasis on local patterns of diversity at a grassland sampling site near Oxford, UK. Individual soil samples (<1 gram soil) from the Oxford site each contained between four and 20 genetically distinct cercomonad 18SrDNA sequences (18S-types), which accounted for up to 66% of the total diversity detected in the 10m x 10m grassland site at a single timepoint. We then used this approach to compare assemblages of cercomonad 18S-types over larger geographical distances, at various timepoints, and from different ecological conditions. In these cases the overlap between sample sets in terms of shared 18S-types was significantly less than that seen within sets collected proximally in space and time. Thus we show that cercomonad communities become more dissimilar with increasing geographical and temporal distance, even though our previous studies have shown that most, if not all, 18S-types can be found on all continents with sufficient sampling effort. Cercomonad assemblages are also influenced by ecological conditions: we recovered cercomonad sequences far less frequently from marine habitats and acid soils than elsewhere, and sets of soil and permanently submerged sediment libraries sampled at the same place and time were quantitatively more similar within than between sets. We suggest that many habitats hold a large pool of dormant or relatively rare cercomonad strains and that observable differences in cercomonad assemblages between sites are determined by a combination of environmental fluctuations and stochastic effects of space and time.

**IN SEARCH OF NUCLEAR ACTIN IN *PARAMECIUM CAUDATUM***

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For a long time actin was regarded only as a cytoplasmic protein. However, recent years have been marked by increasing interest to its nuclear functions. Actin is believed to be an important constituent of the nuclear matrix required for chromatin remodeling and transcription. Actin has been found in the nuclei of several popular model objects, such as *Xenopus* oocytes and slime molds *Dictyostelium* and *Physarum*. Though nuclear matrix might be of fundamental importance in organization and functioning of the amplyploid macronucleus, little if any is known about nuclear actin in Ciliates. The present study is the first attempt to demonstrate the presence of actin in the nuclei of *Paramecium caudatum*. To reveal actin whole cells and paraffin embedded sections were treated with TRITC-phalloidin or polyclonal antibodies to paramecium actin 1-1. Antibody treatment failed to detect actin 1-1 in the nuclei which evidences for the absence of this form of actin in the nuclei. Conversely, TRITC-phalloidin staining was very intensive in both macro- and micronucleus, and was mostly associated with chromatin, as shown in the alternative sections stained with DAPI. Nucleoli in the macronucleus and the achromatin cap in the micronucleus seem to lack actin. Our data argue for the presence of F-actin in the nuclei of *P. caudatum*. Further investigations of actin in the nuclear apparatus of ciliates are underway.

**THE GROUND-PATTERN OF THE HYPOTRICHA (SPIROTRICHA, CILIOPHORA)**

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The Hypotricha Stein (= Stichotrichia Small & Lynn) are a major group of the spirotrichs. The spirotrichs show, mainly according to molecular data, the following structure: (euplotids + (oligotrichs + hypotrichs)). The ground-pattern (= ground-plan) of an evolutionary unit is of fundamental importance for the establishment of a phylogenetic system. It is the combination of all types of features (e.g., morphological, ontogenetic, ecological, molecular) of the stem-species from which the monophylum evolved, that is, it is a summary of apomorphies and more or less young plesiomorphies. Some supposed apomorphies of the hypotrichs are: (1) 18 frontal-ventral-transverse cirri; (2) 3 dorsal kineties; (3) 2 macronuclear nodules; (4) contractile vacuole near proximal end of adoral zone; (5) 2 undulating membranes (?); (6) oral primordium originates on cell surface; (7) parental somatic ciliature completely replaced during cell division. Some plesiomorphies of the hypotrichs are: (1) cirri present; (2) body dorsoventrally flattened; (3) body flexible; (4) frontal-ventral-transverse cirri originate from 6 anlagen; (5) caudal cirri present; (6) cortical granules present; (7) parental adoral zone not reorganised during cell division; (8) lack of kinetodesmal fibre in interphasic specimens; (9) benthic; (10) telomeric repeat sequence TTTTGGGG. For more apomorphic and plesiomorphic features and discussion (including convergences and alternative hypotheses) see poster and Monographs on hypotrichs by Berger (1999, Monographiae Biol., 78: 1-1080: oxytrichids), Berger (2006, Monographiae Biol., 85: 1-1303: urostyloids), and Berger (2007, Monographiae Biol., in press: amphisiellids). Financial support was provided by a grant (APART; Austrian Programme for Advanced Research and Technology; Project 10940) of the Austrian Academy of Sciences, Vienna.

**MYOSIN II AND THE EVOLUTION OF UNIKONTS**

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Based on the taxonomic distribution of myosin domain combinations and the presence of myosin II in particular, it has recently been suggested that Amoebozoa and opisthokonts share a common ancestor, in accordance with their close relationship in several multigene phylogenies. They are grouped together in the unikonts, while all other eukaryotes would comprise the bikonts. However, clear evidence for the monophyly of Amoebozoa is yet missing. Thus, although some members of this phylum are known to belong to unikonts, the phylogenetic position of many lineages of lobose amoebae remains unclear. Besides, relationships between the amoebozoan lineages defined on the basis of morphology and small-subunit ribosomal RNA gene (SSU rDNA) phylogenies are still unresolved. In this study, we use myosin II to try and elucidate some of these important evolutionary questions. The presence of myosin II was tested in about 30 lobose amoebae, representing almost all previously defined lineages of Amoebozoa, and a taxonomically rich phylogeny of unikonts was produced. Our results show that (1) all members of the phylum Amoebozoa belong to unikonts, ruling out the possibility that the root of the eukaryote tree lies among Amoebozoa; (2) myosin II supports the monophyly of Amoebozoa; (3) relationships among lobose amoebae as inferred from myosin II sequences are in striking accordance with the SSU rDNA phylogeny; and (4) a specific duplication of myosin II known in metazoans occurred before the divergence of choanoflagellates and ichthyosporeans, supporting their close relationship to animals.

## ULTRASTRUCTURAL INVESTIGATION OF *CHLAMYDOMONAS CARRIZOENSIS* AND THE VARIABILITY OF TRADITIONAL MORPHOLOGICAL CRITERIA OF *CHLAMYDOMONAS*

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The cell structure of an unicellular green algae *Chlamydomonas carrizoensis* Deason et Bold (strain 46.72 SAG) described as the member of Pleiochloris-group has been studied by light and transmission electron microscopy. As a rule the cells of *C. carrizoensis* are cylindrical, ellipsoid-cylindrical or ellipsoid-egg, slightly asymmetrical, 14-21 μm in length, 5-12 μm in width. The 2 contractile vacuoles are located at the edge of 2 flagella, identical or slightly shorter than cell length. The cell wall consists of 2 layers and bears a prominent keel papilla. Under highly invaginated plasmalemma a great number of homogeneous electron dense globules have been observed. The nucleus is of the complex chromocenter type. Dictyosomes, vacuoles of 3 types and small profiles of mitochondria are located perinuclearly. The chloroplast is cup-shaped, highly lobed, massive, with some perforations. It is formed by short stacks of 3 to 8 (15) thylakoids, stroma, containing some plastoglobules, starch grains and ribosomes. The real chloroplast perforations have not been observed at the ultrastructural level. The stigma is formed by 3 rows of pigmental globules. The number of pyrenoids varies from 1 to -3 or extremely rare - more. The pyrenoids are located in thylakoid lobes by such way that the cells of *C. carrizoensis* can be refered to any of 3 groups of *Chlamydomonas: Chlorogoniella, Agloe* and *Pleiochloris*. The pyrenoids *C. carrizoensis* have the same structural type as the representatives of *Agloe* (*C. radiata*, strain 47.72 SAG) and *Pseudagloe* (*C. mutabilis*, 34.72 SAG) which together demonstrate the nearest to *Chloromonas* localizations in different kinds of phylogenetic trees (Proeschold etal., 2001; Nozaki et al., 2002; Pocock et al, 2004).

## PRIMARY AMOEBIC MENINGOENCEPHALITIS IN MEXICO: CASE REPORT

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A 5-year old male, immunocompetent with history of contact with thermal water in a recreative centre 9 days before the onset of symptoms: fever (38-39°C), increased intracranial pressure, headache, nausea, vomiting, somnolence, anisochoria, restlessnnes, tachycardia, generalized seizures and mental status abnormalities. Initially was diagnosed as bacterial meningitis then treated with vancomycin and cefotaxime without success. CSF (cerebrospinal fluid) was purulent with counts of 47000 cells/mm3, glucose of zero, polymorphonuclear leukocytes (90%), Gram-stain was negative for bacteria. Light microscopy examination of CSF revealed the presence of amoebic trophozoites of free-living amoebae (FLA), next patient was treated with amphotericin B, rifampin, fluconazole, trimethoprim-sulfamethoxazole. However, in spite of treatment, the evolution was fulminant. CAT showed hemorrhage subachnoidea massive and important cerebral edema and died 11 days after the onset of symptoms. Culture of CSF was negative for FLA. Sample of 500 ml of thermal water where the child swam, was culture in NNE medium at 37°C. After 18 hours, amoebic trophozoites morphologically identified as *Naegleria* sp. (*Nw*), were observed. Western blot assay between *Nw* and *N. fowleri* (ATCC-30808) was made. The clinical manifestations, morphological characteristics of the amoebae and the results of Western blot, are compatible with *Naegleria fowleri*, however the isolate was not pathogenic in mice. Even thought Mexico is not considered as endemic zone of meningoencephalitis, there have been reported several cases of this pathology. It is important to consider it since the initial diagnosis in order to determine the real incidence of this pathology in our country.

## LOCOMOTORIAL BEHAVIOR OF PSAMMOPHILIC CILIATES

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We investigated locomotion of 11 species of psammophilic (sand-dwelling) ciliates, using frame-by-frame video analysis. Two-dimensional trajectories were regarded as composition of arcs and straight segments (long lasting elements) and locomotorial patterns which determined their arrangement (short lasting elements). Long lasting elements of three-dimensional trajectories were regarded as helixes with straight or bend axis. Difference in locomotorial behavior between some of the species was observed. The greatest velocities were typical for species which inhabit mainly surface layers of sea sand. Angles of contingence of arcs were correlated with vertical distribution of ciliates. Smaller angles of contingence were more common for ciliates from deeper layers of sand. Fractal dimensions of trajectories were also negatively correlated with depth of distribution maxima. Difference in locomotion between species with similar nutrition characteristics was observed. We suggest that there is no strict connection between locomotion and nutrition of

ciliates. It was found that the values of locomotion characteristics of species with different morphology overlap strongly. It suggests the absence of strict morphological determination of locomotorial behavior for ciliates. Smaller velocities, smaller reorientation angles, and smaller fractal dimensions of trajectories that are characteristic to ciliates from deeper sea sand layers, are consistent with spatial restriction of interstitial capillary system in natural habitats. We suppose also that environmental conditions of psammophilic ciliates from deeper sand layer do not require considerable displacements and reorientations, as in case of superficial species. Difference in locomotion of psammophilic ciliates could be the evidence of various locomotorial tactics for exploring habitats and food seeking.

## THE COMMENSAL SESSILE PERITRICHOUS CILIATES (CILIOPHORA, PERITRICHIA) ON MOLLUSCS FROM WATER BODIES OF UKRAINE AND THE MASURIAN LAKES OF POLAND

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The material was collected in Ukraine during 1981-2006 in fresh and brackish water bodies in the drainage basins of the rivers Dnieper, South Bug, Dniester and Danube, and in the Black Sea (in total, ca. 2500 molluscs were investigated). In Poland (the Masurian lakes) material was collected in 1989; 45 molluscs were examined. The commensal species which were specific to the particular host taxa were attached directly to mollusc bodies. Totally 12 species of *Mantoscyphidia* were observed on gastropod molluscs in Ukraine: *M. physarum, M. limacina, M. hydrobiae, M. acanthophora, M. radixi, M. theodoxis*, and 6 more species that will be described as new for science. On gastropod molluscs from the Masurian lakes of Poland, *M. physarum* (on *Physa fontinalis* and *Theodoxus fluviatilis*), *M. radixi* (on *Lymnaea ovata*) and *Mantoscyphidia* sp.1 (on *L. stagnalis*) were collected. Two species of sessile commensal peritrichs from genera *Mantoscyphidia* and *Epistylis* were found on bivalve molluscs. *M. sphaeriidarum* was found on the leg of *Sphaerium rivicola* from the Upper and Middle Dnieper River. *E. borysthenicus* was widely distributed on the molluscs *Anodonta* spp. and *Unio* spp. in most of investigated water bodies of Ukraine and on the *Unio* sp. in the Masurian lakes. The ciliates were located mostly on the edge of mantle and on the inhalant siphon. It should be noted that *E. entzii, E. balatonica* (attaching to E. *entzii* stalk), *Opercularia gracilis*, and (rarely) *O. plicatilis*, which previously had been known from Hungarian unionid molluscs (Stiller, 1931, 1935, 1941), were universally observed on the inner periostracum of unionids during our investigation.

## *GIARDIA DUODENALIS* WB C6 INDUCES EARLY CASPASE-DEPENDENT APOPTOSIS IN HCT-8 CELL LINE

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*Giardia duodenalis* is a flagellated protozoan which causes enteritis in humans and animals. *Giardia* trophozoites adhere to epithelial cells of the duodenum, causing diarrhoea and often malabsorption, whose pathogenesis remains poorly investigated. Apoptosis is a cell death program executed by intracellular cysteine proteases (caspases) activated through an extrinsic (caspase-8-mediated) and an intrinsic (caspase-9-mediated) pathways, which converge to activate the downstream effector caspase-3, responsible for cell death. Several enteric pathogens, including protozoan parasites, are able to induce enterocyte apoptosis. In particular, some strains of *G. duodenalis* were reported to induce enterocyte apoptosis, but the mechanism involved in this process has not been clarified. Therefore, the aim of this work was to assess whether *G. duodenalis* strain WB clone C6 is able to induce apoptosis in the human intestinal epithelial cell line HCT-8, and to investigate the role of caspases in this process. Results demonstrated that after 16 h from infection, the parasite is able to induce the morphological features of cells undergoing apoptosis, assessed by using the fluorescent dyes YO-PRO-1 and propidium iodide, and confirmed by DNA fragmentation analysis, detection of active caspase-3 and degradation of the caspase-3 substrate PARP. Furthermore, *G. duodenalis* infection induces activation of both caspase-9- and caspase-8-mediated apoptotic pathways, down-regulation of the anti-apoptotic protein Bcl-2 and up-regulation of the pro-apoptotic Bax. Overall, these data suggest that in HCT-8 cells *G. duodenalis* WB C6 is able to induce early caspase-dependent apoptosis, which may contribute to the pathogenesis of giardiasis.

## COMPARATIVE MORPHOLOGY OF PHAGOTROPHIC EUGLENIDS

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Euglenids form a diverse group of protists adapted to a wide range of aquatic environments. Members of the group have different modes of nutrition, including phagotrophy, osmotrophy and phototrophy. Most research on euglenids has been done in photosynthetic species, such as *Euglena gracilis*, because they can be

cultured more readily and are significant as indicators for the health of freshwater ecosystems. However, phagotrophic species found in marine environments comprise the majority of euglenids. Phagotrophic (i.e. bacterivorous and eukaryovorous) euglenids have an elaborate feeding apparatus for capturing prey cells that together with the ability to accommodate large prey particles by means of a plastic pellicle, allowed an ancestral eukaryovore to acquire chloroplasts by secondary endosymbiosis. Aside from a few relatively well-studied phagotrophic species (e.g. *Entosiphon* spp. and *Peranema* spp.), the overall diversity and mechanisms of feeding in phagotrophic euglenids is poorly understood. This basic knowledge is critical for reconstructing the earliest stage of euglenozoan evolution and the origins of complex ultrastructural systems in the group. My research focuses on characterizing new species of phagotrophic euglenids using electron microscopy and molecular phylogenetic approaches. I will present molecular and comparative ultrastructural data on three new species of phagotrophic euglenids: (1) a freshwater *Dinema* sp. with a novel eukaryovorous feeding strategy, (2) a freshwater *Heteronema* sp. with a novel corkscrew-shaped cytoskeleton, and (3) a marine, facultative anaerobic eukaryovorous euglenid covered in episymbiotic bacteria.

**PATTERNS OF THE TESTATE AMOEBAE COMMUNITY STRUCTURE ALONG THE MOISTURE GRADIENT: MICROSPATIAL VIEW**

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Variations in structure of the testate amoebae communities among four moisture micro-gradient series (moss hummocks, shore of boggy lakes, etc.) were investigated in different bogs in the Middle Volga Region in 2006. Each of the four gradient series contained 10 to 12 local communities. Sixty six species and sub-specific forms of testate amoebae were identified. Communities' structure revealed similar trends in all gradients series: abundance and the species richness increased from dry to wet habitats, whereas the species diversity (in terms of Shannon index) remained at the same level. Three community types were distinguished along all microspatial gradients. In the moss hummock of the oligotrophic bog, the driest habitat was occupied by xerophilous species: *Euglypha laevis, Corythion dubium, Trinema enchelys, Assulina muscorum, A. seminulum.* The slope of the hummock was inhabited by *Archerella flavum, Phryganella hemisphaerica, Ph. acropodia, Euglypha ciliata glabra*. In the wet habitats at the base of the hummock, hydrophilous species *Hyalosphenia papilio, H. elegance, Heleopera petricola, Nebela tincta major, Arcella vulgaris, Euglypha ciliata, Difflugia leidyi* were the dominants. At the slope of the boggy lake shore, the driest niche was inhabited by *Assuina muscorum, Nebela tincta*, and the wet zone - by *Cyclopyxis eurystoma, C. arcelloides, Trinema enchelys, Hyalosphenia papilio*; among the submerged mosses in boggy water zone *Arcella arenaria, A. discoides, A. conica, A. gibbosa* were dwelling. The highest heterogeneity of species structure along the moisture gradient was observed in the hummock, the lowest - in the flat ravine in the boggy forest. The observed patterns show that variation in the structure of protozoan communities can be observed at scales measured in centimeters.

**SPATIAL AND TEMPORAL PATTERNS OF THE STRUCTURE OF MARINE INTERSTITIAL CILIATE COMMUNITIES**

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The ciliate community structure in the White Sea estuary was studied from 1991 to 2006. Altogether 143 ciliate species were identified. Two aspects of spatial variability of the community structure were analyzed: organization of an elementary ecosystem (low energy sandy littoral of a shallow bay), and distribution of community along the salinity gradient (a system of alternative communities). Within the elementary ecosystem, ciliates displayed aggregative distribution due to the complex of factors. At relatively large scales (tens to hundreds of meters) spatial heterogeneity of the community was determined by abiotic conditions (type of sediments, amount of organic carbon, Eh, etc.). At the lower scales (centimeters-decimeters), the role of biotic interactions (competition, predation, and food availability) increased. Along the salinity gradient, the community was found to be unified, continual, and two-polar, i.e. it contained marine and brackish-water sets of species. A relative and fuzzy border between the two poles was located in brackish waters of the estuary, characterized by the greatest alterations in redox-potential and salinity which reached occasionally the critical level of 3-8‰. Two aspects of temporal variability were studied as well: seasonal changes, and long term (16-year period) pattern. The dynamics of ciliate community showed clear annual cycle. Complexity of the community increased from the late winter to the early autumn. Increase in available energy in spring presumably triggered this process. In early autumn, amount of light lessened and temperatures fell down, which probably caused simplification of the community structure. Alterations in long-term dyna-

mics of the community structure were characterized by both stochastic and trended changes, alongside with stability of some community parameters. An obvious stability in species richness and evenness was recorded during long-term observations, which suggested certain constancy in general organization of the community niche. On this stable background abundance and biomass as well as trophic type, size, and species structure are more variable. All trended changes of ciliate community structure, probably, reflect changes of littoral ecosystem: silt accumulation and redox-potential decrease.

**MORPHOLOGICAL EVOLUTION, SYMBIOSIS, AND PHYLOGENY OF PARABASALIDS AND OXYMONADS: NEW INSIGHTS FROM THE GUT PROTIST COMMUNITY OF THE WOOD-FEEDING COCKROACH *CRYPTOCERCUS PUNCTULATUS***

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Parabasalids and oxymonads are two of the most unusual, evolutionary divergent, and morphologically spectacular of all organisms but remain poorly studied, partly due to difficulty in cultivation. Members of Parabasalia have evolved enormous structural complexity and bizarre morphologies, while oxymonads apparently lack normal aerobic mitochondria or any derivative organelle, use a non-canonical genetic code, and have a unique single-step meiosis. Most parabasalids and all oxymonads are found exclusively in symbiotic association with animals, particularly termites and wood-eating cockroaches, where they aid in digestion of lignocellulose, a mutualism of enormous ecological importance. We examined members of the gut protist community of the wood-feeding cockroach, *Cryptocercus puntculatus* with light microscopy, transmission and scanning electron microscopy, as well as molecular techniques to improve our knowledge of morphological character evolution in these groups, their associations with symbiotic bacteria, and phylogenetic affiliations. Surface morphology of members of the oxymonad family Saccinobaculidae allies it with families Pyrsonymphidae and Oxymonadidae, according with previously published molecular results. Unlike other oxymonad families, Saccinobaculidae apparently lack bacterial surface symbionts but have a well-developed glycocalyx and other features that may represent synapormorphies of oxymonads as a whole. Examination of members of the parabasalid genera *Eucomonympha, Trichonympha, Barbulanympha, Urinympha*, and *Leptospironympha* reveal previously unrecognized classes of bacterial surface symbionts and ultrastructural features, while molecular results clarify the evolution of the group and support multiple origins of hypermastigote cell form and the monophyly of the order Trichonymphida. Our results allow us to present a new, integrated view of character evolution in these groups.

**A CENTRIOLAR SYNAPOMORPHY FOR CERCOZOA AND AN OVERLOOKED CILIARY/CENTRIOLAR RETICULUM WIDESPREAD IN BIKONT PROTISTS**

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The cercozoan zooflagellate *Sainouron* has a remarkably clear hub-lattice structure that extends from the distal part of the centriolar lumen into the transitional region of the cilium. Both distal and proximal to this is a smooth ciliary/centriolar membrane reticulum. I shall argue that the centriolar part of the hub-lattice structure is the first clear ultrastructural synapomorphy for the phylum Cercozoa. A ciliary or centriolar reticulum is apparently also present throughout the Cercozoa, but both structures were previously overlooked. However, unlike the hub lattice itself, the ciliary reticulum is not a synapomorphy for Cercozoa, being apparently in association with a wide variety of transition region structures in many bikont protists, present but previously overlooked because of its inconspicuousness, asymmetry, and the lack of prior expectation of there being any intraciliary membranes. I shall discuss the possible cell biological and evolutionary significance of these and other ciliary features of Cercozoa.

**ULTRASTRUCTURAL UNITY OF CERCOZOA: THE CENTRIOLAR/TRANSITIONAL HUB-LATTICE, CENTRIOLAR RETICULUM, AND CILIARY CORTICAL FILAMENTS, WITH POSSIBLE ROLES IN NON-CONVENTIONAL CILIARY BEHAVIOUR, USING *SAINOURON ACRONEMATICA* SP. N., A ZOOFLAGELLATE WITH REMARKABLY SIMPLI-FIED AND GEOMETRICALLY DEFINED ULTRA-STRUCTURE, AS MODEL**

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*Sainouron* is a genus of very poorly known but common soil zooflagellates. We sequenced the 18S rDNA and made the first ultrastructural study of *Sainouron acronematica* sp. n. Our phylogenetic analyses indicate that it is specifically related to an unusual non-gliding flagellate, *Proleptomonas*, which groups with heteromitid Cercozoa. *Proleptomonas* has a previously unnoticed unusual cortical nuclear skeleton like *Sainouron*'s and a number of other similarities, so we now classify them together in a new family Sainouridae to distin-

guish them from typical gliding heteromitids. *S. acronematica* has a unique ultrastructure. Each centriole has two fibrous rhizoplasts and a novel centriolar reticulum, with a putative role in centrin-based ciliary bending. The cell has only three cytoplasmic microtubules, all organelles being connected by specific links to them or the nucleus, whose cortex has unusual strengthening tubular inner membrane invaginations and fibrous honeycomb-like lattice. The posterior centriole bearing the motile cilium (with probable receptor) has a novel transitional hub-lattice, and remarkable dense spiral fibre linking its anterior rhizoplast and triplets, and the sole ciliary microtubular root with two microtubules: mt1, underlying the plasma membrane, initiates at the spiral; mt2, laterally attached to mt1 and nucleus, on the anterior rhizoplast. The anterior younger cilium, an immotile stub with novel submembrane skeleton, lacks axoneme, microtubular root, rhizoplast, hub-lattice, reticulum, and spiral and becomes the motile posterior one every cell cycle. We conclude that despite being previously overlooked, the hub-lattice structures and mt1-related roots are phylogenetically widespread in Cercozoa and probably represent synapomorphies for the phylum.

### *HELKESIMASTIX MARINA* SP. N. (CERCOZOA, MONADOFILOSA): A GLIDING ZOOFLAGELLATE LINEAGE OF NOVEL ULTRASTRUCTURE

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*H. marina* differs from *H. faecicola* in that the former was isolated from marine sediment and is unable to grow in non-marine media, whereas *H. faecicola* was isolated from animal faeces and grew well in non-marine media. In the absence of previous ultrastructural work the taxonomic position of *Helkesimastix* has been altogether obscure, though it has been suggested to be related to cercomonads. However, *Helkesimastix* differs radically from cercomonads, lacking their complex microtubular ciliary roots, and our phylogenetic analyses based on 18S rDNA demonstrate that it does not group with them, being either a novel major lineage within Monadofilosa, or specifically related to *Aurigamonas solis* and *Cercobodo agilis*, which form a sister clade to heteromitids. Longitudinal cortical microtubules emanate from a dense apical centrosomal plate to which the tip of the pyriform nucleus and two subparallel centrioles attach by amorphous fibres. A slender striated rhizoplast connects the centrioles: one attached to the centrosomal plate by a complex dense fibrous root bears the long 9+2 posterior cilium; the other has no complex root and no axoneme or just nine very short disorganized singlet microtubules, although its ciliary membrane may extend well beyond any axoneme. Both centrioles have a distal/transitional lattice, with a slender hub in the ciliated one. At least two Golgi dictyosomes and a microbody are attached to the nuclear envelope, which has slender micro-invaginations and probably a cortical lattice. Bacteria are ingested apically, probably via a rudimentary cytostome, and digested posteriorly in association with numerous mitochondria with flat cristae.

### COMPARATIVE ULTRASTRUCTURE OF THE FLAGELLAR APPARATUS OF *PELOMYXA* SPECIES (PELOFLAGELLATA, PELOBIONTIDA)

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Protists attributed currently to the genus *Pelomyxa* (pelomyxoids), are amoeboid, usually multinucleated, micro- or anaerobic organisms with numerous flagella. Flagella of all but one *Pelomyxa* species, are non-motile. Ultrastructure of ten species of this group was described. The structure of the flagellar apparatus of pelomyxoids varied. The axoneme had a non-stable set of microtubules. The transition zone contained a transition cylinder and/or an electron-dense column. The basal body of some species differed from kineto-somes of most protists. The basal body was often asso-ciated with the following microtubular structures: (1) radial microtubules; (2) the lateral rootlet; (3) the basal bundle of microtubules. Each species had a particular composition of microtubular rootlets. All the species had radial microtubules. In some pelomyxoids they formed a cone-like structure. In others radial microtubules were organized in a bundle which lay along the cell surface. In the cytoplasm of some species we revealed free kinetosomes associated with microtubular rootlets, which were structurally different in each species. Structural variability of pelomyxoid flagellar apparatus may be explained by reduction of some of its elements in connection with the loss of flagellar motility. Flagellar apparatus of pelomyxoids demonstrated some structural similarity with that of mastigamoebids.The work was supported by the Russian Foundation for Basic Research (Project 05-04-48166).

### MOLECULAR COLD-ADAPTATION OF AN ANTARCTIC CILIATE

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A huge variety of ciliates inhabit the freezing coastal waters of Antarctica. *Euplotes* species are easy to collect

and expand into stable laboratory cultures. Therefore, they are excellent material to study how life adapted to cold at the molecular and cellular levels. Most research interest was initially focused on *E. focardii*, because of its strictly psychrophilic behaviour associated with a suppression of the heat-shock response, and its unique structural and functional features of the tubulin/microtubule system. *E. nobilii*, a species that is closely allied to *E. raikovi* inhabiting temperate waters (Dini & Di Giuseppe, personal communication), has now been found to provide unique opportunities to study the adaptive modifications that permit the activity of its water-born signal pheromones at freezing temperatures. Like *E. raikovi*, *E. nobilii* constitutively secretes a large family of protein pheromones, three of which were isolated and purified in amounts sufficient to be structurally characterized by NMR spectroscopy. Their three-dimensional conformations show a compact three-helix bundle core that is in common with *E. raikovi* pheromones, and two extended not structured regions that are unique to *E. nobilii* pheromones, one located at the amino terminus and the other linking the first and second helices. The evolution of these two regions is likely to be functionally significant for cold-adaptation, as they appear to substantially improve, locally and globally, the flexibility of the molecular backbone of *E. nobilii* pheromones.

### *ACANTHAMOEBA PALESTINENSIS* PHOTOSENSITISED INACTIVATION AND THE NEED OF NEW TREATMENTS OF PATHOGENIC PROTOZOA

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*Acanthamoeba palestinensis* was chosen as a model for photoinactivation experiments, using a combination of a photosensitiser, visible light, and molecular oxygen, with the aim of decontaminating waters from pathogenic protozoa and, subsequently, of treating diseases caused by pathogenic amoebae. Indeed, some free-living soil and water amoebae, such as species of the genera *Acanthamoeba, Naegleria* and *Balamuthia*, are recognized etiologic agents of mostly fatal amoebic encephalitis in humans, with immunocompromised and immunocompetent hosts among the victims; *Acanthamoeba* spp. are also agents of amoebic keratitis. The life cycle of *Acanthamoeba* includes an active feeding trophozoite and a dormant cyst. In our experiments both trophozoites and cysts undergo extensive inactivation upon photosensitisation by a tetracationic Zn(II)-phthalocyanine (RLP068). The two forms exhibit similar dependency on photosensitiser concentration, although cysts require a significantly longer irradiation time in order to produce a similar degree of inactivation: trophozoites were irradiated for 10 min and cysts for 20 min with 600-700 nm light (50 mW $cm^{-2}$), at photosensitiser concentrations in the range 0.5-5.0 μM and previous incubation of 1 hour in the dark. The location of RLP068 inside non-irradiated trophozoites is particularly evident in the contractile vacuole and becomes more diffuse in photosensitised cells. RLP068 appears to readily cross the external wall of the cysts, in spite of its relatively large molecular weight, and to localise in various subcellular sites, including the double cystic wall. The ultrastructural studies on trophozoites showed a cellular disorganisation, consisting in a large vacuolisation and subsequent mitochondria alteration with loss of cristae, according to the photosensitiser concentration and incubation time. These data encourage a future use of photosensitiser and light against *Acanthamoeba*.

### PROTISTAN MICROFOSSILS IN TRIASSIC AMBER FROM THE DOLOMITES (ITALY)

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In the last few years the largest known deposit of Triassic amber was discovered near the town of Cortina d'Ampezzo in the Italian Dolomites (Southern Alps). Thousands of millimetre-sized drop-shaped pieces of amber per square metre were preserved in a paleosol, which belongs to the Upper Triassic Series and is about 230 million years old. The tiny drops of amber were removed from the sediment, ground and polished using a series of wet silicon carbide papers and observed in water on microscopic slides under an interference-contrast light-microscope. The amber inclusions are representatives of a complex and diverse biocoenosis consisting of both prokaryotic (filiform cyanobacteria, rod-shaped and coccoid bacteria) and eukaryotic microorganisms (fungi, microalgae, protozoa) as well as spores, pollen grains and tissues of higher plants. Here we present some species of testate amoebae of the families Centropyxidae, such as *Centropyxis hirsuta*, and Difflugiidae, such as *Difflugia*-like specimens, some of which are preserved in dividing stage. Several specimens of Ciliophora were also observed: one could be attributed to the genus *Coleps* (Colepidae), others were identified as members of Colpodidae. All protozoa are well preserved and represent the earliest evidence of non-marine testaceans and ciliates. The amber-fossils are similar or identical to some extant species, showing that little or no morphological changes took place in protozoa from the Triassic to the Recent. Investigations

using transmission electron microscope techniques are in progress and could reveal important features of the fine structure of the microinclusions.

### *LEISHMANIA INFANTUM* IDENTIFICATION DURING *LEISHMANIA*/HIV CO-INFECTION IN SOUTHERN EUROPE: STUDY OF 972 STRAINS ISOLATED BETWEEN 1986 AND 2005

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Thanks to the existence of three main Cryobanks and Identification Centres of France (Montpellier), Italy (Roma) and Spain (Madrid), which use the same reference identification technique of starch gel electrophoresis for 15 enzymatic systems, a series of *Leishmania* isolates have been identified in patients with *Leishmania*/HIV co-infections. The majority of the isolates came from four countries of south-western Europe: France, Italy, Portugal and Spain. Between 1986 and 2005, 972 strains isolated from 741 co-infected patients were identified as *L. infantum*. Out of 27 zymodemes detected, two were predominant in the western part of the Mediterranean Basin, namely MON-1 and MON-24. Several zymodemes usually causing CL in non immunosupressed patients are responsible directly for VL when infecting HIV-positive patients. Some zymodemes can be found only during *Leishmania*/HIV co-infection. Enzymatic polymorphism is high in Spain and Italy, and is based on the large number of rare zymodemes. With the exception of Portugal, the numbers of strains isolated from Leishmania/HIV co-infected humans in the countries of Southern Europe have dramatically decreased since 2001, which presumably reflects introduction of highly active antiretroviral therapy (HAART) in treatment of AIDS. Another reason of such a decrease in some countries could also be diffuse application of molecular techniques in the hospitals for diagnosis purpose, making unnecessary *in vitro* isolation of the parasite.

### OCCURENCE OF MICROSPORIDIA AND CRYPTOSPORIDIA IN STOOL SAMPLES FROM HIV POSITIVE PATIENTS WITH CHRONIC DIARRHEA IN BOTKIN CITY HOSPITAL (ST. PETERSBURG, RUSSIA)

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Project on studies of prevalence and clinical manifestation of microsporidiosis and cryptosporidiosis in HIV-infected patients has been recently initiated in St. Petersburg City Hospital No 30. Stool samples from 55 patients with chronic diarrhea were collected and frozen. Seventeen stool samples were examined for presence of cryptosporidia and microsporidia. Neither staining with Carbol-Fuchsin, nor PCR amplification, or indirect fluorescent assay (IFA) with anti-*Cryptosporidium parvum* monoclonal antibodies (mabs) revealed presence of cryptosporidia. Three samples were positive for the microsporidium *Enterocytozoon bieneusi* by PCR-based detection method. Infection of one sample was confirmed also by Calcofluor staining, by IFA with *E. beineusi*-specific mabs, and by staining with FITC-conjugated polyclonal antibodies. The PCR-amplified region of *E. bieneusi* DNA was about 400 bp in length and contained ITS and part of SSU ribosomal gene. It was sequenced and revealed 100% identity to *E. bieneusi*, isolate Peru 13, Genbank Accession # EF014429. Sequencing of amplicons obtained from other PCR-positive samples, as well as search for infection in the rest 38 collected samples, is in progress. Also analysis of 51 clinical reference forms (CRFs) based on patients' medical charts, is underway. These CRFs contain anonymous information on HIV-infection stage, CD4 count, immunological status, presence of other opportunistic infections, antiretroviral therapy, as well as epidemiological data obtained through questionnaires, and will hopefully help to understand pathogenesis and routs of transmission of the studied pathogens. New patients are being permanently included in the survey. Supported by grant No. RUB2-002707-SP-05 from the U.S. Civilian Research and Development Foundation.

### THE ROLE OF ACTIN IN TRANSPORTATION OF *HOLOSPORA*-BACTERIA THROUGH THE CYTOPLASM OF *PARAMECIUM CAUDAUTM* DURING INFECTION PROCESS

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The endocytobiotic system *Paramecium-Holospora* represents by itself the well-developed model for study of host-symbiont relationships. Bacteria of the genus *Holospora* are obligate endonucleobionts of the ciliates of genus *Paramecium*; the bacteria are showing species specificity as well as nuclear specificity. Our research is focused on study of the process of the bacterial transport from the food vacuole of *P. caudatum* into the "target"-

nucleus (*H. obtusa* - to the macronucleus and *H. undulata* - to the micronucleus). The electron microscope observations showed that actin of the host cell may play a role in this process (Gortz, Wiemann, 1989; Fokin et al., 2002). To prove it we use in our work the method of genetical transfection of *P. caudatum* with linear vectors containing actin-GFP fusion constructs. Codon-optimized GFP gene (Takenaka et al., 2001) was fused through the 7 amino acid linker (Wassmer et al., 2005) to the 5' end of the actin of *P. caudatum* (AB070223). This actin is a homologue of actin 1 isoform 1 (AJ537442) of *P. tetraurelia*. Vector was linearised and microinjected into *P. caudatum* macronucleus. In uninfected cells this actin isoform forms comet tails at food vacuoles and accumulates in patches at the surface of food vacuoles. Also it forms a fine ring on the surface of some food vacuoles and we can observe it in the area of cytopharyngal fibers and cytoproct area. It is very similar to the distribution of its homologue in *P. tetraurelia* (Sehring et al., 2007). In infected cells of *P. caudatum* this actin was observed to take part in a specific exit of *Holospora* spp. to the cytoplasm from the food vacuole. We also were able to see a regular layer of actin distributed over the surface of bacteria in the host cytoplasm. Comet tail-shaped actin filaments were also observed at the surface of the bacteria. Thus, we confirm that actin of *P. caudatum* plays a significant role in transport of *Holospora* through the cytoplasm during the infection process. Supported by RFBR grant 07-04-00662.

## EVOLUTION OF DEVELOPMENT, PHOTOBIOLOGY AND BIOGEOGRAPHY OF *VOLVOX*

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In cultures of *Volvox carteri, V. spermatosphaera* and several other species of *Volvox* (as well as in more primitive colonial Volvocaceae) a long period of light-dependent gonidial growth is followed by a series of rapid consecutive divisions, which may occur in darkness. This type of light/dark control is ancestral for the family Volvocaceae. By contrast, in *V. aureus, V. globator, V. tertius* and several other *Volvox* species a period of light-dependent growth is followed by a series of slow and light-dependent divisions. This type of light/dark control is derived. Data on the geographical distribution of all 18 species of *Volvox* have been summarized. An attempt was made to trace a correlation of their latitudinal distribution with the type of light/dark control during asexual development. In high latitudes of the Northern hemisphere (northward of 50-57° north) only 3 above-mentioned derived species of *Volvox* occur, in which slow gonidial divisions start in the morning and are temporarily blocked during the night. These features have adaptive significance under the conditions of long summer day and might have been important for the formation of modern (Holocene) flora of volvocine algae in high latitudes of the Northern hemisphere. According to molecular-phylogenetic data, the family Volvocaceae originated about 50 million years ago (in early Cenozoic). Therefore, *Volvox* evolution predominantly occurred under the warm climate conditions, such as in Eocene and Miocene. Even during winter the temperature in high latitudes might have been favorable for *Volvox* vegetation. However, our experiments show that under the diurnal light/dark regime of 8 h - 16 h (instead of the routine 16 h - 8 h regime) development in cultures of *V. carteri* is blocked, whereas *V. aureus* is able to complete the asexual life cycle. These data imply that evolutionary reorganizations of *Volvox* development, which are primarily connected with changes in the rate and light/dark control of gonidial divisions, might have occurred as an adaptation to warm and short winter day in high latitudes in the past.

## MECHANISMS OF MACROPHAGE-MEDIATED INHIBITION OF *ENCEPHALITOZOON CUNICULI*

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Macrophages host the replication of microsporidia but also can become activated to kill these intracellular organisms. Murine peritoneal macrophages and RAW264.7$^{\gamma NO-/-}$ cells activated by treatment with LPS (10 ng/ml) and IFNγ (100 u/ml) *in vitro* destroyed intracellular *Encephalitozoon cuniculi* via reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI). To further evaluate this *in vivo*, mice unable to generate ROI (B6.129S6Cybbtml), RNI (B6.129SF2/J mice given 2.5% aminoguanidine in drinking water), or both ROI+RNI (B6.129S6 *Cybb*$^{tml}$ given aminoguanidine), along with WT (B6.129SF2/J) mice, were infected with $5\times10^7$ *E. cuniculi*. All mice survived at least 6 weeks, but the percent of infected peritoneal macrophages after 2 weeks of infection was significantly higher in the mice deficient for ROI (2.1+/-0.29; $P<0.001$), RNI (1.6+/-0.73; $P<0.05$), and ROI+RNI (2.4+/-0.37; $P<0.001$) compared with WT mice (0.85+/-0.09). By 4 weeks, no microsporidia were observed in the peritioneal macrophages of the WT or ROI-deficient mice, but were observed in the mice deficient for NOI and ROI+RNI. Addition of ferric citrate to BALB/c peritoneal or RAW264.7$^{\gamma NO-/-}$ macrophages *in vitro* increased replication of *E. cuniculi* to >50% of controls. Furthermore, ferric citrate reversed the inhibition of *E. cuniculi* infection by

activated macrophages from 24.5% (+/-7.7) of controls in the absence of exogenous ferric citrate to 56.02% (+/-6.4) in the presence of 500 μM ferric citrate in a dose-dependant manner. These results suggest that activated macrophages employ mechanisms that, in addition to ROI and RNI, may also utilize iron sequestration for inhibiting *E. cuniculi*.

**MICROSPORIDIOSIS: CURRENT STATUS**

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The phylum Microsporidia includes approximately 1200 species that infect members of all animal phyla, and 14 of these can infect humans. Interest in these organisms grew tremendously during the past 20 years after being associated as a cause of persistent diarrhea and systemic disease in persons with AIDS. Increased awareness and improved diagnostics have broadened our knowledge about the wide demographic, geographic, zoonotic, and environmental range of the species of microsporidia that infect humans. Identification of microsporidia in water sources also led to their inclusion on the National Institutes of Health Category B pathogen list and the EPA microbial contaminant candidates list of concern for foodborne and waterborne transmission. The completion of the *Encephalitozoon cuniculi* genome has led to new insights into the molecular phylogeny and biology of the microsporidia. Additional genomic data on other microsporidia is becoming available and providing additional insights into the biology of these organisms. This presentation will provide an overview of microsporidiosis in humans and highlight new research findings on these pathogenic protists.

**BIPOLAR DISTRIBUTION OF PROTISTAN SPECIES IS NOT A ROSE**

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Literature records the bipolar distribution of some terrestrial and marine species. Even protistan representatives join to this restricted club. Most discussions focus whether bipolar species are isolated inside their own high-latitude areas, or whether trans?tropical shifting occurs that allows intermixing between the gene pools of northern and southern populations. Such a traffic could be favoured by the planktonic life style or intrusion into a transient microhabitat (detritus particle), so as to be conveyed by the oceanic currents, or by cyst-forming ability to be dispersed by some interpolar traveller. Recently, intermixing has been inferred to be a common event in protists, since the same 18S rRNA genotype has been recorded in both the Arctic and Antarctic populations of all the analyzed bipolar morphospecies. We addressed this problem in taxa comprising the genus *Euplotes*, a cosmopolitan, ubiquitous protistan ciliate. Four morphospecies were analysed: *E. euryhalinus, Euplotes* sp., *E. focardii* and *E. nobilii*. In assigning strains to the appropriate morphospecies, the intra- (inside and between strains) and inter-populations variability was considered. Twelve characters, most commonly used for morphological distinctness among *Euplotes* taxa, were chosen. Data produced by the pursued morphometric procedure were analyzed using the multivariate technique of discriminating functions. The 18S rRNA, EF(Elongation Factor )-1α1 and EF-1α2 genes, as well as the Internal Transcribed Spacers (ITS) regions of the rDNA locus, all characterizing the nuclear genotype, plus the mitochondrial 16S rRNA gene were selected. They were amplified and directly sequenced, in order to establish both the phylogenetic relationships among populations and the occurrence of their interbreeding. *Euplotes euryhalinus* included two Arctic population groups, Ar1 and Ar2, and only one Antarctic, An. The Ar1 and Ar2, 18S rRNA genotypes differed for 7 mutations, whereas the Arctic genotype of Ar1 was shared with the Antarctic An. Yet, these last population groups (Ar1 and An) differed in their ITS (3 mutations) and 16S rRNA (87 mutations) genotypes. The fruitful breedings inside the Arctic (Ar1 and Ar2) and Antarctic (An) population groups did not follow the interpolar mating tests: mixed strains failed to mate or if mating occurred, as in mixture between Ar1 × An strains, conjugating mates belonged to Antarctic representatives of the An population group. A far larger number of mutations at selected loci occurred between Arctic and Antarctic population groups of *Euplotes* sp., as well as between Antarctic and equatorial population groups of *E. focardii* (this latter morphospecies has no Arctic representatives). In both morphospecies, conjugating strains of the Antarctic population groups failed to mate with conjugating representatives of population groups of the Arctic or equatorial regions, respectively. Also in *E. nobilii*, Arctic and Antarctic populations cluster in different groups, showing a number of mutations, larger or smaller, whatever gene is considered. Nevertheless, a strain of an Arctic population group, shared one of the 3 mutations at the 18S rRNA locus, differentiating this group from an Antarctic one. Interpolar crosses involving this Arctic strain produced a hybrid although poor viable progeny, suggesting that an irreversible evolutionary divergence between the two polar populations has not yet occurred.

**VIRUSES ECOTYPES IN COLLECTION OF CILIATES WITH DOUBLE VIRAL INFECTION**

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This work is dedicated to the study of PBCV virus (*Paramecium bursaria Chlorella* virus, family Phycodnaviridae, genus *Chlorovirus*) from the triple symbiotic system of *P. bursaria*. We tested the media from *P. bursaria* cultures isolated from the geographic zones, in which water samples were known to be infected with viruses of southern and northern ecotypes, for presence of both viral ecotypes. We demonstrated that after long-term cultivation, only the viruses with the ecotype corresponding to the zoochlorella host survived. To identify the viral ecotype in water samples from Vietnam, we amplified *pol* gene (760 b.p. of length) using PCR with degenerated primer. Amplification of two DNA samples from Vietnam (viruses of northern ecotype from the Nam-Din and Kuang-Bin rivers), as well as from Georgia, Tadjikistan, and Japan (viruses of southern ecotype) provided similar results. Amplification has been preceded in three investigated samples from Vietnam, which confirms that the virus was present in samples. The electrophoresis of Asian (Chinese, Japanese) viruses' amplificates shows that it is closely-related to the southern viruses (PBCV-1). Sequensing of clone DNA was performed. Chinese viruses (from ciliate clones E12 and B7) and Japanese viruses (from KZ1-3) are placed in southern cluster, but inside one they are located closely to the most northern viruses (New-York origin) in dendrogramm of relationship made using the alignment data.

**REVISITING KOFOID AND CAMPBELL TINTINNIDS (PLANKTONIC CILIATES) OF THE SOUTH PACIFIC**

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Tintintinnid ciliates of the plankton are one of the most species-rich groups among all protist groups. Charles Kofoid and Arthur Campbell are responsible for a large part of the tintinnid species catalogue, they recognized 724 species overall. From material gathered during 2 cruises, the Agassiz Expedition in 1904-1905 and the Carnegie Expedition in 1928-1929, they described 221 new species. Most of the new species were found in the South Pacific, an area poorly sampled then, as well as now. Based on their data, species-richness appeared to be extraordinary, and today it provides evidence of a strong latitudinal gradient in tintinnid diversity. However, many tintinnids pictured in their monographs have remained apparently unrecorded since their description. In 2004, the BIOSOPE oceanographic cruise provided an opportunity to obtain material from some of the same areas between the Marquise and Easter Islands where Agassiz and Carnegie expeditions had collected their samples. Many of unusual and ornate morphologies, as well as an extraodinary species richness described by Kofoid and Campbell were confirmed. The marked diversity of tintinnid ciliates may be indicative of a very food-poor environment, in which competitive interactions between species are rare.

**UNIQUE FEATURES OF MICROSPORIDIA PHYSIOLOGY CAUSED BY MINIMIZATION OF PARASITE CELL MACHINERY**

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The ultrastructural, biochemical and mainly genomic studies of microsporidia demonstrate profound minimization of parasite cell machinery. At the same time the loss of many organelles, metabolic pathways and genes by microsporidia was accompanied by acquisition of unique physiological features not found in other eukaryotic cells. Our study was focused on two points: (1) absence of transport vesicles in the parasite cell side by side with presence of genes potentially involved in vesicle formation and fusion with target membranes; (2) a very scanty repertoire of enzymes involved in the last steps of glycolysis suggesting unique mechanisms for pyruvate converting and electron transport. Heterologous expression of proteins of the microsporidium *Paranosema locustae*, as well as immunocytochemical and biochemical approaches was used to investigate these questions. Supported by RFBR grant 05-04-49616.

**PROBLEMS OF IDENTIFICATION OF PILISUCTORID CILIATES (APOSTOMATEA, PILISUCTORIDA)**

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The pilisuctorid ciliates of the order Pilisuctorida are parasites of crustaceans. At present the order involves three families: Conidophryidae, Ascophryidae and Askoellidae, each comprising a single genus with small number of species. In spite of poor species richness, several problems concerning systematic and nomenclature of this group of ciliates arise. Moreover, even species identification is problematic, and often is based rather on host specificity than on the specific characters of the parasite. Our investigations showed that host specificity of pilisuctorid is not so strict, as it had been

assumed earlier, and methods of specific identification based on morphology are needed. The keys for identification of pilisuctorid species, which are based on the macronucleus morphology and mode of attachment of these ciliated to the host body, were developed for the above mentioned problem-solving.

**FIRST REPORT OF ACTINOSPOREANS IN RUSSIA**

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During 2005-2006, actinospore infection of oligochaetes was consistently revealed at five freshwater sites of St. Petersburg and Leningrad district. Totally 36000 specimens of two common oligochaete species, *Tubifex tubifex* and *Limnodrilus hoffmeisteri*, were collected in mud and examined. By using the "cell-well plate method" (Yokoyama et al., 1991) five types of triactinomyxon, one type of raabeia, and one type of siedleckiella were identified. Three different triactinomyxon types were isolated from *T. tubifex*. First type was revealed in midstream of River Okhta, with maximal prevalence of 0.4% in June 2006 samples; the second type was discovered in the mouth of same river (0.44%); and the third type - in a small cold water spring inflowing in Lake Mozhayskoe (0.5%). From *L. hoffmeisteri* collected in midstream of River Slavyanka, the fourth type of triactinomyxon was isolated (0.08%). The fifth type of triactinomyxon was released from the unidentified immature oligochaete collected in midstream of River Okhta (prevalence up to 0.2% in the September 2006 sample). Actinosporean of raabeia type was isolated from *L. hoffmeisteri* collected in the mouth of River Okhta (0.6%). Actinosporean of siedleckiella type was released from *T. tubifex* collected in midstream of River Okhta (0.6%). Five detected types (three triactinomyxon, one raabeia and one siedleckiella) were different from actinosporea forms hitherto described and probably are new. This is the first report on the occurrence of members of Actinosporea in Russia.

**PARTICIPATION OF HOST CELL MEMBRANE RAFTS IN *TRYPANOSOMA CRUZI* INVASION PROCESS**

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Establishment of the infection by *Trypanosoma cruzi*, the ethiologic agent of Chagas' disease, depends on a series of events involving adhesion of the parasite to the cell surface receptors, recruitment of additional receptors to the infection-site, a re-organization of the membrane and, in particular, the parasitophorous vacuole formation. Distinct microdomains in the plasma membrane are responsible for the invasion of some viruses, bacteria, and protozoans. Membrane rafts are small and dynamic regions enriched in sphingolipids, cholesterol, ganglioside GM1 and protein markers like flotillins (1 or 2) forming flatter domains, or caveolins (1, 2 or 3), which are characterized as stable flask-shape invaginations. We explored whether membrane rafts participate in the entry of *T. cruzi*'s trypomastigotes into murine macrophages. Transient depletion of macrophage membrane cholesterol by application of methyl-beta-cyclodextrin, and treatment with filipin caused reduction of trypomastigote internalization. Treatment with a crescent concentration of cholera toxin B that binds GM1, demonstrated the ability to inhibit parasite entry. Using immunofluorescence microscopy we observed a colocalization of GM1, flotilin1 and caveolin 1 in *T. cruzi*'s parasitophorous vacuole. Together our results suggest that membrane rafts are involved in the process of *T. cruzi* invasion of macrophages. Supported by CNPq, FAPERJ and CAPES.

**CORRELATION BETWEEN CORTEX STRUCTURE AND MOTILITY TYPE OF GREGARINES BY THE EXAMPLE OF THREE *UROSPORA* SPECIES**

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We have studied *Urospora travisiae, U. ovalis*, and *U. chiridotae* (Apicomplexa, Eugregarinida, Urostildae) from polychaetes and holothurians of the White Sea. Trophozoites of *U. travisiae* inhabiting the celom cavity of *Travisia forbesii* have a V-like body, with a tip for attaching to the host tissues, and two bead-like rays. Trophozoites glide by one or both leading rays. Numerous epicytic folds extend from the attaching tip to distal ends of rays. The structure of cortex is typical of gregarines. Trophozoites of *U. ovalis* inhabiting the celom cavity of *T. forbesii* are non-attached and oval in shape. They move by metaboly. The cell surface of the non-contracted part of the parasite body is covered with typically structured epicytic folds, whereas that of the contracted part is covered with superfolds bearing several epicytic folds. The skittle-like trophozoites of *U. chiridotae* inhabit the blood-vessels of *Chiridota laevis* attaching to the vessel wall by narrow ends. Gregarines are immobile. Their surface is covered with numerous cytopilia, which are cylindrical, narrowing to the distal end, variable in size and armed with numerous microtubules. The pellicle forms cylindrical invaginations into cytoplasm, at the bottom of which micropores are formed. The data obtained suggest a correlation between cortex structure and type of motility of gregarines: the presence of typical epicytic folds corresponds with gliding; formation of superfolds corresponds with metaboly; disappearance of folds corresponds with

immobility. The mode of motility is probably adaptation of gregarines to parasitizing in a particular hostal biotope.

### *ACANTHOCYSTIS TURFACEA CHLORELLA* VIRUS FROM FRESHWATER PONDS OF SAINT-PETERSBURG

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Since 1984 we have been carrying out the laboratory studies of the tripletic simbiosis *Paramecium bursaria* - *Chlorella* - virus. The related ATCV (*Acanthocystis turfacea-Chlorella*-virus)-type viruses were first found in Germany by Bubeck and Pfitzner. We were interested if we could find this type of viruses in freshwater samples in Saint-Petersburg. Twelve freshwater samples were taken from the ponds of Saint-Petersburg in order to test the presence of the ATCV-type viruses. One ml of the sampled water was added to the suspension ($9\times10^7$ cells/ml) of the virus-sensitive cells of *Chlorella*, the endosymbiont of the heliozoan *Acanthocystis turfacea*. This culture (SAG 3.83) was originally obtained from the Gettingen University collection, and currently is maintained in the Algae collection of the St. Petersburg State University (CALU). Lysis of *Chlorella* cells was observed in 3 samples after a week of cultivation, which suggested occurrence of the ATCV-type virus. To confirm this, and in order to isolate the local virus populations, we cloned the half-liquid mineral medium with the virus-sensitive *Chlorella* (SAG 3.83. culture). To multiply the received clones, one week later we placed the appeared virus plaques into the liquid suspension of the virus-sensitive *Chlorella* cells (SAG 3.83. culture). Lysate was filtered through the nucleopore filter (d=0.4 μm) and stored at 4°C. As a result we got 6 clones of the ATCV-type virus.

### TESTATE AMOEBAE FROM SOILS OF FOREST-STEPPE ECOSYSTEMS

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Species composition and community structure of testate amoebae from black earth soils in forest-steppe zone was studied in "Osrovtsovskaya forest-steppe reserve" (Middle Volga region). Different biotopes representing all types of forest-steppe transition were studied: meadow-steppe, steppe-meadow, broom bush, cherry-tree bush, blackthorn bush, bird cherry tree forest and aspen forest. Thirty-two species and forms were identified. *Centropyxis sylvatica* was the most common species, which inhabited all biotopes studied. Other common species were *Cyclopyxis kahli, Trinema complanatum*, and *Centropyxis aerophila*. Members of families Centropyxidae, Cyclopyxidae, Euglyphidae, Trinematidae were predominant in the species list. The most peculiar structure of species community was observed in broom bush, where *Plagiopyxis penardi* and *Euglypha strigosa glabra* dominated, as well as in cherry-tree bush, where *Centropyxis aerophila sphagnicola* was especially abundant. In other biotopes *Centropyxis sylvatica* was a part of the dominant species complexes, as well as *Cyclopyxis kahli* in the meadow-steppe, *Centropyxis aerophila sphagnicola* in the steppe-meadow, and *Centropyxis aerophila* in the blackthorn bush, bird cherry tree and aspen forests. Thus, a few dominant species formed diversity of communities due to rearrangement of the same set of dominant species. Species richness, species diversity, and abundance of amoebae decreased along the soil profile, whereas Pielou evenness index seemed to be rather constant both between different biotopes and along the soil profile. The cherry-tree bush and bird cherry tree forest exhibited the maximal species richness. The lowest number of species was observed in the meadow-steppe, the driest habitat. The largest abundance (more than 400 ind. per gram of dry soil) was recorded in the cherry-tree bush where population of *Centropyxis aerophila sphagnicola* reached the highest abundance. Micro-spatial heterogeneity (within a single biotope) was rather low. On average, only 35% of total species richness in a biotope was affected by the beta-diversity component (i.e. differences between samples within one biotope). On the contrast, meso-spatial heterogeneity within "Osrovtsovskaya forest-steppe reserve" region was higher: approximately 65% of the regional species richness was affected by the beta-diversity component.

### HOLOSPORACEAE, RICKETTSIALES, AND MITOCHONDRIAL ORIGIN: RICKETTSIAE IMPORT MITOCHONDRIAL PORIN

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Huge numberof protists are well known to contain different endosymbiotic bacteria. From an evolutionary perspective, the most interesting among them are α-Proteobacteria in view of their apparent connection to mitochondrial origin. Phylogenetic data based on rRNAs, whose genes reside in mitochondrial genomes, and chaperonin 60 (Cpn60), encoded by a nuclear gene, indicate that mitochondria and Rickettsiaceae assemblage, i.e. true rickettsiae, share last common ancestor (LCA) to the immediate exclusion of a group of rickettsia-like endosymbionts, or family Holosporaceae. These in turn diverge after free-living α-Proteobacteria. Holosporaceae encompasses mostly endosymbiotic bacteria of protists such as *Acanthamoeba*, paramecia and acidophilic Protozoa. Rickettsiaceae (rickettsiae

and anaplasmas) and Holosporaceae are classified with the order Rickettsiales. The above data reveal a paraphyletic nature of Rickettsiales and strengthen an idea that organelle has derived from an already reduced rickettsia-like endosymbiotic bacterium whose closest extant relatives are the species of Holosporaceae. Two major evolutionary events that transformed endosymbiont into mitochondrion were the acquisitions of ATP/ADP carrier (AAC) and protein import machinery. Earlier on I suggested that both systems have already been present in the above LCA and inherited by Rickettsiaceae vertically. Phylogenetic analyses of non-mitochondrial type AAC are consistent with this view, the classic endosymbiont theory, and suggest that it may have first appeared in Holosporaceae during mitochondrial origin to provide the host with respiration-derived ATP. It was shown that the species of the genus *Rickettsia*, but not a facultative intracellular α-Proteobacterium *Bartonella henselae* import mitochondrial porin VDAC1. Cell fractionation experiments suggest that porin localizes to Bayer's adhesion zones, which may thus be a prototype of mitochondrial contact sites between inner and outer membranes which contain VDAC1 (membrane heredity). The more porin is contained in rickettsial cells, the easier they are permeabilized, suggesting a functionality of mitoporin in *Rickettsia*. It is thought that Rickettsiaceae do possess some primitive protein import machinery, yet having some components in common with sophisticated mitochondrial protein import. The data presented may also shed light on a nature of obligate rickettsial symbiosis. Supported by the European Commission under Marie Curie Incoming International Fellowship, grant MIF1-CT-2006-039819.

## INSERTIONAL MUTAGENESIS GENERATES OSMOREGULATORY MUTANTS IN *CHLAMYDOMONAS REINHARDTII*

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*Chlamydomonas* cells have developed elaborate and sensitive protection systems that enable them to rapidly signal, respond and adapt to osmotic changes. However, genes and proteins responsive to osmotic shock, especially to hypo-osmotic shock, are practically unknown in *C. reinhardtii*. Existance of organisms with mutations at the loci responsible for the specific steps of metabolism is a valuable tool of functional genomics. Heterologous integration occurring during transformation with a selectable marker in *C. reinhardtii* has been used to generate osmoregulatory mutants. A wild-type strain was transformed with the plasmid bearing the paromomycin-resistant *AphVIII* gene to generate insertional mutants defective at regulatory steps of the adaptive responses to hypo-osmotic shock. Two osmoregulatory mutants, osr1 and osr2, were isolated on the basis of the requirement for growth in medium of high osmotic strength. In normal medium of low-osmotic strength the mutant cells swell and burst. The mutants exhibited defects in the contractile vacuole cycle. Wild-type recombinant progeny were obtained from the crosses among two mutants, indicating that these two mutations implicated two different genes. Insertional mutagenesis has thus permitted the generation of novel osmoregulatory mutants that will be of value in molecular dissection of the osmoregulatory system in the unicellular organisms.

## CHEMOTAXIS TO AMMONIUM AND AMMONIUM TRANSPORT IN *CHLAMYDOMONAS*

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Ammonium is the preferred nitrogen source for most unicellular organisms, including the soil green alga *Chlamydomonas reinhardtii*. Ammonium transport is a key process in its metabolism. The current evidence strongly suggests that eight of the *CrAmt1* genes encode the high-affinity transport systems (HATS) for ammonium in *Chlamydomonas* cells. These represent the largest set of *Amt1* genes described so far in any organism. The importance of ammonium as a nitrogen source also means that many motile microorganisms have evolved an additional adaptation such as chemotaxis that allows them to move towards ammonium. *Chlamydomonas* vegetative cells are attracted to ammonium, which in the absence of nitrogen source initiate the program of sexual differentiation. A new methylammonium-resistant mutant hat1 has been isolated by insertional mutagenesis. This mutant was affected at multiple loci and, at physiological level, seems to be affected in the activity of HATS component for ammonium/methyl ammonium. The transcription of the *CrAmt1*.(1-8) genes in hat1 strain appeared to be normal except for a slight and general lower expression than in the wild type that was significant for *Amt1*.5, 6 and 8. Treatment with the potassium channel inhibitor tetraethylammonium (TEA) blocked chemotaxis to ammonium/methylammonium and [$^{14}$C]-methylammonium uptake. Our results suggest that hat1 mutant could be affected at genes encoding regulatory elements of AMT1 activity and that chemotaxis in response to ammonium/methylammonium is mediated by ammonium transporters sensitive to TEA inhibition related to one class of potassium channels - voltage-independent non-selective cation channels. Supported by the grant N05-1000008-8004 from INTAS.

## A MODEL FOR THE MORPHOGENESIS AND EVOLUTION OF THE EUGLENID PELLICLE

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The articulating protein strips of the euglenid pellicle are novel cytoskeletal elements that vary considerably in number, surface patterns and ultrastructure. While pellicle characters have been used previously to make inferences about euglenid evolutionary history, taxon sampling is poor and little is known about the development and evolution of the characters themselves. I will present comparative data that provide insights into the developmental processes involved in pellicle duplication and maturation during mitosis. These data support a model of pellicle morphogenesis and evolution that explains the observed variation in characters like strip number, strip reduction and rows of pellicle pores. For instance, examination of dividing cells of *Euglena gracilis* has shown that the posterior whorls of strip reduction in phototrophic euglenids are each composed of strips that were synthesized during different rounds of cell division. During subsequent cell divisions, the component strips of a single whorl of reduction increase in length to form the next posterior whorl of reduction. These results demonstrate that strips of the euglenid pellicle are "multigenerational" and variation in whorled patterns of reduction is the result of evolutionary modifications in the relative timing of strip development (i.e. heterochrony). Moreover, examination of the highly differentiated strip lengths in the benthic marine phototroph *Euglena obtusa* indicate that pellicle pores are formed in the most mature strips and that parent strip identity (i.e. maturity and length) plays an important role in determining the identity of nascent strips.

## LONGITUDINAL STUDY OF MICROSPORIDIA IN CATTLE ON A DAIRY FARM

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Feces from 30 pure-bred Holstein female cattle on a dairy farm in Maryland were examined consecutively at weekly, biweekly, or monthly intervals from 1 week to 24 months of age for the presence of microsporidian spores. Fecal specimens were sieved and subjected to density gradient centrifugation to remove debris and to concentrate spores. The presence of spores was determined by PCR/gene sequence analysis of the internal transcriber spacer (ITS) region of *E. bieneusi* rDNA which has extensive genetic diversity. It has been used to indicate that some genotypes have zoonotic potential whereas others possibly represent host-specific genotypes or even different species from *E. bieneusi*. In this dairy herd the cumulative prevalence of *E. bieneusi* was 100% since all 30 calves shed spores at some time during the study. Of 990 specimens collected, 234 were infected with microsporidia (23.5%). Differences in prevalence of infection appeared to be related to the age of the animals. The prevalence was lower in pre-weaned calves (less than 8 weeks of age) (33.3 %) than in post-weaned calves (3-12 months of age) (100%) and heifers (13-24 months of age) (70%). Four genotypes of *E. bieneusi* were identified. EB2 was the most prevalent genotype (100%), while EB4, EB3, and EB1 were found in 30, 10, and 0.3%, respectively. None of the infected animals had diarrhea or appeared ill. Based on previous reports genotypes EB1-4 have been found only in cattle and appear to present no danger to humans.

## CILIATES AS NATURAL HOSTS OF MANY NOVEL *RICKETTSIA*-LIKE BACTERIA

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*Rickettsia*-like organisms (RLOs) are Gram-negative prokaryotes known as obligate intracellular parasites of arthropods, annelids, mollusks and vertebrates; some species are human pathogens. Recent studies reported the presence of these organisms also in protists, although no specific researches were accomplished up to now. In this study we assess the presence of RLOs infecting some brackish-living ciliate protists, by using the "full-cycle rRNA approach" (16S rDNA characterization and use of specifically designed oligonucleotide probes for in situ detection) and TEM techniques to perform ultrastructural analysis. At present, six kinds of RLOs were identified in five different ciliates, namely *Pseudomicrothorax dubius* (Nassophorea), *Spirostomum minus* (Heterotrichea), *Euplotes octocarinatus* (Spirotrichea), *Paramecium* cfr. *multimicronucleatum* (Oligohymenophorea) and *Diophrys oligothrix* (Spirotrichea), which harbors two different symbionts. Phylogenetic analysis based on 16S rDNA sequences revealed that the symbionts of *S. minus* and *Paramecium* cfr. *multimicronucleatum* are associated to the genus *Rickettsia* (fam. Rickettsiaceae); the symbionts of *E. octocarinatus* and one of that of *D. oligothrix*, despite the host difference, form a monophyletic clade that probably represents a new genus within Rickettsiaceae; the symbiont of *P. dubius* and the other symbiont of *D. oligothrix* form distinct basal clades within Rickettsiaceae: they can also represent new genera. In some cases, ultrastructural analysis showed peculiar morphological features. These preliminary results revealed an unexpected, intriguing phylogenetic and morphological diversity among the RLOs; the frequency of occurrence

of such kind of association suggests that protists could play the role of natural reservoir for some potentially hazardous pathogens.

**EVOLUTIONARY PATHWAYS IN FRUITING AMOEBAE (MYCETOZOA)**

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The phylum Amoebozoa includes numerous aerobic amoebae that lack cytoplasmic microtubules and the clade Conosa, comprizing the free-living aerobic Mycetozoa (slime-molds) and the amitochondrial Archamoebae (e.g. *Entamoeba, Mastigamoeba*), which typically have a microtubular skeleton. Mycetozoa are common soil organisms with a life cycle having a trophic stage of amoebae or plasmodia alternating with a dispersal phase culminating in the formation of often-stalked fruiting bodies. Traditionally they include three major groups: Myxogastria, Dictyostelia, and Protostelia. We have examined the phylogenetic relationships among Conosa using sequences of 18S rRNA and protein synthesis elongation factor genes. Previously the position of *Ceratiomyxa fruticulosa*, which has a plasmodium like myxogastrids but single-spore sporangia on stalks like protostelids, has been very unclear; some taxonomists put it in Myxogastria and others in Protostelia. We find that it is clearly sister to other Myxogastria, not to any more typical protostelids. We also find strong support for a new clade, grouping Myxogastria (including *Ceratiomyxa*) with Dictyostelia. Protostelia appear to be split into two groups, and therefore might be polyphyletic; however weak bootstrap support and some differences among trees make further data essential to test further the possible monophyly of Protostelia and Mycetozoa. However the position of *Ceratiomyxa* and grouping of Myxogastria and Dictyostelia seems very robust. We will discuss the characters that appear to be most meaningful and possible evolutionary scenarios.

**THE KINETOPLAST GENOME OF *LEISHMANIA MAJOR* CONTAINS SEVERAL MAXICIRCLE CLASSES UNDERGOING DIFFERENTIAL AMPLIFICATION AT THE AMASTIGOTE STAGE**

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The mitochondrial (kinetoplast) genome of Trypanosomatidae has a unique structure. It is composed of thousands of interlocked circular molecules, maxicircles and minicircles. Minicircles (1000-10000 copies in a kinetoplast) are represented by different sequence classes coding for guide RNAs. Usually a few classes are much more abundant than the others. Minor minicircle classes may be eliminated after long maintenance of cells in culture, or, on the contrary, amplified after acquisition of drug resistance. In contrast, maxicircle population has been supposed to be homogeneous and stable. We have demonstrated that maxicircle population in clonal cultures of *Leishmania major* is composed of at least 10 major and minor sequence classes. Each class has a specific number of long repeats in the non-coding region. Nearly all investigated clones (10 of 11) have an identical set of major maxicircle classes, but some minor classes appear to be absent in some clones. *Leishmania major* is a digenetic parasite with two life cycle stages: promastigotes (with active mitochondria) and amastigotes (anaerobic). We have demonstrated that amplification of minor maxicircle classes and/or elimination of major classes always occurs during promastigote-amastigote transformation (after infection of hamsters with clonal promastigote cultures). Remarkably, patterns of maxicircle amplification and elimination differ from clone to clone. Amastigote-promastigote transformation is accompanied by complete reversion to the promastigote-specific pattern of maxicircle classes in all clones. The significance of these drastic genome rearrangements remains unknown. Our results provide a new outlook on the kinetoplast genome structure and functioning.

**DIVERSITY AND GEOGRAPHIC DISTRIBUTION OF SOIL PROTOZOA**

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Soil is inhabited by most main groups of protists, ranging from heterotrophic to autotrophic species, the latter being especially frequent in the litter layer. Two groups of soil protists have been investigated in considerable detail, viz., testate amoebae and ciliates. However, still all data refer to morphospecies because molecular investigations are difficult in the soil habitat. Data on flagellates are still rare, but one species, *Hemimastix amphikineta*, is a highly conspicuous flagship with restricted Gondwanan distribution. About 600 testate amoebae species have been recorded from terrestrial habitats globally, and rather many of them are palaeoendemics (break of Pangaea) or continental endemics, for instance, the genera *Apodera, Certesiella, Lamtopyxis* and *Matsakision*. Many of these are size flagships and

thus provide indisputable evidences for a restricted distribution of protist morphospecies. Over 1000 species of soil ciliates have been reported. We show by faunistic and statistical analyses restricted distribution patterns, especially restricted Gondwanan/Laurasian occurrence. In sum, protist biogeography is similar to that of plants and animals, but with an increased proportion of cosmopolites, favouring the moderate endemicity model proposed by Foissner. Supported by FWF grants 19699, 15017 and by the Taiwan National Science Council, projects NSC-94-2118-M006-001, 95-2118-M007-003.

**A UNIQUE ASSOCIATION BETWEEN BACTERIA AND CILIATES: SILICIOUS RESIDUES FROM FOOD BACTERIA ARE THE MAIN COMPONENT OF THE CYST WALL OF *MARYNA UMBRELLATA* (CILIOPHORA, COLPODEA)**

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*Maryna umbrellata* is an about 150 µm-sized, globular ciliate typically inhabiting ephemeral pools. It belongs to the class Colpodea, family Marynidae. *Maryna umbrellata* can quickly encyst and excyst and feeds on bacteria. The resting cyst, which has an average diameter of 105 µm, has a 10 µm thick wall. The interior half of the wall is of ordinary fine structure, while the exterior half consists of countless, minute globules with a size of 0.5 - 2 µm. Transmission electron microscopy (TEM), X-ray analysis, and treatment with hydrofluoric acid (HF) reveal the globules to be composed of amorphic silicon. The silicon globules are recognizable in the interphase specimens, where they appear as minute, strongly sparkling "crystals" when observed with interference contrast. Detailed TEM investigations showed that the silicon globules are not produced by the ciliate, but taken from certain food bacteria containing minute silicon spheres (proven by TEM, X-ray, and HF!). These spheres are agglomerated by the ciliate and extruded during the very early phase of encystment. To our best knowledge, such mechanism (slave silicon spheres) has not been described in any other protist, but likely occurs in several species of the family. Supported by the FWF, grants P-19699 and P-15017.

**SOME FACES OF RUSSIAN PROTISTOLOGY IN ST. PETERSBURG**

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The first Russian protozoological research dates back to the late XVIII century. But visible protistological line was started from the Russian follower of Ehrenberg - S.S. Kutorga (1805-1861), who compiled the "Natural History of Infusoria" (1839). Then he switched completely to geology. However, one of Kutorga's students, L.S. Cienkowsky (1822-1887) was a real protistologist. He discovered and described several dozens of protists and traced life cycles of many of them. He payed particular attention to the cyst formation in ciliates and phenomenon of symbiosis in lower organisms. His protistological ideas and works inspired a number of students and followers. Among of them A.S. Famintzin (1835-1918), plant physiologist, was always interested in protists. He probably inspired for the field his student - K.S. Merezhkovsky (1855-1921) although the latter graduated from the Zoological Department. Merezhkovsky was the one to study protists most systematically in Russia during that period: in the beginning - ciliates (1877-1886) and then - diatoms. In his own turn, Merezhkovsky started to teach W.T. Schewiakoff in St. Petersburg University (1881-1884). Later Schewiakoff (1859-1930) graduated from Heidelberg University under the supervision of O. Butschli (1889). In 1896 Schewiakoff presented in St. Petersburg the monography "Organisation and systematics of Infusoria Aspirotricha (Holotricha auctorum)", which was a brilliant conclusion of a series of monographs started by F. Stein. From this point all St. Petersburg's protistological "forces" grew up from Schewiakoff directly (S.I. Metalnikoff, S.V. Awerinzev, A.S. Schepotiev, I.K. Dembovsky) or from his best student, V.A. Dogiel (1882-1955): G.N. Gassovsky, A.A. Strelkov, G.I. Polyansky, E.M. Cheisin, and finally, L.N. Seravin and I.B. Raikov.

***HOLOSPORA* AND *HOLOSPORA*-LIKE ENDOCYTOBIONTS IN CILIOPHORA**

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The life cycle of highly infectious endocytobionts (Eb) as *Holospora* includes infective (I) and reproductive (R) stages. These Eb are widespread in several *Paramecium* spp. However, Eb with similar life cycle are also present in some natural populations of other ciliates: *Stentor, Frontonia, Metopus, Trithigmostoma, Balantidium, Prorodon, Zoothamnium, Trichodina* and *Vorticella*. Such *Holospora*-like Eb are, as a rule, represented by small R-forms (1-3 µm) with homogeneous cytoplasm, whereas the I-forms are often much larger (10-30 µm) and differentiated into the cytoplasmic, the periplasmic and the recognition tip zones. Three *Holospora*-like Eb were newly discovered in native populations of *S. minus, F. leucas* and *F. salmastra* in Italy. Those Eb populated the macronucleus of ciliates. Both Eb from *Frontonia*

spp. manifested FISH signal using *Holospora*-specific probes. The Eb from *S. minus* also belong to alpha-proteobacteria. The Eb from *F. leucas* were typical *Holospora* with dimensions: 1-2.5×0.9-1.0 μm (R) and 5-12×0.7-0.8 μm (I). Eb from *F. salmastra* had size 2-4×1.3-1.5 μm (R), but I-forms could reach 8-30×2-5 μm with peculiar spindle-shape form. The Eb of *S. minus* differed from "classical" *Holospora*: 3-6×2-3 μm (R) and 5-8×4-5 μm (I) and had some kind of extrusive device. Aposymbiotic cells can be experimentally infected by the homogenate of infected ones, and only I-formes could enter the macronucleus. Phylogenetical position of the Eb from *F. salmastr*a was confirmed by 16S rRNA gene characterization. These findings for the first time definitely recorded at least two species of *Holospora* out of the *Paramecium* genus.

## ANALYSIS OF THE β-TUBULIN GENE FROM *VITTAFORMA CORNEAE* SUGGESTS BENZIMIDAZOLE RESITANCE

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*Vittaforma corneae* is a microsporidium causing corneal and disseminated infections in humans. Benzimidazoles are widely used as antihelmintic drugs in veterinary and human medicine and as antifungal agents in agriculture, and albendazole is one of the most commonly used drugs for treating microsporidiosis in humans. However, clinical data show poor response of *Vittaforma* infections to albendazole. We amplified, cloned, and sequenced the β-tubulin gene of *V. corneae*, and the sequence showed 72% similarity with the β-tubulin of *Enterocytozoon bieneusi* and 72 to 74% similarity with β-tubulins of *Encephalitozoon* spp. Analysis of the obtained sequence provides an explanation for the observed clinical resistance of *V. corneae* to albendazole. The β-tubulin gene encoded by the sequence has a substitution at $Glu_{198}$ (with glutamine), which is one of six amino acids reported to be associated with benzimidazole sensitivity. Benzimidazoles were assayed for antimicrosporidial activity using an *in vitro* assay with *V. corneae* and *Encephalitozoon cuniculi* (as control). Microsporidian spores and drugs were added to monolayers of MRC-5 cells on day 0, and the numbers of organisms were measured on day 10. Albendazole expressed significant higher MIC50s for *V. corneae* than for *E. cuniculi*. Molecular data explain the clinicaly observed resistance of *V. corneae* to benzimidazoles, that could be shown also *in vitro*.

## COMPARATIVE MORPHOLOGY OF PELOBIONTS

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The pelobionts (Pelomyxidae + Mastigamoebidae) are a group of mostly free-living amoeboid and flagellated protists which inhabit microoxic environments. The group can be defined by reference to ultrastructural characters: a single basal body and absence of Golgi dictyosomes and cristate mitochondria. A major impediment to progress in determining the phylogenetic position of pelobionts is the fragmentary nature of structural data for the group and taxonomic tangle. In the present work the original ultrastructural data from 10 studied *Pelomyxa* species (*P. palustris, P. belevskii, P. binucleata, P. corona, P. flava, P. gruberi, P. prima, P. sigara, P.stagnalis* and *P. tertia*) and 3 mastigamoebids species (*Mastigamoeba aspera, M. setosa* and *Mastigella nitens*) are compared to data from previously examined pelobionts. The comparisons are documented and summarized as parsimony analyses. Our results show that pelobiontids extremely varied in their cellular organization. The most considerable differences concerned the basal parts of flagella, nuclear organization and cell surface of the pelobiontids. Cladistic analyses using morphological characters support the monophyly of the pelobionts but do not favour monophyly of main genera of pelobionts. On the basis of these data we now regard the genera *Pelomyxa* and *Mastigamoeba* as paraphyletic groups. The work was supported by the Russian Foundation for Basic Research (Project 05-04-48166).

## INFECTION OF *HOLOSPORA* IS CONTROLED BY 89-KDA PERIPLASMIC PROTEINS AND THE HOST ACTIN

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*Holospora* species are endonuclear bacteria symbionts of the ciliate *Paramecium* species. Infectious forms of this bacterium shows distinctive structure, one half of which contains the cytoplasm and the other half of a periplasmic lumen with an electron-translucent tip. When the infectious form is engulfed into the host digestive vacuole (DV), the bacterium escapes from the latter to appear in the host cytoplasm, migrates to the target nucleus, and penetrates the nuclear envelope with the special tip ahead, but not with the other tip. To investigate the underlying molecular mechanism of this infection process, we raised a monoclonal antibody against the special tip-specific 89-kDa protein of *H. obtusa*, sequenced this protein partially and identified the corresponding complete gene. The deduced amino acid sequence carries two actin-binding motifs near N-

terminal of the protein. Indirect immunofluorescence microscopy shows that during the escape from the host DV, the 89-kDa proteins translocates from the inside to the outside of the tip. In the host cytoplasm, bacteria keep the 89-kDa proteins outside the tip. When the bacteria invades the macronucleus, the 89-kDa proteins are left behind at the entry point of the nuclear envelope. Monoclonal antibodies specific for *P. caudatum* actin, labeled bacterial 89-kDa proteins in the host cytoplasm and penetrated the target nuclear envelope. Latrunculin B, an inhibitor of actin polymerization, inhibited the bacterial infection. These results show that the 89-kDa proteins and the host actin play a role in *Holospora*'s migration through the host cytoplasm and the invasion into the target nucleus.

## LIFE CYCLE AND PHYLOGENETIC POSITION OF NOCTILUCID DINOFLAGELLATE (*NOCTILUCA SCINTILLANS*)

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*Noctiluca scintillans* is an unarmed heterotrophic dinoflagellate that inhabits world's oceans and is sometimes responsible for harmful red tides. The life cycle, in particular the developmental process after gamete fusion, has not been fully elucidated. In addition, the phylogenetic position of *N. scintillans* has been still disputed because of two alternative views deduced from a few morphological characters and phylogenetic analyses of SSU rDNA. Here we show new details of various stages in a whole life cycle, leading to the idea that some characteristics in noctilucid gametes might reflect the ancestral state of dinoflagellates. In fact, the gametes retain not only two flagella that differ in length and motion (although the extent of differentiation is quite low), but also longitudinal and transverse grooves, as is typical of dinoflagellates, indicating that dinoflagellate-like characteristics are conserved only in the gametes, but not present in the specialized trophonts. This is also supported by our phylogenetic analyses using two protein-coding genes (beta-tubulin and hsp90), in which *N. scintillans* is one of the most ancestral dinoflagellates, i.e. the next earliest branch after *Oxyrrhis marina* within the dinoflagellates. Given the phylogenetic position of *N. scintillans*, its extremely specialised diploid trophont, and the primitive dinoflagellate-like characteristics of its haploid zoospore, we conclude that noctilucids might be a possible evolutionary link between ancestral diploid dinoflagellates and most other haploid core dinoflagellates. This implies that the transition from diploid to haploid in trophonts occurred in the ancestor of core dinoflagellates, via neoteny of a noctilucid-like zoospore.

## EPIZOOTIOLOGY OF *THELOHANIA SOLENOPSAE* (MICROSPORIDIA) IN THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*

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Epizootiology of the microsporidium *Thelohania solenopsae* was investigated in red imported fire ants, *Solenopsis invicta*. The microsporidium was detected at 16% of 165 sites and in 10% of 1309 colonies surveyed throughout Louisiana. Its distribution was clumped with 32% of nests infected in northeastern Louisiana, 18% in the southwestern corner, and 3% elsewhere. The microsporidium infected 2.3% of monogyne (single-queen) and 55.9% of polygyne (multiple-queen) colonies. The highest prevalence rates were near marshes and waterways, and the lowest were in agricultural and forested areas. Prevalence decreased as distance from commercial waterways and ports increased. Occurrence of *T. solenopsae* was positively correlated with the number of nests at a site and with pH, calcium, and sodium content of the soil. Microsporidium-infected colonies were less likely to have brood than healthy colonies. *T. solenopsae* epizootics were also monitored over time. In a natural epizootic, 89-100%-infected polygyne ants gradually disappeared, possibly because they were at a competitive disadvantage to 15-26%-infected monogyne ants. The monogyne form did not sustain the pathogen after polygyne ants disappeared. Long-term epizootics developed when the microsporidium was released in two predominantly polygyne populations but not at two monogyne sites. Prevalence peaked at >75% in both social forms; the form suffering higher prevalence decreased proportionally to the other. Prevalence averaged 47-57% and did not vary seasonally. The microsporidian rate of spread was 0.8-9.4 m/month. *T. solenopsae* in these epizootics weakened ant populations only sporadically, through decreases in numbers of foragers, colony numbers, colony size, or brood.

## PREVALENCE OF CRYPTOSPORIDIA IN THE FARM ANIMALS OF AZERBAIJAN

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Various kinds of farm animals were examined in Azerbaijan for presence of protozoan invasion by observation of thin fecal smears stained by Zeel-Neelsen technique with optical microscope. The extensivenesses of invasion (EI) with cryptosporidia amounted in cattle 20.8% (216 animals were examined), zebu - 10% (30), sheep - 24.2% (335), goats - 32.4% (127), buffalos - 20.2% (148), horses - 17.4% (23), donkeys - 5% (20). In total 899 animals were examined in the farms situated

in lowland, foothill and mountain areas of Azerbaijan. The same animals were also examined for oocysts of *Eimeria* in fecal smears and, excluding horses and donkeys, for *Cryptosporidium* cysts in skeletal muscles. The joint invasion by *Eimeria* and *Cryptosporidium* was observed in all the studied kinds of animals. The highest values of EI were noted in the goats of the lowland farms (60.8%) and sheep of the foothill ones (53.3%). In cattle and buffalos in all farms, regardless of the altitude above sea level, the EI values were quite high: from 31.8 to 46.1%. The zebus manifested rather low EI - 21.4%. The value of joint EI with tissue cysts of *Sarcocystis* and *Cryptosporidium* were 1.5-2 times higher than that with *Eimeria* and *Sarcosystis*. The *Cryptosporidium* oocysts were found for the first time in the farm animals of Azerbaijan in the 1980s. In the subsequent 10 years of investigation significant prevalence of cryptosporidia in cattle, sheep and pigs was revealed. About one fifth of all the animals examined released oocysts. Till the end of the 1990s, the trend to the reduction of EI with cryptosporidia in cattle, sheep and pigs was evident, presumably due to dismantling of large livestock farms. However, the present study demonstrated that all the studied kinds of farm animals were invaded by cryprosporidia and EI of cattle with cryptosporidia rested at the same level.

## NEW DATA ON DIVERSITY AND DISTRIBUTION OF THE CENTROHELID HELIOZOA (CENTROHELIDA) IN UKRAINE

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It is believed that the Centrohelida is a cosmopolitan group of protists. However their distribution and species composition in different regions of the world has not yet been adequately investigated. Twenty-five species of heliozoans were registered in Ukraine. Only eleven species of heliozoans recorded in Ukraine have been studied by electron microscopy (Mikrjukov, 1995, 1997, 1999); identification of other 14 species needs additional confirmation. We collected fifty-five samples in subbenthic water layers and inside clumps of aquatic macrophytes from thirty fresh-water reservoirs (shallow waters, rivers, streams, ponds, lakes and channels, etc.) in the environs of Kiev, Ukraine. Isolated heliozoans were examined by light microscopy and scanning electron microscopy (SEM) with special attention to ultrastructure of the periplast and scales. So far 6 species have been identified and studied morphologically: *Acanthocystis myriospina* Penard, 1890, *A. pectinata* Penard, 1889, *Choanocystis aculeata* (Hertwig et Lesser, 1874), *Raphidocystis* sp., *Polyplacocystis ambigua* (Penard, 1904) and P. *coerulea* (Penard, 1904). One species of *Raphidocystis* and two species of *Polyplacocystis* were new species for the fauna of Ukraine. Three species (*Acanthocystis myriospina, A. pectinata* and *Choanocystis aculeata*) previously studied only by light microscopy, were examined by SEM.

## DIVERSITY OF BACTERIAL SYMBIONTS IN *PARAMECIUM*

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In the middle of last century many bacterial symbionts in *Paramecium* have been described; much information about them was reviewed by Preer, Preer and Jurand (1974). However, since then many new symbionts were found in *Paramecium* and in other ciliates. New observations emphasize old views that interactions between killer bacteria and paramecia are extremely complex, and it seems hardly acceptable to regard all these endocytobioses as examples of mutualism. Largely based upon rDNA-sequences, the phylogenetic relationships of some symbionts have been studied. The phylogenetic position of the old kappa-particles, *Caedibacter taeniospiralis* was found to be different from that of other caedibacters showing that the genus is not monophyletic (Beier et al., 2002). As *Caedibacter* and related bacteria have been found in protozoa other than ciliates, the question about host specificity of some of the symbionts is open. A low or even zero host specificity of intracellular bacteria may point to the potential risk for animals and humans. Most bacterial symbionts known, however, appear to be host specific. As many new symbionts of different bacterial taxa have been found in ciliates recently, we have to accept that many if not most of the bacterial endocytobionts in ciliates and other protists are not yet known. The extended and more systematic search for intracellular bacteria in protists appears to be necessary.

## COMMUNITY STRUCTURE OF BENTHIC FORAMINIFERA FROM A GLACIATED FJORD OF SPITSBERGEN

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Tidewater glaciers in subpolar fjords play an important role in structuring the benthic communities. The glaciers feed icebergs and meltwater in the fjords. Sequential change of the dominant species along the fjord and decrease in the foraminifera abundance seawards was recorded previously in Tempelfjorden (Western Spitsbergen) (Korsun, Hald, 2000). In the present study we examined whether this pattern has been reproduced over years and whether the community structure has changed following the surge of the glacier in 2004. Sampling was performed on the transect of 9

stations along the fjord in July or August 2001-2005. Surface sediment samples were retrieved by RV Jan Mayen using a 50x50-cm box-corer. Samples were preserved in ethanol with Rose Bengal stain. Living foraminifera were identified to the species level where possible, and counted. The species richness, the Shannon-Weaver diversity index, and the total density of foraminifera increased seawards. Cluster analysis based on the absolute abundances revealed two groups of stations corresponding to the innermost and the outer parts of the fjord. At the inner-fjord stations *Elphidium excavatum* f. *clavata, Quinqueloculina stalkeri*, *Cassidulina reniforme* dominated. However the density of *E. excavatum* f. *clavata, Q. stalkeri*, as well as most other taxa increased seawards. At the outer-fjord stations *Nonionellina labradorica, Labrospira crassimargo* and *Recurvoides* spp. dominated. Community structure was stable during the whole period of observations, and no significant effect of the glacier surging in 2004 could be detected from our data.

## GENES OF THE MEVALONATE PATHWAY IN *GIARDIA INTESTINALIS*: HOW MANY DO EXIST, WHICH ARE TRANSCRIBED AND HOW THEIR EXPRESSION CHANGES DURING ENCYSTATION?

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The protist *Giardia intestinalis* is a human parasite which causes diarrheal disease throughout the world. It is frequently described as an ancient protist, member of the primitive group Diplomonads. Thus, it becomes an interesting model to search for the origin of eukaryotic pathways. In the present study we looked for the genes that encode the enzymes of the mevalonate pathway in *G. intestinalis*, especially those specific for biosynthesis of cholesterol, ubiquinone, dolichol and prenylated proteins. Firstly, we made a bioinformatic search into the parasite's genome using consensus sequences. We found one gene of the dolichol biosynthesis, and four - of the prenylated proteins biosynthesis. Their existence in the parasite was proved by the polymerase chain reaction (PCR), and their transcription was evaluated by reverse transcription PCR (RT-PCR). Finally, we studied the expression profile of the genes during encystation, a process in which a trophozoite becomes a cyst, with real time RT-PCR. We found differences in the transcripts quantity during the different stages of encystation. In conclusion, the genes coding the enzymes of the dolichol and prenylated proteins biosyntheses, as well as their transcripts were found in *G. intestinalis*, suggesting that these products which participate in posttranslational modifications are necessary in one of the most ancient eukaryotes. Moreover, the differences in the quantity of the transcripts show that they are regulated transcriptionally during encystation, suggesting that they play an important role in this differentiation process.

## MICROSPORIDIAN PARASITES OF ROTIFERS FROM THE VOLGA DELTA

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The study of rotifer infestation in water bodies of the Volga delta was based on light microscopy analysis of quantitative zooplankton samples collected during the years 1986-2006. In addition, qualitative samples were also taken and living material was examined. The most frequently found microsporidian parasites of rotifers were *Microsporidium asperospora* (Fritsch, 1895). Mass infestation by *M. asperospora* was most common in *B. calyciflorus*. Severely infested rotifers were completely filled by these sausage-shaped parasites. When the infested rotifers died, small round spores escaped from the body cavity of the rotifers through the head region into the surrounding water. Single cases of infestation of *Brachionus urceus, B. diversicornis, B. quadridentatus, Polyarthra longiremis, P. dolichoptera, Epiphanes brachionus, Conochilus unicornis, Conochiloides coenobasis, Sinantherina semibullata* and some other rotifer species with the parasites similar to *M. asperospora*, were recorded. Another parasite, *Microsporidium polygona* (Fritsch, 1895), was recorded in *A. priodonta, A. brightwelli* and *S. semibullata*. Cysts similar to *Bertramia beuchampi*, as described by Stempell (1921), were found in *Trochosphaera solstitialis*. *Polyarthra luminosa* was infected with cysts resembling the ones of metchnikovellids, possibly, of the genus *Amphiacantha*. Some other protozoan parasites were observed in rotifers from the Volga delta, but those have not been identified. This investigation showed that many species of rotifers inhabiting the Volga delta are subject to infestation with microsporidia. For most rotifer species only single cases of infection were recorded through 20 years of observations. However, infestation of some abundant species was observed every year.

## PHOSPHOLIPIDS OF THE MICROAEROPHILIC PROTOZOA FLAGELLATE, *TRICHOMONAS VAGINALIS*

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Lipid composition of the sexually transmitted flagellated protist, *Trichomonas vaginalis*, showed the following (%

total) distribution of polar lipids: phosphatidyl-ethanolamine (30%), phosphatidylcholine (9%), phosphatidylglycerol (7%), phosphatidylserine (7%), phoshpatidic acid (3%), dimethyl-phosphatidylethanolamine (2%). Two unusual constituents were acylphosphatidylglycerol (26%) and ceramide phosphorylethanolamine (7%). The two unusual lipids were subjected to both single-stage electrospray ionization mass spectrometry (ESI-MS) and tandem ESI-MS for structural analysis. For acylphosphatidylglycerol, the peak at m/z 1013 gave fragments at m/z 281, 283, and 255 representing stearic, oleic and palmitic acids, respectively. A fragment ion at 749 corresponded to 16:0/18:1 phosphatidylglycerol, and those at 757 and 729 indicated neutral losses of palmitic stearic acids respectively. For ceramide phosphoryl-ethanolamine (m/z 661), palmitic acid was the amide-linked fatty acid of the phosphoceramide (m/z 617), whereas stearic acid and ethanolamine were the other fragments detected. The structure finally assigned corresponded to N-(hexadecanoyl)-sphing-4-enine-1-phosphoethanolamine. Cardiolipin was not detected on thin layer chromatography (TLC) plates, nor by ESI-MS of total lipid or of eluates from TLC plates, although an authentic standard gave the expected negative ion (M-H)- at m/z 1470. These data are discussed in relation to the unusual lifestyle of the organism and its phylogenetic status.

## HEMOPARASITES OF FISH AND AMPHIBIA IN THE GANYHCHAY RIVER (NORTHWESTERN AZERBAIJAN)

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The objective of our investigation was the study of the hemoparasites of fish and amphibia in the Ganyhchay river (Alazan) that originates in the mountains of the Greater Caucasus, flows through the territory of Georgia, and enters Mingechavir reservoir, the latter being the dwelling place of valuable gamefish. Material for the study was collected in the upper reach of the river, at the border with Georgia, and in the lower one, 5 kilometers from the point where it enters Mingechavir reservoir. Forty six fish specimens (*Varicorhinus capoeta, Barbus lacerta cyri, Chalcalburunus chalcoides, Abramis brama orientalis, Cyprinus carpio, Lucioperca lucioperca, Silurus glanis)* and 14 specimens of *Rana ridibunda* were examined for hemoparasite invasion. Blood for examination was taken by routine methods developed for fish and amphibia. All the examined fish specimen were found to be free of hemoparasites. In the blood of 12 amphibian specimens *Trypanosoma loricanum, T. neveulemairei, T. mega* (Mastigophora: Trypanosomidae) and also some trypanosomes that were not identified to the species level were found. The extensiveness of the amphibian invasion with the hemoparasites was 85.7% and intensity - 10-38 hemoparasites per a blood smear. Apart from that, coccidians (Apicomplexa, Coccidia) of Haemogregarinidae and Lankesterellidae families were found in the amphibian blood samples. Representatives of the genus *Lankesterella* were found within the Caucasus's territory for the first time. The multicellular parasites, the larvae of the tissue nematodes - microfillaria (Nematoda: Filariidae) were found as well. Absence of hemoparasites in fish from the Ganyhchay river may be due to the complex of abiotic- and biotic factors, characteristic for the mountain rivers, such as lack of aquatic vegetation and speedy flow which blocks development and reproduction of the leeches *Piscicola geometra*, the main vectors of the fish hemoparasites. The amphibia had abundant hemoparasite fauna, presumably, because of the semi-aquatic lifestyle facilitating contacts with the hemoparasite vectors: leeches and blood-sucker insects (mosquitoes, gnats etc.).

## CIMES: CILIATES AS MONITORS FOR LATERAL GENE TRANSFER

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We had postulated that ciliates are ideal monitors to detect lateral gene transfer (LGT) from genetically modified organisms to ciliates thriving in the gastro-intestinal tract of ruminants or cockroaches. Unique nuclear dimorphism and the intriguing structure of the macronuclear genomes of ciliates should allow excluding any artefacts due to contamination by traces of DNA from GMO. This could be substantiated. *Nyctotherus ovalis* and several rumen ciliates have been used to study lateral gene transfer from bacterial and plant sources both for short term (3 years) challenges with transgenic Bt 176 maize and LGT in evolutionary time. While the short term experiments did not reveal any LGT from Bt 176 maize, we identified substantial amounts of LGT in evolutionary time. The bioinformatic analysis of more than 4000 cDNAs from rumen ciliates and more than 4000 minichromosomes and 5000 cDNAs from *N. ovalis* revealed 148 bacterial to ciliate transfers in the rumen ciliates, and about one order of magnitude less in *N. ovalis*. Also, genes obtained by LGT differed depending on the ciliate species. It will be discussed, whether different macronuclear genome architecture, i.e. midichromosomes *vs.* minichromosomes, or dissimilarities in lifestyles could be responsible for these differences. In addition, we will discuss the significance of the frequent potential plant-ciliate transfers.

## *TRICHOMONAS VAGINALIS* AND *GIARDIA INTESTINALIS* PRODUCE NITRIC OXIDE: DOES NITROSYLATION INDUCE CARBOHYDRATE METABOLISM?

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The arginine-dependent production of nitric oxide (NO) in *Trichomonas vaginalis* and *Giardia intestinalis* was studied. Potentiometric measurement of whole cell suspensions of *T. vaginalis*, monitored by a NO electrode, indicated that the basal production rate of NO (15.3 nmol $min^{-1}/10^6$ organisms) increased eight-fold after 4 h starvation. *G. intestinalis* monitored by NO electrode produced NO, which was stimulated by the addition of arginine. When the granular fraction of *G. intestinalis*, which contained marker enzyme activity similar to that found in hydrogenosomal fractions of T. *vaginalis*, was monitored, NO was produced at a rate of 415.6 nmoles/mg protein/min. Fluorimetric detection of both organisms by confocal microscopy, after pre-incubation with the NO-specific fluorogen 4-amino-5-methylamino-2'7'-difluorescein, indicated population heterogeneity of NO production in freshly harvested organisms. Brightly fluorescent *T. vaginalis* (25%) showed fluorescent reaction product uniformly throughout the cytosol, whereas less intensely fluorescent organisms (70%) showed organellar localization. *G. intestinalis* showed localization of NO production in the periphery of the cytosol; organisms stained with 5μM tetramethylrhodamine ethyl ester showed localization in circular organelles with membrane potential along the periphery of the organism, suggesting NO production by these membrane potential generating organelles. NO synthase activity measured in cell-free extracts by NO electrode and a colorimetric assay indicated the presence of both particulate and non-sedimentable activities in *T. vaginalis,* and localized NOS activity to the granular fraction in *G. intestinalis*. The apparent $K_M$ for arginine for the non-sedimentable NOS in *T. vaginalis* was $2.5 \times 10^{-4}$ moles/L, and for *G. intestinalis* $8.28 \times 10^{-3}$ moles/L, respectively. We conclude that the granular fraction of *G. intestinalis* contains nitric oxide synthase which may be localized in redox-balancing organelles. Bioinformatic searches confirmed the presence of two potential NOS genes in *T. vaginalis* and one in *G. intestinalis*, which contain protein motifs typically associated with NOS sequences. Bioinformatic searches also located nitrosylation sites in specific enzymes of bioenergetic pathways. Implications of NO production in the evolution, biology and pathogenicity of these parasites are discussed.

## TOTAL INTRACELLULAR BACTERIUM SPECTRA FROM DIFFERENT CLONAL CULTURES OF *ARCELLA* SPP. COMPARED BY DGGE

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Several species of intracellular bacteria which are considered as endocytobionts of free-living amoebae have already been reported in earlier studies; however, our understanding of the total composition of bacterial species within one host organism is very poor. Furthermore, a comparison of total bacterial symbiont spectra among different host clones may also provide new significant data for symbiosis studies. In this experiment we examined the total bacterial spectra of different species of *Arcella*, a lobose testate amoeba. The host cells were fed with pure *Enterobacter aerogenes* cultures in order to reduce the contamination by external bacteria and were washed in sterile water before molecular analyses. The 16S rRNA gene-based molecular technique was applied to reveal the complete bacterial species composition and variability of *Arcella* cells. Molecular cloning was carried out to identify most of the colonizing bacterial species, and denaturing gradient gel electrophoresis (DGGE) was used to compare the symbiotic spectra of different host cells. We examined both different cultures kept up under laboratory conditions for a longer period, and newly established clonal cultures from several environmental samples.

## TESTATE AMOEBAE (PROTISTA) COMMUNITIES IN *CALLUNA VULGARIS* LITTER DIFFER WITH INCREASING ALTITUDE

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Species richness of higher organisms decreases generally with increasing altitude and latitude, but it remains unclear whether this pattern also holds true for microorganisms. If this is the case, does the observed decrease result from the effect of altitude or rather from declining diversity of habitats? To disentangle the effects of altitude and habitat diversity, we only considered communities of a single habitat occurring along a large altitudinal gradient. We studied testate amoebae communities in *Calluna vulgaris* litter along the vertical transect crossing the forest- and timber-lines from 1800 to 2400 m in the Swiss Alps. The study area is characterized by siliceous bedrock and by a timberline located at about 2200 m. The pH of each sample was also measured. A total of 31 testate amoebae taxa belonging

to 14 genera were identified. The number of taxa observed per sample of 100 individuals varied from 12 to 21. The dominant species *Corythion dubium, Assulina muscorum* and *Trinema lineare* were present in all samples. The sum of their relative abundance was always higher than 35%. Species richness varied only slightly across the samples, but nevertheless it decreased significantly with the altitude. Although this trend was not strong, it supported the hypothesis about positive correlation between the testate amoeba diversity and temperature. In a canonical correspondence analysis, the variation in the relative abundance of the testate amoeba taxa was explained, firstly, by altitude, and, secondly, by the litter pH. Presence or absence of forest was not significantly correlated with the composition of the testate amoebae communities; the altitudinal trend is therefore gradual and does not reflect a threshold effect.

**PHYLOGENY AND BIODIVERSITY OF HETEROMITID CERCOZOA: ABUNDANT, DIVERSE, AND UBIQUITOUS ZOOFLAGELLATES REVEALED**

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Heteromitids form a clade of diverse and abundant heterotrophic flagellates that includes *Heteromita, Bodomorpha, Allantion, Proleptomonas*, and many unnamed and previously unplaced taxa. They occupy freshwater and soil habitats globally. However, intensive screening of ecologically and globally diverse DNA samples using group-specific primers suggests that heteromitids are not found in marine environments. We created 18S rRNA gene libraries from individual (c. 1 gram) soil samples from a sampling site near Oxford. All samples tested contained heteromitid sequences. The number of genetically distinct lineages (18S-types) in each soil sample ranged from 12 to 40, and species richness estimators suggest that some such samples may harbour more than 50 18S-types. Our sampling of multiple 18S rDNA libraries from the UK, Australia, South America, and Europe, reveals many novel heteromitid groups and suggests that total global heteromitid diversity exceeds 500 species. Community comparisons suggest that heteromitids are more homogeneously distributed on a global scale than Clade A cercomonads. Here we present a new and highly sampled heteromitid phylogeny of over 200 (mostly novel) sequences, and describe many new strains. We show that the morphological diversity of heteromitids is far greater than previously realized, and demonstrate that robust species definitions must include a description of the mode of movement and behaviour of strains over time, as well as an 18S rDNA sequence signature unique to each strain.

**MULTIPLE LOSSES OF SEX WITHIN A SINGLE GENUS OF MICROSPORIDIA**

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Most asexual eukaryotic lineages have arisen recently from sexual ancestors and contain few ecologically distinct species, providing evidence for long-term advantages of sex. Ancient asexual lineages provide rare exceptions to this rule and so can yield valuable information relating to the evolutionary forces that underlie the maintenance of sex. Among the Microsporidia there are many asexual species which have traditionally been grouped together into large, presumably ancient taxonomic groups. However, these putative ancient asexual lineages have been identified on the basis of morphology, life cycles and small subunit ribosomal RNA (16S rRNA) gene sequences, all of which are of questionable value in accurately inferring phylogenetic relationships among Microsporidia. The hypothesis of a single, ancient loss of sex within the *Nosema/Vairimorpha* group of microsporidia was tested using phylogenetic analyses based on alignments of rRNA and RPB1 gene sequences from sexual and asexual species. Neither set of gene trees supported ancient asexuality, instead indicating at least two, recent losses of sex within the *Nosema/Vairimorpha* group of microsporidia. The absence of ancient asexual lineages indicates that sex confers important long-term advantages even upon highly simplified eukaryotes such as microsporidia. The rapid evolution of microsporidian life cycles indicated by this study also suggests that even closely related microsporidia cannot be assumed to have similar life cycles and the life cycle of each newly discovered species must therefore be completely described. These findings are relevant to the use of microsporidia as biological control agents, since several species under consideration as potential agents have life cycles that are incompletely described.

**PARASITIC SYSTEMS OF MICROSPORIDIA**

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Co-evolution of microsporidia and invertebrates resulted in formation of two types of parasitic systems, which we refer to as "tight" (stable) and "loose" (unstable). Microsporidia, infecting hosts with high abundance and population density like aquatic forms of dipterans, develop "tight parasitic systems". Stability of these systems is sustained by alteration of horizontal and vertical transmission of the parasites, both finely tuned to particular characters of the microsporidium and its insect host, i.e., narrow specificity of the parasite, type

of the insect activities at the imaginal and larval stages, different susceptibility/resistance to infection expressed by males and females, etc. Prevalence of infection in such systems seldom exceeds 10%, and the number of members of the system is strictly limited. "Loose parasitic systems" emerged through interactions of microsporidia with the hosts with scanty natural populations and low densities. Wide spectrum of systematically or ecologically related hosts, and diverse routes of horizontal and vertical transmission usually maintain stability of these systems. Number of the members of such parasitic system may vary. Solar activity rhythms or human activity impact causing outbreaks and increasing host population densities, favor development of pathogenic forms of microsporidia and emergence of epizooties with 100% infection prevalence. Epizooties of microsporidiosis may occupy vast territories, including isolated spots. Such epizooties usually lead to elimination of the majority of the host population together with the most highly pathogenic microsporidia isolates, destabilizing thus the parasitic system. However, eventually the system recovers due to existence of low pathogenic forms of microsporidia. Because pest outbreaks are considered as a result of anthropogenic pressure onto nature, expression of wide epizooties of microsporidiosis may also be regarded as a consequence of human impact.

## THE LIGHT MICROSCOPIC EVIDENCES OF SEXUAL PROCESS IN LOBOSE AND FILOSE TESTACEAN AMOEBAE

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Life cycle stages of different terrestrian testacean amoebae were investigated with light microscope in clonal cultures and samples. Both, the lobose and filose testaceans have the same main stages: asexual part includes trophozoite undergoing binary fission, precyst, and resting cyst; sexual - trophozoite copulation (hologamy), zygocyst, spore, and trophozoite. The most complete life cycle has been shown for *Corythion delamarei*: trophozoites contact by pseudostomes; cytoplasms of both partners fuse; the nucleus and cytoplasm of one cell move into the partner's test; the following nuclear fusion gives a synkaryon; a zygote produces a thick wall and becomes a zygocyst inside the test. At the next stage, zygocyst covering disappears, and the synkaryon undergoes 2 meiotic divisions. This results in 4 separate haploid cells, which incyst inside the mother shell and typically produce 4 spores. Sometimes the zygote produces more than 4 spores: the tests with 5, 6, 8 and even with 15 spores (as a result of asynchronous divisions in some species) were found. Spores come out from the test and release small amoeboid cells - young trophozoites forming the tests. The formation of a new test during asexual binary fission was also demonstrated. Separate stages including copulation and spore formation were observed in the following species: *C. orbicularis, C. dubium, Trinema lineare, T. complanatum, T. enchelys, Euglypha strigosa, E. cristata, E. ciliata, Nebela bohemica, N. tincta, Assulina seminilum, A. muscorum, Heleopera sylvatica, Arcella vulgaris, Tracheleuglypha dentata*. The EM studies of nuclear behaviour are in the schedule.

## NEWLY DISCOVERED SYMBIOTIC ASSOCIATION BETWEEN AMOEBA AND FUNGUS

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A large number of fungi are known to parasitize on different protists: ciliates and there cysts, flagellates (especially algae), and amoebae. Here we provide the data on organization of newly discovered symbiotic association between a large naked lobose amoeba and a fungus. In outward appearance the association looks like an amoeba with a tuft of fungi hyphae protruding from its surface. During amoeba locomotion, the hypha tuft is located on the amoeba uroid. The tuft includes several separate (up to 15) filamentous talli, each consisting of single frequently branched hypha 2.6 ?m wide and up to 300 ?m long. Each thallus forms the lobed haustorium within the amoeba cell (in special invagination of amoeba plasmalemma), which also functions as a holdfast. In association with the amoeba, the fungus produces zygospores with ornamented thickened walls that arise from zygogamy, consisting in conjugation of equal parts of talli. After zygospores' maturation, the whole tuft of fungi hyphae is detached from the amoeba cell, leading either to amoeba's death or to its further free-of-fungus endurance. The fate of the detached hypha tuft is unknown. Asexual reproduction most likely proceeds by means of detachment of short parts of hypha from the tallus. Life cycle and morphological data suggest that the described fungus belongs to a new genus within the family Cochlonematacea (Zygomycota). At least four species of amoebae of the family Amoebidae (Lobosea, Gymnamoebia) form association with fungi. Three of them appeared to be new species of the genera *Amoeba, Trichamoeba* and *Polychaos*, and another one is likely to be *Amoeba proteus*.

## MACRONUCLEAR INTRA S-PHASE CHECK POINT IN *TETRAHYMENA THERMOPHILA* INDUCED WITH LOW DOSES OF APHIDICOLIN, ROLE OF "PSEUDO SPINDLE" IN MACRONUCLEAR DNA SEGREGATION AND APOPTOTIC-LIKE DEGRADATION OF "EXTRUSION BODIES"

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Low doses of aphidicolin induced cell division arrest in *T. thermophila*. Cells advanced in oral morphogenesis completed one cell division in the presence of the drug, while the other did not progress in cortical morphogenesis. They increased macronuclear DNA content (3-4×), size (>2 × cell surface) and number of ciliary basal bodies during aphidicolin treatment. These data suggest an intra S-phase check point mechanism in *Tetrahymena* due to successive rounds of abnormal DNA replication in presence of the drug. *In silico* of *Tetrahymena* genome many genes coding for PI-3 kinases were found, but we were not able to specify direct homologs of genes coding for mammalian ATM and ATR proteins. The putative homolog of mammalian CHFR protein, involved in $G_2$/M transition, was identified in *Tetrahymena*. Cells shifted to the drug-free medium, resumed their divisions. Then a "central chromatin granule" appeared in the middle of dividing, elongating "giant" macronucleus. This chromatin was first surrounded by microtubule bands involved in the macronuclear division ("pseudo spindle") and next disposed as an extrusion body (EB), containing up to 1/3 of the previous macronuclear content. It suggests an active role of intra macronuclear "pseudo spindle" both in segregation of the "normal" mini chromosomes into daughter macronuclei and segregation of the defective chromatin into EBs. EBs underwent an "apoptotic-like "degradation (as old macronuclei in conjugants): they consisted of highly condensed chromatin, were stained with the TUNEL method and showed DNA fragmentation in gel electrophoresis. EBs were also stained in vivo with acridine orange, which is a marker of autophagy.

## ON THE IMMUNOLOGICAL MECHANISMS OF *TRYPANOSOMA CRUZI* ANTI-TUMORAL PHENOMENON

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The recent studies suggest that inhibitory influence of *T. cruzi* on cancer growth has got a dual mechanism. The following observations show that direct selective effect of live *T. cruzi* (and its preparations) on the cancer cells *in vivo* is supplemented by indirect influence on the whole cancer process by means of immunological mechanism: (1) organisms which survived the *T. cruzi* infection, were significantly protected against the transplanted tumor; (2) intentional immunization of mice with avirulent live trypanosomes resulted in reliable inhibition of sarcoma-180 and Ehrlich-adenocarcinoma growth; (3) preparation of lysed *T. cruzi* epimastigotes was immunogenous, it possessed immunomodulatory activity, and immunization with this preparation created a definite degree of protection against tumor implantations. In all these experiments the antitumoral effect coincided with appearance of mice' antibodies to *T. cruzi*, and it positively correlated with the level of considered antibodies. The antitumoral effect is based on the known antigens community of *T. cruzi* and the host. This antigens community of *T. cruzi* and cancer cells was shown by indirect immunofluorescence. Thus, the participation of humoral immunity in *T. cruzi* antitumoral phenomenon is evident. Its role is rather significant. In vivo effect of *T. cruzi* preparation is accompanied by substantial pool of following antibodies against *T. cruzi*: (a) spontaneous ones, discovered in the intact mice; (b) the ones induced by tumor transplantation; (c) antibodies induced by *T. cruzi* itself, as a result of injection with *T. cruzi* preparation. Occurrence of these antibodies is correlated with inhibition of cancer growth. The results presented are likely to make possible the onco-prophylactic use of *T. cruzi* extraordinary properties.

## REGULATION OF AMINO ACID TRANSPORT BY CALCIUM ION AND SUGARS IN *PARAMECIUM* SYMBIONT

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The green ciliate, *Paramecium bursaria*, has several hundred chlorellas. Symbiont F36-ZK isolated from Japanese *P. bursaria* F36 showed notable characteristics on nitrogen utilization, i.e., the alga lacked nitrate reductase activity and had three constitutive amino acid transport systems. Interestingly, the algal Ser uptake was inhibited by polyvalent cations, especially $Ca^{2+}$, although the cation generally activates amino acid transport in many organisms. The inhibition was

noncompetitive, since $Ca^{2+}$ affected only the $V_{max}$ of Ser transport. Effect of sugar on amino acid was also evaluated, because symbionts were known to release sugar. Uptake of Ser was increased by glucose even at low concentration ($EC_{50}$=3 μM). Non-metabolizable glucose analogues also accelerated Ser uptake, indicating that sugar did not supply energy for transport. The $V_{max}$ of Ser transport was doubled by treatment with glucose, however the response was sustained even when protein synthesis of cells was inhibited, thus, the phenomenon was not due to new protein synthesis. Surprisingly, uptake of radiolabeled glucose was not detectable, therefore, it was considered that the acceleration by glucose occurred via glucose sensing and signaling pathway. The response to glucose-related compounds was measured, and the results revealed an importance of stereochemistry at carbon 1, 2 and 5, and hydroxyl group on carbon 3 and 6 for the response. Glucose recovered the inhibition of Ser uptake by $Ca^{2+}$, thus, amino acid flux is easily controlled by these compounds, implying a possibility of regulatory system in *Paramecium* symbiosis.

## *OPHRYOGLENA* SP., A PARASITE OF *DREISSENA POLYMORPHA* (PALLAS) IN POLAND, AND REMARKS ON ITS POSSIBLE REPRODUCTION IN THE HOST

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The interest in parasites of *Dreissena polymorpha* (Pallas) expanded when it became known that this invasive mollusc has successfully spread within Europe and North America. The first ciliates included in the genus *Ophryoglena* were found by the senior author in the lakes Lichenskie and Goslawskie near Konin (Poland) on November 9, 1970. Over the next 5 years, more than 2200 zebra mussels from that region were investigated, and many of them were infected with *Ophryoglena*. During that time many slide preparations from tissue smears were stained and silver-impregnated, and morphology of more than 600 specimens of *Ophryoglena* sp. was investigated in details. The results of these investigations will be published in a joint paper with Dr. V.I. Yuryshynets. In the present communication, the authors want to draw attention to a group of small *Ophryoglena* ciliates found in 15 specimens (about 2.5% of silver impregnated specimens examined). These ciliates differ by the form of the body and the pattern of the argentophilic system at the posterior end of the cell. The *Ophryoglena* sp. ciliates normally have a regular course of kineties in this region, but in these specimens, the kineties were curved and formed "a scar", which may have arisen at the place of splitting the cell during palintomy, suggesting reproduction of ciliates inside the host body. Occurrence of large and wide specimens, which can be considered as protomonts, in the examined population, confirms this suggestion. Presence of one small, pear-shaped specimen, with regular pattern of kineties on its posterior end, presumably a theront, supports the hypothesis that reproduction of the ciliates parasitizing the inner organs of their hosts may occur both in the external environment and inside the host organism.

## COMPARATIVE GENOMICS OF MICROSPORIDIA

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Genomic compaction has occurred many times in evolution, but we generally do not know why. Nuclear genomes are typically quite spacious, but in several lineages compaction has occurred, the most spectacular being microsporidian (intracelluar parasites) and nucleomorphs (endosymbionts). We have used a comparative approach to investigate the distribution of compaction in microsporidia, and variations in its effects on genome function. The best studied microsporidian genomes (*Encephalitozoon* and *Antonospora*) are all relatively small and fit the expectations for a compacted genome. We have carried out genome sequence surveys (GSS) on species with larger genomes, and these paint a very different picture. In *Brachiola algerae* and *Edhazardia aedis* gene density is four to five-fold lower than observed in *Encephalitozoon* and *Antonospora*, and transposons abundant. In both surveys, all genes identified are also found in *Encephalitozoon*, suggesting the three species contain similar proteomes despite genome size differences. One of the interesting effects of compaction described in *Antonospora* is the high frequency of overlapping transcription. We have now determined that *Encephalitozoon* transcripts overlap at a similar frequency, but in different ways. *Antonospora* transcription tends to initiate in the upstream intergenic region and terminate beyond the downstream intergenic region, but *Encephalitozoon* transcriptions more often initiate within the upstream gene and terminate within the downstream intergenic region, suggesting compaction affected the two genomes differently. We also show there is little conservation of transcript types between *Antonospora* and *Encephalitozoon*, suggesting the process is very fluid over time, and that transcription overlap has not stabilized the genome.

## NOVEL COMPARTMENTALIZATION OF PYRUVATE: NADP+ OXIDOREDUCTASE IN SPOROZOITES OF *CRYPTOSPORIDIUM PARVUM*

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Using pyruvate dehydrogenase, most eukaryotes oxidatively decarboxylate pyruvate within mitochondria. Anaerobic parasitic protists use instead the $O_2$-sensitive enzyme pyruvate:ferredoxin oxidoreductase (PFO), which is localized within the cytosol, hydrogenosomes, or mitosomes. In contrast, both *Cryptosporidium parvum* and *Euglena gracilis* encode and express a unique $O_2$-sensitive fusion enzyme, pyruvate: $NADP^+$ oxidoreductase (PNO), the N-terminal PFO domain of which is fused to a C-terminal NADPH-cytochrome P450 reductase. Unlike *E. gracilis*, the PNO of *C. parvum* lacks a mitochondrial targeting peptide. *C. parvum* does possess a small mitosome sandwiched between the nucleus and crystalloid body (CB). Transmission electron microscopy (TEM) and tomographic reconstructions reveal a complex arrangement of membranes outside and within this organelle. The inner mitosomal membrane lacks tubular "crista junctions" typical of metazoan, fungal, and protist mitochondria. This is congruent with the loss through reductive evolution of the entire mitosomal genome and the capacity for oxidative phosphorylation. Nevertheless, the *C. parvum* mitosome shares with other anaerobic protists the machinery to assemble [FeS] clusters, an essential function of eukaryotic mitochondria. Western blot analysis shows that sporozoites of *C. parvum* express the entire fusion protein, and both immunofluorescent and immunogold electron microscopy confirm that *Cp*PNO is primarily cytosolic. Unexpectedly, however, *Cp*PNO was also distributed within the crystalloid body, an enigmatic organelle whose function is unknown. Because *Cp*PNO is compartmentalized in a novel way, there is an intriguing possibility that *C. parvum* has an unusual type of energy metabolism that it might be exploited for drug development against human cryptosporidiosis.

## MORPHOLOGY, MORPHOGENESIS AND PHYLOGENETIC ANALYSIS OF MARINE CILIATE *FAVELLA EHRENBERGII* JORGENSEN, 1924 (CILIOPHORA: CHOREOTRICHIA)

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*F. ehrenbergii* was collected from Incheon coastal water, Korea and cultured in the laboratory. Although this species is widely distributed in marine water, its infraciliature is still unknown. The taxonomical description of this species has been based on lorica shape. However, several studies have been reported the variation of lorica shape within the *Favella*. In this laboratory culturing study, the morphology and morphogenesis of *F. ehrenbergii* were observed using the protargol staining method, and small subunit rDNA sequences were also analyzed. Our morphological data showed 16-18 of oral kinetids and almost 100 of somatic kineties. Compared with other species, oral kinetids and somatic kineties of *F. ehrenbergii* certainly differed from those of *F. panamensis* (22 of oral kinetids and 74 of somatic kineties) and *F. novaeangliae* (24 of oral kinetids and 35 of somatic kineties).

## CYST MORPHOLOGY AND ENCYSTMENT OF THE PLANKTONIC OLIGOTRICH CILIATE *STROMBIDIUM CAPITATUM*

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Cysts of an oligotrich ciliate were isolated from natural sediment samples collected in Onagawa Bay of Japan and in Masan Bay of Korea, and incubated under laboratory culture conditions. Excysted vegetative cells were observed after protargol staining and were identified as *Strombidium capitatum*. Cysts of *S. capitatum* have a spherical shape, with a papula and cyst wall ornamented with spines. Size varied from 60-62 μm in total length and 50-55 μm in width. Seasonal changes in the vegetative population and sedimentation of newly formed cysts were investigated *in situ*. Planktonic vegetative cells were abundant during the cold season from December to April, when the water temperature was lower than 15°C. Mass encystment occurred in March, when the vegetative population flourished. Living cyst abundance in the sediments increased in January and April and empty cysts largely increased from October to March. These results indicate that *S. capitatum* is well adapted to cold water and aestivates during the warm period in summer.

## FOOD SELECTION AND FEEDING STRATEGIES OF PROTOZOA IN PLANKTON OF RIVER DANUBE

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Food consumption and feeding strategies of protozoa were investigated in plankton of River Danube during one year. Feeding behaviour and food vacuole contents were studied in living and preserved samples. Two hundred protozoan species - heterotrophic flagellates, naked amoebae, heliozoans and ciliates - were included into study. Half of species were mainly bacterivorous, one quarter - algivorous, and smaller proportions - flagellativorous, mixotrophic and omnivorous. Annual

distribution of protozoan biomasses constituting these feeding categories showed different patterns. Bacterivores and algivores comprised 80% of total biomass, 20% were mainly flagellativory. Algivory was most important in growing seasons, while in winter bacterivory and flagellativory dominated. The most common feeding strategy among heterotrophic flagellates was suspension feeding. Greater part of the flagellates was free swimming, smaller - sedentary. One third of them were substrate associated raptors, 15% - free swimming raptors, and 10% - osmotrophic. Regarding the annual distribution of flagellate biomasses, suspension feeders dominated, with high peaks of free swimming raptors in growing seasons. Substrate associated raptors were sparse. Among ciliates half of species were raptors and another half - suspension feeders, few were diffusion feeders. Half of suspension feeders were free swimming, and half - sedentary, with few loricated free swimming tintinnids. Regarding to ciliate biomasses, suspension feeders were dominant, with growing season peaks of raptors, and small summer peak of diffusion feeders. Many feeding niches can be distinguished according to food type, food size and feeding strategies of protozoans, but overlapping is obvious and resource utilisation may be low some times during seasonal succession.

**PROTOZOAN COMMUNITY IN PLANKTON OF RIVER DANUBE: TEMPORAL DYNAMICS, TROPHIC INTERACTIONS AND ROLE IN MICROBIAL FOOD WEB**

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The planktonic protozoan community of River Danube (Hungary, 1669 river km) involving all major groups, was investigated during one year. We examined temporal dynamics, trophic interactions between protozoa and their food organisms, and structure of the microbial food web. Protozoan groups with the largest biomass were heterotrophic flagellates, ciliates and naked amoebae. We determined the type and size of consumed algae for many protozoan species using *in situ* food vacuole content analysis. Examination of the coupling of temporal dynamics of investigated groups may reveal indirect trophic relations between many protozoa and their food organisms. We found no coupling between bacteria and heterotrophic nanoflagellates, edible algae and *Paraphysomonas vestita*, but significant coupling was detected between edible algae and *Collodictyon triciliatum*, many edible algae and algivor ciliate groups, and between prey ciliates and predatory ciliates. We revealed the structure of microbial food web and the quantity of intercompartmental carbon flow summarising the species specific trophic relations. Growth rates were assumed from literature. In most parts of the year the microbial loop proved to be dominant, nanoflagellates were the most productive protozoan group. At high phytoplankton abundances the classical food web based on phytoplankton was likewise important, sometimes channelling larger fluxes than the microbial loop. Most important herbivores were ciliates and large heterotrophic flagellates. The main proportion of primary production was usually not utilized directly in the microbial food web, although at high algivor protozoan biomasses the half of the primary production might have been consumed.

**DIFFERENT MODES OF STOP CODON RESTRICTION BY THE *STYLONYCHIA* AND *PARAMECIUM* ERF1 TRANSLATION TERMINATION FACTORS**

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In universal-code eukaryotes, a single translation termination factor eRF1 decodes three stop codons: UAA, UAG and UGA. In some ciliates, like *Stylonychia* and *Paramecium*, eRF1s exhibit UGA-only decoding specificity, while UAG and UAA are reassigned as sense codons. Since variant-code ciliates may have evolved from (a) universal-code ancestor(s), structural features should exist in ciliate eRF1s that restrict their stop codon recognition. In omnipotent eRF1s, stop codon recognition is associated with the amino terminal domain of the protein. Using both *in vitro* and *in vivo* assays we showed that chimeric molecules composed of the N-terminal domain of *Stylonychia* eRF1 fused to the core domain (MC domain) of human eRF1, retained specificity towards UGA; this unambiguously associates eRF1 stop codon specificity to the nature of its N-terminal domain. Functional analysis of eRF1 chimeras constructed by swapping the ciliate N-terminal domain sequences with the matching ones from the human protein, highlighted the crucial role of the tripeptide QFM in restricting *Stylonychia* eRF1 specificity towards UGA. Using the site-directed mutagenesis, we showed that *Paramecium* eRF1 specificity towards UGA resides within the NIKS (amino acids 61-64) and YxCxxxF (amino acids 124-131) motifs. Thus, we established that eRF1 from two different ciliates relies on different molecular mechanisms to achieve specificity towards the UGA stop codon. This suggests that eRF1 restriction of specificity to only UGA might have been an early event occurring in independent instances in ciliate evolutionary history, possibly facilitating the reassignment of UAG and UAA to sense codons.

**PHOSPHATIDYL-INOSITOL SPECIFIC PHOSPHOLIPASES OF *PARAMECIUM TETRAURELIA* INVOLVED IN ANTIGENIC VARIATION**

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Surface antigens of several protist species are Glycosyl-Phosphatidyl-(GPI)-anchored and Phosphatidyl-Inositol specific Phospholipases (PI-PLCs) are strongly assumed to be involved in antigen release in the medium, especially during antigenic switching. We report here the analysis of six different PI-PLCs present in *Paramecium tetraurelia*. Transcriptional analysis shows that all six genes, including two paralogs, are up-regulated during antigenic switching. Additionally to the typical X-, Y- and C2-domains of previously described PI-PLCs of other organisms, two PI-PLCs exhibit calcium binding motifs. Interestingly, these two PI-PLCs were those which are shown to be not involved in antigenic variation, as silencing of these genes did not alter antigenic switching. On the contrary, silencing of the other PI-PLCs was characterized by a decelerated antigen shift: the old antigen was present much longer on the cell surface, compared to control cells. Moreover, our data suggests that PI-PLCs in *Paramecium* are not involved in the normal antigen turnover in stable serotypes. Therefore, their role in cutting GPI anchors seems to be a special mode during antigenic switching. Apart from the discrimination in function of PI-PLCs we have shown that not a single PI-PLC is responsible for GPI-cleavage during salt-alcohol extraction of surface proteins, and therefore that all PI-PLCs in general are able to cut GPI-anchors. With this PI-PLC variety, *Paramecium* seems to become the organism of choice to study PI-PLC activity, its influence on GPI-anchored proteins and role in the signal transduction pathway.

**SIGNALLING PATHWAYS AND ACTIN DYNAMICS IN *AMOEBA PROTEUS***

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The results of our recent studies indicated that Rho family-based regulation played a key role in motility of free-living *Amoeba proteus*. Blocking Rac- or Rho-related signalling pathways with C3 transferase, antibodies against human RhoA and Rac1, and Y-27632 (a specific inhibitor of Rho-associated kinase) caused distinct and irreversible changes in amoebae morphology and significant inhibition of their migration. We revealed that protein(s) from the amoeba cytosolic fraction facilitated the nucleation step of actin polymerisation, and blocking Rac-like protein abolished this effect. The Rho-related signalling pathway exerted its effect on migration and endocytosis by myosin inactivation and probably by inhibition of cofilin-like protein. We also observed that Arp2/3-like protein complex played a role in actin filaments branching in different areas of migrating amoeba (cortical layer, perinuclear cytoskeleton, adhesion structures), but not in the fronts of advancing pseudopodia and the uroid. This might suggest that in *A. proteus* Arp2/3-dependent actin polymerisation was not engaged in the frontal membrane proggression. Cofilin-like protein was involved in actin depolymerisation within the middle-anterior region of the cell but not in the processes of the cortical network disorganisation occurring in the uroid. In migrating amoebae, the course of changes in filamentous (F)/total (T)-actin ratio corresponded to the distribution of the tension in the cell cortex, as the areas of maximal isometric tension were located in the middle-posterior region behind the adhesion area, and in the distal part of the uroid and retracting pseudopodia. These results aided in elucidation of mechanisms of actin cytoskeleton dynamics in *A. proteus.*

**TIMING OF DIFFERENTIATION OF PERIALGAL VACUOLE MEMBRANE FROM DIGESTIVE VACUOLE MEMBRANE OF THE CILIATE *PARAMECIUM BURSARIA* DURING INFECTION WITH SYMBIOTIC ALGAE *CHLORELLA VULGARIS***

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Each symbiotic *Chlorella* cell of the ciliate *Paramecium bursaria* is enclosed in a perialgal vacuole derived from the host digestive vacuole to be protected from lysosomal fusion. To know timing of differentiation of the perialgal vacuole from the host digestive vacuole, algae-free *P. bursaria* cells were fed with the symbiotic *C. vulgaris* cells for 1.5 min, washed, chased, fixed at various times after mixing, and an acid phosphatase activity in the vacuoles enclosing the algae was detected by Gomori's staining. This activity appeared in 3-min old vacuoles, and all vacuoles with algae showed activity in 30 min. Algal escape from the digestive vacuoles began at 30 min by budding off the digestive vacuole membrane into the cytoplasm. In the budded membrane, the algal cell was surrounded by a Gomori's staining-positive thin layer. The vacuoles with a single algal cell soon moved to and attached at just behind the host cell surface. Such vacuoles were Gomori's staining-negative. These results indicate that the perialgal vacuole mem-

brane differentiates soon after the algal escape from the host digestive vacuole, and differentiation completes prior to the algal localization just beneath the host cell surface. This is the first report to show the timing of differentiation of the perialgal vacuole membrane during infection of *P. bursaria* cell with the algal cell.

## SQUARE NUMBER OF FOOD VACUOLES AS A NUTRITION INTENSITY INDEX IN PERITRICHS (CILIOPHORA, PERITRICHIA) OF THE ACTIVATED SLUDGE IN THE SEWAGE TREATMENT PLANT

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The square number of food vacuoles is a sensitive index of the nourishment intensity of peritrichia-sedimentators. We studied the possibility of the square number of food vacuoles as the index of nutrition intensity in five species of peritrichs (*Vorticella striata* Dujardin, 1841; *Epistylis plicatilis* Ehrenberg, 1831; *E. bimarginata* Nenninger, 1948; *Opercularia phryganeae* Kahl, 1935. *V. convallaria* (Linnaeus, 1758)) and modified this method with taking into consideration the peculiarities of Peritrichia biology and the conditions of activated sludge tank in the sewage treatment plant. The square number of food vacuoles depends on the temperature determined with the help of the one-way ANOVA. For all species the authentic connection between the temperature and the square number of food vacuoles was fixed. The square number of food vacuoles is maximal under optimal conditions of the hydrochemical parameters of the activated sludge in the sewage treatment plant. The usage of the square number of food vacuoles permits to check up the effectiveness of sewage cleaning, to react effectively on the technological disbalance in the process of cleaning, and to determine the optimal technological regimes during the exploitation of the sewage treatment plant.

## NEW LIFE FOR OLD COLLECTIONS OF CILIATES

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Ciliates collected from the intestine and caecum of *Equus hemionus kulan* in 1987, from Yakut horse in 2001, and from faces of *Elephas maximus* in 2003, all fixed in 4% neutral formalin, were postfixed in osmium tetroxide and studied in transmission electron microscope (TEM) 2006 - 2007. Observation of thin sections of some species of Buetschliidae, Cycloposthiidae and Ditoxidae revealed good preservation of cell structures. Fine morfpology of the concretion-vacuoles, kinetids, skeletal plates, hydrogenosomes and other organelles was thoroughly examined. Twenty year old samples of buetschliids were washed with water and prepared for osmicating with or without prefixation in 2.5% buffered glutaraldehyde. In both cases, the results of TEM were equally satisfactory. All other formalin samples of ciliates were postosmicated directly after washing in water.

## *LEPTOMONAS JACULUM* (LEGER 1902) WOODCOCK 1914: A *LEPTOMONAS* OR A *BLASTOCRITHIDIA*?

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The genus *Leptomonas* Kent, 1880 contains homoxenous trypanosomatids with promastigotes as the main morphotype in their life cycle. Systematics of the genus needs to be revised, since its type species (*L. butschlii*) is seemingly not a trypanosomatid, and molecular phylogenetic studies showed the genus to be polyphyletic. One of the first steps of this work should be the choice of the type species in the substituting taxon. Since the most of leptomonads parasitize insects, *Leptomonas jaculum*, as a first species described from this host group, could be considered as a name-bearing type. Meanwhile this species has not been used so far in molecular phylogenetic studies due to failure to cultivate it. We determined sequences of the 18S rRNA gene of *L. jaculum*, amplified directly from the infected bugs, and revealed the phylogenetic proximity of this species to blastocrithidias. The cells of this species resemble promastigote, nevertheless they possess a cryptic undulating membrane (multiple desmosomes form an extensive contact between the flagellum and the cell body inside the flagellar pocket), making them similar to epimastigotes, which have such contact extended to the outer cell surface. Moreover, presence of the cyst-like amastigotes in the life cycle, and absolute absence of the cytostome also bring *L. jaculum* and blastocrithidias together. *L. jaculum* should obviously be removed from the polyphyletic group named so far the genus *Leptomonas*. However, the displacement of this species into the genus *Blastocrithidia* Laird, 1959 is premature until the inclusion of its type species (*B. gerridis*) in the phylogenetic analysis.

## COMMUNITY OF PLANKTON CILIATES OF THE AZOV SEA

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Ciliates are one of the most widespread and numerous groups, though very poorly known. Nevertheless, they are one of the most perspective groups as bioindicators. Data on the ciliates community of the Azov Sea have

been fragmentary so far. Some vast regions were not investigated at all. Large-scale, regular research of the Azov Sea ciliate plankton was held for the first time during the period of 1999-2006. In total, 91 ciliate taxa were identified during this period in the water body, including 72 taxa being reported from the Azov Sea for the first time. Reliable data on distribution of the quantitative characteristics of the ciliate plankton were obtained. The structure of the community of plankton ciliates, and ecological peculiarities of the most abundant species were examined for the first time. Dynamics of the ciliate community structure and quantitative characteristics of the ciliate plankton under the influence of different biotic and abiotic factors have been studied.

**THE SPHAGNUM PONDS OF SIMMELRIED IN GERMANY: A BIODIVERSITY HOT-SPOT FOR MICROSCOPIC ORGANISMS**

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This study describes 656 species of bacteria, protists, and micrometazoa occurring in the Simmelried, a three hectare-sized moorland in southern Germany. Each species is shown by an average of two colour micrographs. Further, the surface organization of most main groups is demonstrated by scanning electron micrographs. The Simmelried formed after the last ice-age, that is, about 15,000 years ago. The investigations indicate that the 656 species documented represent only two thirds of the taxa actually present. Thus, a considerable diversity was accumulated over 15,000 years, emphasizing the great distribution capacity of microorganisms. On the other hand, some common species are lacking (e.g., the ciliate *Colpidium colpoda*, the euglenid *Phacus pleuronectes*, and rotifers of the genera *Proales* and *Floscularia*), and many undescribed species were discovered. While a mass of undescribed species is comprehensible in the poorly studied amoebas, flagellates and ciliates, this is surprising in well-known groups, such as euglenids and chrysophytes. Thus, some of the undescribed species might be regional or local endemics. The book has been published by Shaker, Aachen and is available in printed and electronic form: http://www.shaker.de/Online-Gesamtkatalog/Details. asp? ID=0&ISBN=3-8322-2544-7&Reihe=0.

**SURVEY FOR MICROSPORIDIOSIS IN HIV/AIDS PATIENTS IN ST. PETERSBURG, RUSSIA: SEROLOGICAL IDENTIFICATION**

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Microsporidia, world-widely distributed opportunistic parasites described predominantly from immunocompromised patients, have been in the focus of attention of physicians and researchers since the onset of HIV/AIDS pandemics in the 1970s. Nothing is known so far about occurrence of microsporidiosis in Russia. Recently a project funded by CRDF, has been initiated to survey prevalence of microsporidiosis and cryptosporidiosis among HIV/AIDS patients in Russia. As a part of this survey we evaluated sera from 28 HIV/AIDS patients of City Municipal Hospital St. Petersburg, Russia suffering from lasting diarrhea, for reactivity to major microsporidian species infecting humans, i.e. *Encephalitozoon cuniculi, E. intestinalis, E. hellem and Enterocytozoon bieneusi* by enzyme-linked immunosorbent assay (ELISA) and by indirect immunofluorescence assay test (IFAT). We found one stool sample positive for *E. bieneusi* detected by *E. bieneusi*-monoclonal antibodies (mabs) and by rabbit *E. bieneusi* polyclonal sera. Three sera samples were positive for *E. cuniculi* in ELISA, and three - for *E. bieneusi* and *E. hellem*, both by ElISA and IFAT. Our study is in accord with the results of PCR identification of *E. bienusi* by specific primers. Testing of the samples by other available methods, including PCR identification with other specific and universal primers and staining techniques, as well as examination of new samples is underway. Supported by grant No. RUB2-002707-SP-05 from the U.S. Civilian Research and Development Foundation.

**A HIGHER SPECIES DIVERSITY IN THE FAMILY MICROCHLAMYIIDAE OGDEN, 1985 (AMOEBOZOA, TESTACEALOBOSIA): TWO NEW SPECIES OF THE GENUS *SPUMOCHLAMYS* KUDRYAVTSEV AND HAUSMANN, 2007**

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Two species of testate amoebae isolated from the epiphytic moss have flexible plate-shaped tests. Dorsal parts of these tests have spongious structure. They are thicker in the center and become thinner at the margin to the end in a delicate membrane forming a flexible aperture. The cell body attaches directly to the dorsal part of the test. Amoebae move by gliding on a flattened hyaline sheet, expanding from the aperture. When the cells are suspended in the medium and turned upside down, they twist their test margins towards the ventral surface, and produce several long lobopodia that turn the tests back to their natural orientation by dragging them to the substratum. The species differ from each other in the test size and structure of its dorsal part. With the ultrastructural data showing the test structure and

relations between the test and cell body, both amoebae can be unambiguously identified as new species of the genus *Spumochlamys* (family Microchlamyiidae), so far comprising only one species *S. iliensis*. By contrast, when the largest of the two species was observed only by light microscopy, it ideally matched the existing descriptions of another microchlamyiid amoeba, *Microchlamys patella*, however, the structure of the dorsal part of the test wall resembled *Microchlamys sylvatica* Golemansky et al., 1987. The presented results confirm previous suggestion that the family Microchlamyiidae contains more species, which can be reliably distinguished only by combination of light- and electron microscopy. Supported by the RFBR grant 06-04-49387.

**LOCOMOTION AND F-ACTIN DISTRIBUTION IN FLABELLINEAN AMOEBAE OF COCHLIOPODIIDAE, *FLAMELLA* AND *PELLITA* (AMOEBOZOA)**

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We performed a comparative study of locomotion in four species of *Cochliopodium*, Cochliopodiidae n. gen., *Flamella* n. sp. and *Pellita digitata*, and revealed the distribution of F-actin in the moving cells by TRITC-phalloidin staining. Although Cochliopodiidae and *Flamella* show a very similar pattern of movement accompanied by an expansion of a broad peripheral hyaloplasmic sheet, while the rest of the cell is dragged forward, the spatial organization of their actin cytoskeleton is different. They both have a fine actin network in the leading hyaloplasm, but in addition cochliopodiids possess pronounced bundles of actin, oriented transversally or obliquely to the direction of movement and resembling stress fibers of motile metazoan cells. *Pellita digitata* shows a different pattern of movement and actin distribution. It forms a smooth anterior hyaloplasm and a posterior spherical granuloplasm. The ventral surface of hyaloplasm produces short fine subpseudopodia; they penetrate a thick cell coat of this amoeba and adhere to the substratum. In contrast to Cochliopodiidae and *Flamella*, the cell surface of *P. digitata* demonstrates a typical rolling movement. These amoebae have a thin actin cortex in the granuloplasm, from which numerous actin bundles originate. They are directed forward into the hyaloplasm and end in the ventral subpseudopodia. The revealed differences in the distribution of F-actin confirm an earlier idea of a high diversity in the patterns of movement among Amoebozoa and indicate a possible correlation between the spatial structure of the cytoskeleton and phylogenetic relations within Amoebozoa. Supported by DAAD fellowship A/05/00103 and RFBR grant 06-04-49387.

**AN UNUSUAL COMBINATION OF LIGHT- AND ELECTRON-MICROSCOPICAL FEATURES IN "*THECAMOEBA*" *VERRUCOSA* (EHRENBERG, 1838) (AMOEBOZOA)**

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*Thecamoeba verrucosa* (Ehrenberg, 1838) is often mentioned, when the genus *Thecamoeba* is discussed in the literature, but its ultrastructure is unknown and this species does not exist in culture. We isolated and studied an unusual amoeboid protist that has an elongated triangular shape during locomotion, with the flattened anterior part, a tapering uroid raised over the substratum and irregular dorsal longitudinal wrinkles. A single nucleus contains a central coarsely granular endosome and an additional smaller homogeneous body located near the endosome. An ultrastructural study shows that this amoeba has a prominent microtubular cytoskeleton with irregular bundles of microtubules in the cytoplasm. Microtubules are most numerous near the nucleus and converge towards a dumbbell-shaped MTOC located near the invagination of the nuclear membrane. The central part of the nucleus is occupied by the coarsely granular electron-dense body surrounded by a less dense layer of homogeneous material, sometimes forming a smooth spherical body. Amoebae have mitochondria with tubular cristae, numerous dictyosomes and a typical plasma membrane without any visible glycocalyx. All light-microscopical features of this amoeba perfectly correspond to the earlier descriptions of *Th. verrucosa* and allow its inclusion in Thecamoebidae; by contrast, its ultrastructure shows that its assignment to the family Thecamoebidae is not correct. The revealed combination of light-microscopical and ultrastructural features does not allow a certain inclusion of this species in any of the amoebozoan taxa; gene sequence data are necessary to clarify the taxonomic position of this amoeba. Supported by DAAD fellowship A/05/00103 and RFBR grant 06-04-49387.

**SALINITY TOLERANCE OF LOBOSE AMOEBAE AND CILIATES**

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Salinity tolerance zone of 13 lobose amoebae and 16 ciliate species were estimated. Potential salinity tolerance zone was also evaluated using stepwise acclimation method. It was either identical to the zone of salinity tolerance, or certain differences were observed. For two amoebae and two ciliate species, several isolates were investigated. Several ecological groups of species differing by their relation to salinity were distinguished in amoebae and ciliates. The first group comprises the absolutely freshwater species, which have shown growth only in the range from freshwater medium to 2.5 ppt. The second group consists of species, which grow in the range from freshwater medium to 6-8 ppt. The third group is made up of the euryhaline species, they have shown successful growth in the range from freshwater medium to at least 35 ppt, which is the average ocean salinity. The fourth group consists of the strictly marine species, which cannot exist under salinity less than 5-10ppt. It is interesting that in the case of two amoebae and one ciliate species studied, different isolates had their own zone of salinity tolerance. Therefore, this feature is not a species attribute and may be determined by the conditions in the local habitats. Combination of these data with those on occurrence of species in different habitats leads to conclusion that freshwater and marine faunas of amoebae and ciliates can partially interfere. It can be supposed that an evolutionary trend within protists' taxa is occupation of new types of habitats with different salinitiy.

## A SYNOPSIS OF THE BLACK SEA FAUNA OF PLANKTONIC CILIATES

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Since 1871, more than 500 species of ciliates were discovered in the Black Sea. Basic attention was given to studying of benthic ciliates, ectocommensals and parasites. Using plankton nets ciliates were studied only as a component of zooplankton. As a result, the species lists of pelagic ciliates in most of the Black Sea areas are incomplete and usually limited to tintinnid species. In Georgia only 9 taxons have been found, for Turkey - 12, Bulgaria - 23, Romania - 14, and Russia - 27. Special taxonomic studies nearshore and in the open sea were few. The most complete studies of species composition have been made along Ukrainian coasts: 184 taxons (201 if limans and lagoons are included). Overall, 205 taxons of ciliates (including common species) were recorded in the Black Sea plankton. Analysis of species composition shows, that this list is still far from being complete. Here are some possible ways of its augmentation: special faunal studies in the above mentioned areas; due to new benthic and fouling species, which may compose from 5 up to 60% of the total number of species in the sample; due to alien species, with regard to increasing level of "mediterranization" of the Black Sea fauna and transmission via ballast waters; due to description of new species. Such information will allow to add data on biodiversity and to conduct comparative analysis for ecological zoning of the Black Sea area.

## INTRONS IN 18S RNA GENE AS A TAXONOMIC CHARACTER OF *CHLORELLA* ENDOSYMBIONTS OF *PARAMECIUM BURSARIA*

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Since 1996 (X International Congress of Virology) we discuss the problem of taxonomic difference of two (southern and northern) ecotypes of *Chlorella* sp., the endosymbionts of *P. bursaria*. Southern ecotype is characterized by *ts* phenotype (sensitive to 32°C), which differs from the northern one by isozyme spectra of 8 enzymes, contrasting serological patterns, and different types of viruses (NC64A& Pbi-types of *Chlorovirus*, Phycodnaviruses). Genomic dactyloscopy by UPPCR patterns also allows dividing zoochlorellae strains into the northern and the southern ecotype, corresponding, probably, to two different species. In our last publications (and in papers of Hoshina et al., 2004, 2005) it was shown that all strains of northern and southern ecotypes could be distinguished from free-living *Chlorella vulgaris* by occurrence of introns in the anterior (5'-) region of the 18S RNA gene; the southern zoochlorellae had two additional introns in the central and posterior (3'-) regions of the gene. By sequences of 18S rDNA exon zoochlorellae from *P. bursaria* (northern and southern ecotypes) are closely related to *Ch. vulgaris, Ch. sorokiniana* and *Ch. lobophora*. According to "Intron early" hypothesis, such difference can arise very early, and therefore we suggest that two groups of *P. bursaria* endosymbionts belong to different species. A small number of cultivable strains of these groups are the only fact that prevents us from the final decision. By new methods (see theses by Vorobyev et al.) we hope to get information about more northern and southern populations of *P. bursaria* endosymbionts, which will allow us to solve the problem.

## RE-EVALUATION OF DORSAL SILVER LINE SYSTEM IN CHARACTERIZATION OF *EUPLOTES NOVEMCARINATA* (WANG, 1930) (CILIOPHORA: HYPOTRICHIDA)

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A population of *Euplotes novemcarinata* was isolated from the debris of sesame plants collected at Andong, Korea. Its morphology of each developmental stage was studied by observing a live, protargol and silver-nitrate impregnated specimens. It was discovered that the dorsal silver line system varied according to the developmental state of dorsal ridges of this species as follows. When the dorsal ridges were protruded extremely from the dorsal surface, more than four rows of small polygons were observed to make the highly irregular complex type of the silver line system. In contrast, when the dorsal ridges were protruded subtlety from the dorsal surface, 3~4 rows of the small polygons were found to make a multiple type of the silver line system. These facts demonstrate that *Euplotes muscicola* is a potential synonym of *E. novemcarianta*, and it is suggested that dargyrome patterns that characterize *Euplotes* species has to be re-evaluated especially in the case of the species having the distinct dorsal ridges.

## POTENTIAL RISK OF CONTAMINATION OF HOSPITAL ENVIRONMENT BY *CRYPTOSPORIDIUM* OOCYSTS FROM PATIENTS WITH ACTIVE CRYPTOSPORIDIOSIS

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Background: *Cryptosporidium* causes prolonged and severe diarrhea in immunocompromised patients, with no effective specific treatment available. As *Cryptosporidium* oocysts in stools are directly infective, there is a potential risk of person to person transmission and occurrence of nosocomial cryptosporidiosis in the hospital setting. Aim: To study the potential sites of contamination by *Cryptosporidium* oocysts in the close hospital environment of patients presenting active *Cryptosporidium* infections. Methods: In the rooms of 7 patients with cryptosporidiosis, surface samples were collected using previously humidified swabs. Additionally, swabbing of patients' anus and fingernails was performed. A nested PCR assay amplifying a fragment of *Cryptosporidium* spp. 18S rRNA gene was then performed, followed by molecular typing by restriction fragment length polymorphism and/or sequence analysis. Results: *Cryptosporidium* DNA was evidenced in anus swabs from all 7 patients. For each patient, the same species was obtained in anus swab and in stools (*C. hominis* in 4 patients, *C. parvum* in 2, *C. felis* in 1). *Cryptosporidium* DNA was evidenced in one of the 42 swabs sampled in patients' rooms; this swab was sampled from the toilet button in a patient's bathroom. Genotyping identified *C. parvum*, as for the patient who was occupying this room. Conclusion: This study showed that *Cryptosporidium* DNA can occasionally be demonstrated on surfaces touched by *Cryptosporidium*-infected patients, suggesting a possible spread of oocysts in patient's environment. This underlines the need for a strict application of hygiene measures for patients with active cryptosporidiosis, and possibly isolation of these patients to prevent nosocomial transmission of cryptosporidiosis.

## LIVING IN THE DEAD ZONE: MICROZOOPLANKTON COMMUNITIES DURING SEASONAL HYPOXIA IN LAKE ERIE AND THE GULF OF MEXICO

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The northern Gulf of Mexico (Louisiana shelf) and the central basin of Lake Erie experience seasonal hypoxia, covering broad regions in summer and impacting their living resources. However, little is known about its effects on microzooplankton, a key component of the pelagic food web in both systems. This study examined the composition, distribution, and dynamics of ciliates and dinoflagellates and other planktonic microbes (bacteria, heterotrophic nanoplankton, phytoplankton, and rotifers) using microscopy, single-cell and community 18SrDNA analyses, shipboard incubations, and real-time flow-cytometry. During the early to mid-stratification, epilimnetic microzooplankton was dominated by oligotrich ciliates and removed ca. 80% of daily primary production and 30% of phytoplankton biomass in Lake Erie. The hypolimnion was occupied by the remnants of the spring community (e.g. *Gymnodinium helveticum* and *Histiobalantium bodamicum*). The dinoflagellate *Ceratium herundinella* and rotifers underwent diel vertical migrations extending to the hypolimnion (dissolved oxygen, DO, 2-3 mg/l). During the late stratification in September 2005, microzooplankton biomass in the hypolimnion (DO < 1 mg/1) was formed by choreotrich ciliates, which were grazing on nano-sized cryptophyte flagellates. Examination of plankton distribution and composition along several meridional transects and a 220 nm cross-shelf transect in the Gulf of Mexico in August 2006 revealed significant spatial heterogeneity. Heterotrophic micro-

bes and diatoms peaked in a mid-shelf warm front and the Mississippi River plume, respectively. Their diel vertical patterns were linked to the fluctuating DO concentrations in the bottom layer, which were apparently influenced by the currents. The hypoxic waters were dominated by choreotrich ciliates and gymnodiniid dinoflagellates.

## A NEW CILIATE SPECIES OF THE GENUS *AMPHISIELLA* (CILIOPHORA, HYPOTRICHA) FROM ESTUARY IN KOREA

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The *Amphisiella* ciliates collected from the estuarine littoral water and weeds (salinity 2‰, location 35° 32'43"N, 129° 20'18"E) in Korea. The description was based on the observation of living specimens, protargol impregnated specimens and morphometric analysis. The present species is most similar to *A. annulata* (Kahl, 1928) sensu Berger (2004) but differs from it as follows. Body size 125-175 × 25-40 μm *in vivo*, elongated, elliptical. Two ellipsoidal macronuclear nodules. On average three spherical or ellipsoidal micronuclei. 43 adoral membranelles on average. Amphisiellid median cirral row extended from right frontal cirrus to near transverse cirri, slightly to distinctly sigmoidally, consisting of 30 cirri on average. Six transverse cirri J-shaped, with two pretransverse cirri. 30 left and 28 right marginal cirri. Five types of colorless granules: (1) usually 8-14 ring-shaped or doughnut-shaped granules in cytoplasm, colorless and gray, about 5-7 μm in diameter, (2) 14-16 small (ca. 1-1.5 μm in diameter) colorless or vitreous cortical granules were arranged as a longitudinal band of 2-oblique-rows on dorsal surface, 5-7 dorsal kineties running along these longitudinal bands, (3) small (ca. 0.4 μm in diameter) colorless or vitreous cortical granules regularly arranged in longitudinal rows between dorsal kineties, (4) small (ca. 0.5 μm in diameter) colorless granules irregularly arranged in dorsal and ventral surfaces, (5) spindle-shaped or ellipsoidal granules (3-4 μm long and 1.5-2 μm wide) colorless granules densely arranged beneath the ventral surface.

## FREE-LIVING HETEROTROPHIC FLAGELLATES FROM DEEP-SEA SEDIMENTS OF GIPPSLAND BASIN, SOUTH-EASTERN AUSTRALIA

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In order to contribute to understanding of geographic distribution of free-living marine heterotrophic flagellates, the diversity of heterotrophic flagellates occurring in a number of deep-sea sites in Gippsland Basin (Australia), was investigated. This community of organisms from marine bottom sediments has not previously been studied in Australia. 82 species are described with uninterpreted records based on light-microscopy of living cells in natural communities. The records include two new species: *Sphenomonas altus* nov. spec. and *Platychilomonas planus* nov. spec. Of the 82 species, 11 species are new to Australian marine sites, but the majority of the species encountered here also were found at other locations worldwide, including Australian freshwater sites. The new records for Australian marine sites are: *Acanthocorbis unguiculata, Cercomonas* sp.2, *Gweamonas unicus, Helkesimastix faecicola, Notosolenus* sp.1, *Petalomonas* sp.2, *Petalomonas* sp.3, *Platychilomonas planus, Salpingoeca amphoridium, Spironema multiciliatum, Sphenomonas altus*. The relative lack of novelty provides little support for the existence of endemic biota among this group of organisms which has been shown also for freshwater species.

## DISTRIBUTION OF HETEROTROPHIC PROTISTS AND FACTORS CONTROLLING THEIR DISTRIBUTION IN MASAN BAY, KOREA

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In order to understand the distribution of heterotrophic protists and factor controlling their distribution in Masan Bay, which is heavily polluted, this study was conducted during Feb. 2004 - Nov. 2006. During the study, abundance of heterotrophic bacteria and bacterial production averaged at $2.14 \times 10^6$ cells/ml and 84 mgC/m$^2$, respectively. Abundances of cyanobacteria and photosynthetic nanoflagellates averaged at $6.17 \times 10^3$ cells/ml and $3.18 \times 10^3$ cells/ml. Protists consisted of heterotrophic nanoflagellates (HNF), heterotrophic dinoflagellates (HDNF) and ciliates, and their abundances averaged $1.22 \times 10^3$ cells/ml, $1.20 \times 10^4$ cells/l and $0.13 \times 10^4$ cells/l, respectively. Generally, the chl-*a* concentration and the abundances of heterotrophic bacteria, photosynthetic nanoflagellates and protists were higher in the inner zone of the bay with high concentration of organic matters, than in the middle and outer zones. The temporal patterns of heterotrophic bacteria, cyanobacteria and photosynthetic nanoflagellates showed seasonality (i.e., in summer high density and in winter low density). Unlike those microbes, protists did not show seasonality. Using the grazing rates of heterotrophic nanoflagellates on bacteria previously reported for this area, it was calculated that about 69% of bacterial production was removed by HNF grazing activity. Also about 24% of initial chlorophyll-*a* concentration was removed by microzooplankton

grazing activity. In conclusion, this study suggests that in Masan Bay heterotrophic protists control the growth of bacteria and phytoplankton, and heterotrophic protists represent an important link between bacterial & microalgal biomass and higher trophic levels.

**COMMUNITY STRUCTURE AND DYNAMICS OF CILIATED PROTOZOA ALONG SALINITY GRADIENTS AT SALTPANS OF THE YELLOW SEA**

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The community structure and dynamics of ciliates along salinity gradients ranging from 28 psu to 311 psu at eight saltpans of the Yellow Sea, were investigated in April, June, August and September 2001. A total of 98 species of ciliates were identified using live observation and protargol staining techniques. The highest number of species (19) was found from the sample collected in April with the salinity up to 50 psu. With the salinity increasing beyond 265 psu, only one species, viz., *Fabrea salina*, could be observed. The ciliate abundance varied from 44 to 210,929 inds. $l^{-1}$ and the maximum value was mainly contributed by *Strombidium styliferum* in August with the salinity of 36 psu. The biomass ranged between 2.39 and 9,866.63 μg C $l^{-1}$, and the maximum occurred in June due to the dominance of *Fabrea salina* when the salinity reached 148 psu. Both abundance and biomass decreased to the minimum with the salinity increasing to 311 psu in June. Statistical analyses show that neither ciliate abundance nor biomass was correlated to salinity, while species richness and diversity significantly decreased ($p < 0.01$) along the increased salinity gradients. Meanwhile, our data indicate that there were shifts of ciliate groups from oligotrichs towards hypotrichs to heterotrichs dominance with the increase of salinity. Supported by the National Science Foundation of China (No. 40576072) and the '100 Talents Project' of Chinese Academy of Sciences.

**THE BIODIVERSITY OF HELIOZOANS AND HETEROTROPHIC FLAGELLATES OF SEVERAL BOGS AND SMALL RIVER**

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Heliozoan and heterotrophic flagellate biodiversity in several bogs and one small river was investigated. Centrohelids *Raphidiophrys intermedia, Acantocystis penardi, A. nichollsi, A. takahashii, Pterocystis pinnata, Raineriophrys echinata*, desmothoracid *Clatrulina elegans,* rotosphaerid *Rabdiophrys* sp., actinophryids *Actinophrys* sp., and *Actinosphaerium eichhornii* have been found. 31 species of flagellates from 12 taxonomic groups were observed in the samples from acid bogs, but only 4 species were found in the plankton of the small river. The representatives of kinetoplastids and cercomonads were dominated in the samples in terms of species richness. *Spumella* sp., *Bicosoeca lacustris, Bodo saliens, Spongomonas uvella, Allantion tachyploon, Cercomonas agilis*, and *Protaspis simplex* were the most common species. Some rare species such as *Rhipidodendron splendidum, Dimastigella trypaniformis, Protaspis verrucosa, Apusomonas proboscidea, Aurigamonas solis, Reclinomonas americana* have been identified. The majority of observed flagellates were bacterivores, but *Rhynchobodo* sp., *Allantion tachyploon, Protaspis verrucosa, Aurigomonas solis* were predators. The bogs and river biodiversities considerably differed from each other.

**IDENTIFICATION OF TWO *NOSEMA* SPP. ISOLATED FROM *PIERIS RAPAE* AND *HEMEROPHILA ATRILINETA***

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Microsporidia are a group of obligate intracellular parasites. Some microsporidia play an important role in biological control of the silkworm Pebrine disease. This paper is focused on life cycle studies, ultrastructural identification, and genetic variations (in SSU-rDNA and ITS region sequences) of two microsporidia, isolated from *Pieris rapae* (CFD) and *Hemerophila atrilineta* (SCH). Following results were obtained. (1) Life cycles of CFD and SCH, were similar, except for duration of life cycles, which were obviously shorter in CFD (72h) than in SCH (96h). (2) The spores collected from original hosts varied in shape. The spore shape was tending to become uniform (oval and smooth) after propagating the isolates in the experimental host (silkworm) repeatedly. Both isolates had three-layered spore wall, isofilar polar filament, bipartite polaroplast, and two nuclei. The number of polar filament coils and the size of posterior vacuole varied depending on generation. (3) Phylogenetic analyses of CFD and SCH isolated from original hosts, based on partial SSU-rDNA sequence put these microsporidia into *Nosema/ Vairimorpha* clade. If microsporidia were passed through silkworms 4 times, the sequences obtained from harvested spores fell into the clade of true *N. bombycis* group. (4) ITS region of two isolate displayed high variations. Different clones from the same PCR amplication also significanly varied. Multiple alignment revealed three highly varied sites in the ITS region of the studied isolates. Our results suggest that both isolates belong to the genus *Nosema*, and that microsporidia might possess a selective adaptability and potential to differentiate in the alternative host. Supported by NSF grant 30671588.

**EVOLUTION OF EUKARYOTIC LIFE: OSCILLATIONS, RYTHMS AND CLOCKS ARE A NECESSITY**

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Experiments with ameboid and ciliate protozoa (eg. *Acanthamoeba castellanii* and *Tetrahymena pyrformis*) indicate that the cytosolic metabolism produces $H_2S$, while the mitochondria oxidise it. This is an echo of primeval organisation in the first eukaryote, which was formed when an Aarcheon host engulfed a proteobacterial endosymbiont and initiated a syntrophic interaction with mutually beneficial characteristics. Energy generation accompanies the disposal of this toxic common intermediate. Evidence that this interaction occurs even today comes from studies of a spontaneously self-synchronised culture of yeast, where the two processes are separated in time, as phase-related metabolic steps in the cellular network. In yeast this temporal organisation is the output of a 40 min ultradian clock; in *Acanthamoeba*, it is a 69 min clock, and in *Tetrahymena* it is 50 min. $H_2S$ is also the synchroniser that enables spontaneous behaviour with respect to intracellular redox potential. Waves of synchrony spread from individual mitochondria through the entire cell and then to neighbouring cells, so that the whole population acts almost as a tissue. We suggest that these processes are recapitulating early events in the establishment of eukaryotic organisation at a cellular and multicellular level. $H_2S$ is pivotal as an evanescent, volatile, highly diffusible, messenger molecule.

**PHYLOGENETIC POSITION OF THE ODONTOSTOMATIDS INFERRED FROM THE SMALL SUBUNIT RRNA GENE SEQUENCE OF *EPALXELLA ANTIQUORUM* PENARD, 1922 (PHYLUM CILIOPHORA; ORDER ODONTOSTOMATIDA)**

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The odontostomatid ciliates have remained a homogeneous order of ciliates since the 1930s when they were recognized as a monophyletic assemblage. Since that time they have been placed with the heterotrich ciliates, and more recently transferred as incertae sedis to the new "riboclass" Class ARMPHOREA. We were able to obtain the small subunit rRNA gene sequence of the odontostomatid *Epalxella antiquorum* Penard, 1922, collected from the meromictic alpine Lake Alatsee in Germany, in July 2005. An alignment with representatives of all 11 classes of ciliates unambiguously places the *Epalxella* sequence with other representatives of the Class PLAGIOPYLEA with 100% support in both maximum likelihood and Bayesian analyses. *Epalxella* is the basal lineage with trimyemid and plagiopylid ciliates forming the two terminal sister clades. While this molecular support is strong and unambiguous, there are no obvious morphological features to unite these three clades. Thus, the Class PLAGIOPYLEA must continue to be referred to as a "riboclass." Using the *Epalxella* sequence as a basal marker, we tentatively identified 20 environmental isolates to the terminal plagiopylean clades: eight to the genus *Trimyema*; four to the genus *Plagiopyla*; and eight to two new species, one of which might represent a new plagiopylean genus.

**MICROAEROPHILIC AND ANOXIC CILIATES DOMINATE A WARM-MONOMICTIC HYPOSALINE LAKE**

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Four-year-data on the ciliate assemblage structure in a high altitude, athalassohaline maar-crater lake Alchichica, Mexico (18°10'N; 93°10' W, altitude 2340 m) were analysed. Experimentally, growth rates of ciliates were evaluated. DAPI staining was employed to count ciliates while the Quantitative Protargol Staining for their identification; a total of 40 taxa was identified. Peritrichs often numerically dominated the ciliate assemblage; a maximum of 54 cells $ml^{-1}$ (mainly *Rhabdostyla* sp.) was observed in the surface layer at the end of the mixing period, during the development of diatoms (*Cyclotella alchichicana*), cyanobacterial bloom (*Nodularia* sp.) and its decay. Minute spirotrichs (particularly *Halteria grandinella*, maximum 7 cells ml-1) and a haptorid, *Belonophrya pelagica* (2.8 cells $ml^{-1}$) occasionally dominated in the epilimnion, while scuticociliates were numerically dominant within the hypolimnetic assemblages (*Cyclidium glaucoma*, 8 cells $ml^{-1}$; *Uronema nigricans*, 18 cells $ml^{-1}$; and an anaerobic cf. *Isocyclidium globossum*, 46 cells $ml^{-1}$). Mixotrophic *Euplotes* cf. *daidaleos* (29 cells $ml^{-1}$) and *Pelagothrix* sp. (6.4 cells $ml^{-1}$) were important around the oxycline, along with haptorids, particularly *Phialina* sp. (19 cells $ml^{-1}$). Strictly anaerobic ciliates of genera *Caenomorpha* (1.5 cells $ml^{-1}$), *Epalxella* and *Trimyema* (36 cells $ml^{-1}$) were found in considerable numbers at the end of the stratification period. Calculating the contribution of the ciliate biomass from the microaerobic metalimnion (dissolved oxygen < 2 mg $l^{-1}$) and from the anaerobic hypolimnion (May/July through December) suggested these layers to be much more important than the epilimnetic/oxygenated ones; however, ciliate growth

rates were lower there. Apparently, microphilic and anaerobic ciliates were not related to higher bacteria numbers.

## EARLY BIOSPHERIC EVOLUTION, THE ORIGIN OF EUKARYOTA AND FUTURE PERSPECTIVES OF LIFE ON THE EARTH

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According to contemporary data the Solar System and the Earth appeared about 4.6 - 4.5 billion years ago. The first indications of photosynthetic activity were discovered in the most ancient sediments dated at 3.8 billion years ago. Apparently water, sediments and life appeared on our planet almost at the same time - about 3.8-4.0 billion years ago. During the first two billion years there were only prokaryotes in the biosphere. The prokaryotes have no ability to phagocytosis, therefore enormous quantity of biomass was buried in sediments. Oil pools, gas-fields, and a lot of minerals are the products of prokaryote activity in the early biosphere. Appearance of eukaryotic protists (between 2 and 1 billion years ago) led to decrease of biogenic elements burial in sediments. After origin of Metazoa (about 600 million years ago), burial rate of biogenic carbon was reduced three times in comparison with Archaeozoic era. Nevertheless organisms can not use the already buried carbon. Oil, gas, coal, shale and other sources of biogenic carbon remain almost inaccessible for them until arising of human civilization. From the biosphere standpoint the destination of the human civilization is the extraction and incineration of oil, gas and coal, production of ores and their dissolution in waters of the great oceans. After this predestination will be accomplished the human civilization will die in a natural way, but the biosphere will receive new resources and get a new impulse to develop. So, life will continue to develop after human extinction, but its existence is limited by the seeping of oxygen from the Earth nucleus in the future. It is estimated that the oxygen seep will start in 600 million years. In 800 million years, oxygen-nitrogen atmosphere pressure will reach 5 atmospheres and temperature - 76 degrees centigrade (that means the destruction of almost all eukaryotes). In 1000 million years, oxygen-nitrogen atmosphere pressure will reach 15 atmospheres and temperature will rise up to 110 degrees centigrade, which means destruction of all prokaryotes.

## DYNAMICS OF MICROSPORIDIAN INFECTION IN POPULATIONS OF HARMFUL ARTHROPODS

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Dynamics of microsporidian infection in natural insect populations is well studied for some lepidopteran pests like corn borer (Siegel et al., 1988), cabbage butterfly and gypsy moth (Issi, 1991). In populations of phytophagous insects, diverse routes of parasite transmission are available. Microsporidia develop intensively and may regulate host densities, preventing pest outbreaks to some extent. Four-year observations over the population of meadow webworm *Pyrausta sticticalis* L. during its depression phase showed that low pest density is correlated to the high level of microsporidian infection, this dependence being especially prominent when the insect host abundance is compared to microsporidian prevalence in the previous generation. Deliverance from microsporidian infection facilitates insect population growth and under favorable hydrothermal conditions, creates necessary prerequisites for the pest outbreak (Malysh et al., 2006). This parasitic system may be referred to as a "loose". On the other hand, in blood-sucking arthropods like ixodid ticks, horizontal transmission is limited as compared to lepidopteran insects. Vertical transmission is more probable way for parasite's maintenance in populations of hematophagous hosts. Microsporidian infections in ixodid ticks are characterized either with low prevalence rates (Rehacek, Weiser, 1978) or with extremely low intensity of infection (Tokarev, Movila, 2004; Tokarev et al., in press). For a "tight" parasitic system, consisted of microsporidia and ixodid ticks, significant role of parasite in host density regulation is highly unlikely. Supported by RFBR (04-03-49629, 06-04-90814) and by a grant from President of Russian Federation for Y.S.T. (MK-653.2007.4).

## ULTRASTRUCTURAL ORGANIZATION AND FORMATION OF CYST-LIKE CELL IN HOMOXENOUS TRYPANOSOMATIDS OF THE GENERA *LEPTOMONAS* AND *BLASTOCRITHIDIA*

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Cyst-like stages of two trypanosomatid species, *Leptomonas oncopelti* from the bug *Oncopeltus fasciatus* and *Blastocrithidia gerridis* from the water strider *Gerris lacustris*, were studied. Formation of cyst-like cells of *L. oncopelti* begins with separation a small daughter cell from the promastigote. This small cell remains associated with the flagellum of the parental promastigote and divides twice to give rise to rosette of 4 "straphangers". "Straphangers" contact each other by the modified flagella and form zonal desmosomes. One of the modified flagella attaches to the parental flagellum by formation of zonal desmosome. Formation of *B. gerridis* "cysts" starts with nuclear division. One of the daughter nuclei becomes a nucleus of the "bud", which is formed on the body of the parental epimastigote. This nucleus divides twice. Flagellum-free "bud" with 4 sets

of organelles is formed. "Bud" separates from the parental cell and divides to produce 4 cyst-like amastigotes. Formation of "cysts" is accompanied by increase in the electron density of cytoplasm and cellular organelles. Nuclear divisions in *B. gerridis* "bud" are associated with progressive condensation of the chromatin, which masks microtubules and kinetochores. *L. oncopelti* chromatin is transformed into "labyrinthine structure". In mature "cysts" chromatin is not structurized. DNA fibrils of the kinetoplast lack circular configuration; they are condensed and do not exhibit any regular organization. Changes in envelopes begin with thickening of all three layers of plasmalemma. The layer of submembranous cytoplasm is condensed and masks subpellicular microtubules. Cortical complex consisting of thickened plasmalemma and dense granular submembranous cytoplasm is formed. In the electron-dense mature "cysts" the following zones could be distinguished: the zone of densely packed ribosomes, the modified kinetoplast, and the nucleus.

### *AMOEBORADIX GROMOVI* GEN. ET SP. NOV. - ENIGMATIC PARASITE OF THE ALGA *TRIBONEMA GAYANUM*

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Small amoebae (2.5 μm) with stiff flagellum (8 μm), composed of 9 microtubular singlets, contain a nucleus, contractile vacuole, and mitochondria with lamellar cristae, but no dictyosomes or phagosomes. Amoebae attach to the cell wall of the alga and transform into oval cysts with spines. Rhizoid appears from the base of the cyst and penetrates into the algae cell through the cell wall. A round thin-walled cyst is transformed into an oval (16 × 9 μm), thick-walled, sessile, monocentric, and epibiotic sporangium with granular contents. Cysts have a thin long rootlet, which penetrates the algal cell. Sporangia produce amoebae. Parasite develops on live algae *Tribonema gayanum* only. *A. gromovi* resembles at most the chytrid *Phlyctidium anatropum*, which was observed on different algae including *Tribonema*. *P. anatropum* also forms flagellated amoeboid zoospores. But it has an asymmetrical, strongly arched sporangium, and its haustorium is very small and rounded. Another similar chytrid, *Amoebochytrium rhizidioides*, also produces amoeboid spores, but without flagella. Moreover it has polycentric thalli, its sporangia are pyriform, basally apophysate with more or less prolonged discharge tubes. *A. rhizidioides* differs significantly from *A. gromovi* in morphology and habitat as a saprophyte growing in gelatinous matrix of *Chaetophora elegans*. On the basis of LM and EM data we are describing here a new genus and species *Amoeboradix gromovi*, named in honor of B.V. Gromov, who made a bulk of this study. The molecular investigation of this strain is in progress. Supported by RFBR grant № 05-04-49667.

### TWO SPECIES OF EPIBIONT CILIATES (SUCTORIA) ON *HESPEROCORIXA* SP. (HEMIPTERA: CORIXIDAE) FROM A POND IN HIDALGO, MEXICO

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Epibiont ciliates associated with some Arthropod groups, such as Crustacea and Insecta, were recorded all over the world. Concerning the hexapods, few orders were studied (Hemiptera, Trichoptera and Diptera). In Mexico the studies of epibiont ciliates were performed on crustaceans *Cambarellus patzcuarensis* and *Hyalella azteca*, but there were no data for insects. We collected 158 individuals of Hesperocorixa (Hemiptera: Corixidae) in a pond of Hidalgo, Mexico, between February and August 2005, with aquatic net. We found two species of suctorian epibionts, *Discophrya cybistri* and *Acineta tuberosa* attached to the legs of the corixid. *Discophrya cybistri* was found on 113 (71.5%) of the basibionts. From total 7203 individuals recorded, 6057 (84%) were observed attached to the middle legs. Few individuals of *Acineta tuberosa* were observed on 12 (7.6%) corixids. We conclude that preference of *D. cybistri* for the middle legs can be due to their architecture. These appendages are covered with numerous setae, which provide a shelter to the ciliated epibionts.

### PATTERNS OF PROTOZOAN COMMUNITY STRUCTURE: LINKING PROCESSES AND SCALES

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Patterns of the species diversity formation at different spatial scales were studied using protozoan communities as model systems. Three community types at three spatial scales were investigated. Testate amoebae communities and heterotrophic flagellate communities inhabiting sphagnum bogs were studied at the (i) megascale in different regions, such as northern tundra in Karelia, southern tundra in Yaroslavl region, forest-steppe in Penza region; (ii) mesoscale in different biotopes within one region, such as bogs at various successional stage, with different vegetation and levels of anthropogenic disturbance; and (iii) microscale in different microhabitats within one biotope, such as hummocks, lawns, hollows, sphagnum species etc.

Marine interstitial ciliate communities were studied at the megascale (in Barents, White, Black seas), mesoscale (in biotopes with various salinity, depth, sediment characteristics within one sea), and microscale (microhabitats within one type of sediments at the same depth). We examined the way in which total (gamma) diversity at different scales is partitioned into alpha and beta components. Alpha diversity, also referred to as within-habitat diversity, is the component of total diversity that can be attributed to average number of species found within homogenous sampling units, named habitats. Beta-diversity, referred to as between-habitat diversity, is the component of total diversity that can be attributed to differences in species composition among the homogenous units in the landscape. Beta-diversity is determined by (i) variation in environmental characteristics among local habitats, and (ii) the degree of habitat specialization of the biota. So, we try to understand, how contributions of alpha- and beta- to gamma- diversity change as a function of a spatial scale. Analysis of contribution of alpha and beta components into total species richness suggested that the community at the microscale as well as at the megascale was alpha-dominant. In other words, at these scales community is constrained by local factors. At the microscale, first of all biotic interactions (competition, predation, mutualism, etc.), as well as some abiotic parameters, affected the size of the niche space (biotope heterogeneity, limiting factors, disturbance, etc.). At the macroscale it was history of climate, migration, and evolution. At the mesoscale community seems to be beta-dominant. Total diversity is affected by differences between habitats along environmental gradients and among different type ecosystems within the region. Ciliate community displays the most clear cross-scaling pattern. Testate amoebae community is rather alpha-dominant, whereas heterotrophic flagellate community seems to be beta-dominant at all spatial scales.

## RESOLVING THE POSITION OF RHIZARIA ON THE BASIS OF MULTIGENE ANALYSIS

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Our present knowledge of the first step in the radiation of eukaryotes may be better illustrated not by a tree but by a multifurcation that spawned around a dozen of "supergroups", a condition that is referred to as the "Eukaryotic Big Bang". The taxon Rhizaria is one such supergroup. The monophyly of Rhizaria was established on the basis of rRNA gene sequences and the sequences of the main components of the cytoskeleton, but the phylogenetic position of the taxon itself remained a mystery. In order to address this question we have searched the chlorarachniophyte *Bigellowiella natans* database for the presence of ribosomal protein cDNA sequences. A set of 61 full and partial ribosomal protein sequences of the host cell was extracted from the database and aligned with the homologues sequences from over sixty representatives of other eukaryotic groups. All analysis yielded a sister group relationship between *Bigellowiella natans* and another protist group, Heterokonta, with high statistical support. The discovered monophyly of a group uniting Rhizaria and Heterokonta allows us to reconstruct the last common ancestor of this group as a heterotrophic amoebaflagellate with a complex life cycle. Even further, this result may lead to a revision of our current views on the subject of plastid loss and gain as it prompts us to reconsider the central hypothesis of rhodophyte-derived plastid evolution, which assumes a single plastid gain event in the last common ancestor of Heterokonta, Dinophyta, Haptophyta and Cryptophyta. Supported by the Russian Foundation for Basic Research (grants 05-04-49705 and 06-04-49288).

## MAIN EVOLUTIONARY TRENDS IN THE FORAMINIFERAL TEST AND TEST WALL DEVELOPMENT

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The main evolutionary trends in the phylum Foraminifera were transition from the unilocular to the multichambered test as a result of the process of polymerization (in the classes Miliolata, Spirillinata, Nodosariata, Rotaliata), and to the supermultichambered test in the more advanced groups (in the classes Miliolata, Rotaliata), then the differentiation of the chambers by their size and later by their function in some higher representatives (in the classes Miliolata, Spirillinata, Rotaliata). The polymerization of apertural openings and inner elements of apertural structures also took place in parallel in each of these classes. The next step of the multichambered test evolution was development of integrative systems: the inner communication between multiple chambers was going from simple foramens to stolones, and then to apertural integrative systems (class Rotaliata) and systems of canals (classes Spirilinata, Rotaliata). According to a new concept of foraminiferal evolution (Mikhalevich, 1992-2005) the agglutinated test wall developed into different types of the calcareous secreted wall. This process occurred independently in the different phyletic lines (classes) of Foraminifera: in Miliolata, Spirillinata, Nodosariata, Rotaliata). Appearance of the light and strong bilamellar (bifontinal) test wall of Rotaliata can be regarded as aromorphosis. The most primitive class Astrorhizata includes

exclusively unilocular representatives with tectinous or agglutinated test walls. The complexity of the multichambered foraminiferal test with its unique integrative canal system represents strikingly high level of organization among Protista.

## $H_2$ PRODUCTION IN THE FISH PARASITE *SPIRONUCLEUS VORTENS*

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The fish parasite *Spironucleus vortens* causes major problems in aquaculture of ornamental fish. The organism studied here was isolated from an angelfish (Sarah Poynton, 1995) and grown in bile supplemented (1%) TYIS33 medium. A membrane-inlet mass spectrometer was employed to monitor, in a closed system, m/z peaks at 2, 32 and 44 for $H_2$, $O_2$ and $CO_2$, respectively. When introduced in air saturated buffer, *Spironucleus vortens* consumed $O_2$ at the average rate of 34 ±15 nmoles/min/$10^7$ cells. $CO_2$ was produced at 25 ± 12 nmoles/min/$10^7$ cells. $H_2$ production started under microaerophile conditions ($O_2$ = 60 μM) with a rate of 20 ± 11 nmoles/min/$10^7$ cells. KCN (15 mM) inhibited H2 production by 85% and 20 mM by 96%, indicating that an Fe-only hydrogenase is responsible for $H_2$ production. Metronidazole (1mM) inhibited $H_2$ production by 50%, while $CO_2$ production was not affected. A higher concentration (1.5 mM) inhibited $H_2$ production by 87% and $CO_2$ production by 36%, suggesting that metronidazole is reduced by an enzyme of the $H_2$ pathway, thus competing for electrons with $H^+$. The question of the source of $H_2$ requires discrimination between the various organelles/inclusions evident in confocal and Normarski direct images. Antibodies raised to *Trichomonas vaginalis* hydrogenosomes and their enzymes have failed to reveal hydrogenosomes in this organism. We have used 15 nm diam. quantum dots (streptavidin coated) as markers for ingested materials in phagocytotic/pinocytotic vacuoles and vesicles. 16s rDNA probes will be used to investigate the possible presence of endosymbionts (archeal/bacterial). The authors thank Prof. J. Kulda for providing the organism and his expertise on its culture.

## *PNEUMOCYSTIS JIROVECII* INCREASES PROINFLAMMATORY CYTOKINES LEVEL IN COPD PATIENTS

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Chronic obstructive pulmonary disease (COPD) is characterized by a permanent air flow limitation caused by chronic inflammation of the bronchial wall. Many studies showed an increase in IL-8, MCP-1 and TNF-alpha in induced sputum of these patients. Local and systemic responses are likely related to such factors as genetic and immunological characteristics, smoking, and infectious agents. It is known that COPD patients can be colonized by P. *jirovecii*. Studies of animal models showed that infection with *Pneumocystis* induced a rise of proinflamatories cytokines. Therefore, it is possible to think that *P. jirovecii* colonization plays a role in COPD physiopathology. The aim of the study was to identify changes induced by *P. jirovecii* in systemic inflammatory response in patients with COPD. COPD was diagnosed in 51 patients according to GOLD classification. Identification of P. jirovecii was performed by nested PCR of DNA extracted from respiratory samples. The systemic inflammatory response was analyzed in serum using a commercial ELISA (R&D systems) for IL-6, IL-8, TNF-alpha and MCP-1. Patients with COPD colonized by *P. jirovecii*, showed a higher level of proinflammatory cytokine than non-colonized subjects.

| Cytokine levels (pg/ml) | COPD without *P. jirovecii* (N=23) | COPD with *P. jirovecii* (N=28) | P-value (Student-T) |
|---|---|---|---|
| IL-8 | 13,89 | 21,26 | 0.028 |
| TNF-alpha | 3,57 | 8,15 | 0.047 |
| IL-6 | 5,34 | 16,95 | 0.038 |
| MCP-1 | 726,99 | 1012 | 0.048 |

No statistically significant differences in age, sex, tobacco consume, respiratory function, lymphocytes and leucocytes counts were found. The data suggest a relationship between P. jirovecii colonization and high level of systemic inflammatory response in patients with COPD. Supported by ERA-NET *Pneumocystis*-PathoGenoMics.

## LONG-TERM SURVIVAL OF THE RESTING CYSTS OF THE HALTERIID CILIATE *MESERES CORLISSI*, AND IMPLICATIONS FOR ITS MAINTENANCE IN LABORATORY CULTURES

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*Meseres corlissi* is a rare freshwater ciliate adapted to small, ephemeral aquatic habitats. It forms desiccation resistant cysts to survive dry periods. Long-term survival of these cysts was studied in a 250 g soil sample from the type locality, an astatic meadow pond. The soil was air-dried and stored at 18 to 22°C in the dark for 3 years. At irregular intervals, 4 g subsamples were tested for viable cysts of *M. corlissi*, following a standard procedure. These tests were positive up to 24 months of storage, but negative later on. Viable cysts were also

detected in a subsample which for 2 weeks had been stored at -25°C. Based on these findings, a technique was developed to cultivate clonal strains of *M. corlissi* in alternating phases of active populations and desiccated cysts, thus imitating the life history of field populations. Commercial garden soil was used as a substrate. Cysts embedded in dry soil remained viable for up to 7 months at 18 to 22°C; they also tolerated storage at -25°C. Cysts formed in the absence of soil, in contrast, did not survive desiccation periods longer than one week. These results indicate that the *M. corlissi*-type population is well adapted to the ranges of temperature and humidity occurring in its natural habitat. They also suggest that dispersal of cysts by wind is unlikely, due to their low tolerance to desiccation when not embedded in soil. This may to a certain extent explain the rare and disjunct occurrence of this ciliate.

## ERVIN BAUER, A HUNGARIAN-SOVIET PIONEER OF THEORETICAL BIOLOGY AND PROTISTOLOGY

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Ervin Bauer (1890-1938) is a legendary figure of Soviet biology of the 1930s. He developed deductively a consistent theoretical biology starting from his principle of "permanent disequilibrium of living matter." Work in his laboratories in Moscow (Obukh Institiute of Professional Diseases and Timiryazev Biological Institute) and Leningrad (All-Union Institute of Experimental Medicine) was directed to provide experimental evidence of this disequilibrium. Among many biological objects this work included studies on protists. With A.M. Granovskaya (1903-1958?) he explored the effects of injury on hypotrich ciliates in various stages between cell divisions, focusing on nuclear processes and respiration. These were among the first measurements of respiration in single protist cells. With A.M. Lunts (1900-1977), who was a student of Max Hartmann in Berlin and returned to the USSR in 1932, he explored tactic phenomena in green algae. With V.S. Brandgendler (1901-1941), who joined Bauer briefly after work in mammalian physiology, he investigated serum toxicity in *Paramecium*. These efforts made significant contributions to protozoology of the time and, while seemingly disparate, they all formed integral part of Bauer's theoretical constructs. After Bauer's repression in 1937, Granovskaya was also repressed, Lunts became head of the Biology Department of the Saratov Medical University, Brandgendler returned briefly to mammalian physiology and was killed in the defense of Leningrad.

## THE DIVERSITY OF MITOCHONDRION-RELATED PROTIST ORGANELLES

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Trichomonad flagellates were shown in the 1970s to contain double membrane-bounded hydrogen producing organelles, the hydrogenosomes, instead of mitochondria. Since then all currently recognized major clades of eukaryotes - except the Rhizaria - were found to include some groups that do not harbor typical aerobic mitochondria but contain other forms of double membrane-bounded organelles. These organisms were sometimes called amitochondriates and regarded to be ancestral eukaryotes. Such notions are no longer in favor. The morphology and biochemistry of these organelles differs dramatically from group to group. The so far recognized major types are the hydrogen producing hydrogenosomes and the smaller mitosomes with no major role in metabolism. Their major differences notwithstanding these organelles share a number of biological characteristics with each other and with typical mitochondria. In contrast to earlier opinions, today they are all regarded as members of a monophyletic family of organelles, derived from the unique mitochondrial endosymbiotic event. The observed diversity reveals the extreme evolutionary plasticity of these characteristic organelles of the eukaryotic cell.

## *PNEUMOCYSTIS JIROVECII* COLONIZATION ALTERS PULMONARY SURFACTANT-ASSOCIATED PROTEINS IN SUBJECTS WITH IDIOPATHIC INTERSTITIAL PNEUMONIA

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Data obtained on *in vitro* and animal models with *Pneumocystis pneumonia* showed that this patogen bound to surfactant proteins and could alter their expression and distribution. Pulmonary surfactant-associated proteins, SP-A and SP-D, play an important role in lung host defence and in surfactant homeostasis.It was demonstrated that SP-A and SP-D regulated NF-kB and, by this pathway, were able to enhance or suppress inflammatory mediators production. Idiopathic Interstitial Pneumonia (IIP) is a heterogeneous group of poorly understood diseases. All of them include an initial inflammatory response that could be triggered by an infectious agent. The objective of the study was to identify possible alterations in pulmonary surfactant-associated proteins in patients with IIP colonized with

*Pneumocystis*. Bronchoalveolar lavage fluid (BALF) from 40 IIP patients was collected, and identification of *P. jirovecii* was performed by nested-PCR of mt LSU RNAr gene. SP-A levels were determined by densitometry, ELISA (BioVendor) and western-blotting. All data were normalized with total protein concentration (quickstart Bradford dye reagent, Biorad) for each sample. *P. jirovecii* was identified in 14 out of 40 (35%) IIP patients. The results obtained for surfactant-associated proteins are shown in the table. Conclusions: (1) high rate of *P. jirovecii* colonization was found in IIP patients; (2) statistically significant SP-D decrease was observed in the *P. jirovecci*-colonized IIP patients; (3) *P. jirovecci* could play a role in the physiopathology of this disease through interaction with pulmonary surfactant-associated proteins. Supported by ERA-NET Pneumocystis-PathoGeno-Mics.

| | IIP colonized | IIP no colonized | p |
|---|---|---|---|
| SP-A (CN*/mg prot) | 11.77 | 15.25 | 0.295 |
| SP-D (ng/mg prot) | 406.24 | 904.86 | 0.026 |

* Normalized relative concentration

## ORGANISATION AND EXPRESSION OF THE DINOFLAGELLATE MITOCHONDRIAL GENOME

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Protist mitochondrial genomes are extremely diverse in size, gene content and organisation. Although the mitochondrion of all aerobic protozoans studied contains a genome, the number of genes present is highly variable. This situation is thought to have arisen by the differing extents of gene transfer to the host nucleus between lineages over the course of evolution. Within the alveolates, a group comprising the ciliates, Apicomplexa and dinoflagellates, the ciliate and apicomplexan mitochondrial genomes have been well studied, and several complete sequences from each are now available. However, dinoflagellate mitochondrial DNA (mtDNA) has hitherto defied characterisation due to its unusually complex structure. We have recently shown that the dinoflagellate mitochondrial genome encodes the three respiratory complex subunits *cox1, cox3* and *cob* and we have found small rDNA fragments similar to those observed in Apicomplexa. Unlike Apicomplexa, however, the genome appears to consist mostly of non-coding DNA containing pseudogenes, short coding region fragments, and closely-packed stem-loop structures. Several differently-sized transcripts are produced for each gene, and comparison of sequences from EST projects and genomic clones implies extensive RNA editing. Although the highly reduced mitochondrial gene content supports the relationship of the dinoflagellates and Apicomplexa as sister groups, the absence of large regions of non-coding mtDNA in Apicomplexa suggests that this feature arose after the two lineages diverged.

## MINIATURE CHROMOSOMES OF MICROSPORIDIA: STRUCTURE, SEGREGATION AND COMPACTION

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Genomes of microsporidia consist of a small number of miniature chromosomes, ranging from 120 to 2700 kbp and showing extensive length polymorphism of homologs. It is conceived that microsporidian chromosomes, similarly to many other parasitic protists, contain a conserve core and repeat-rich variable extremities mediating frequent interchromosomal recombinations. Conserved restriction patterns of homologous chromosomes identified in monomorphic diplokaryotic microsporidium *Paranosema grylli*, evidence for the existence of internuclear genome homogenization. The meiosis was recently found in this species and is long known in *Nosema rivulogammari*, but most of monomorphic diplokaryotic microsporidia still are believed to be agamous. It is unknown if any kind of internuclear exchange leading to genome homogenization, takes place in these species. Microsporidia divide by closed intranuclear pleuromitosis, which involve neither the disintegration of the nuclear envelope, nor the chromosome arrangement in a metaphase plate. Chromosomes remain weakly condensed; probably such a simple and fast mitosis does not require high degree of compaction of small chromosomes. Spreading of interphase chromatin in a low ionic strength solution revealed 10-nm nucleosomal and 20-nm chromatin fibers, but none of the higher order structures. *In silico* analysis of core histone genes in *Encephalitozoon cuniculi* and *Antonospora locustae* demonstrate that they are the most divergent eukaryotic histone sequences of all known to date. However, positions of aliphatic amino acids critical for histone folding are conserved, and histone fold domain can be recognized unambiguously. Most of potential sites of post-translational modifications can be also tentatively identified. Hence, despite the high degree of histone sequence divergence, the overall structure of nucleosome in microsporidia is similar to that in other eukaryotes. Data used were in part obtained in cooperation with the team of Prof. C.P. Vivares, Universite Blaise Pascal, Clermont-Ferrand, France. Supported by RFBR grants 05-04-49222 and 07-04-00662.

## MACRONUCLEAR GENOME ORGANIZATION CHANGES AT THE INITIAL STAGES OF SPECIATION: *PARAMECIUM AURELIA* COMPLEX AS A MODEL

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Pulsed-field gel electrophoresis (PFGE) was applied to analyze and compare macronuclear genome organization of all fifteen species of the *Paramecium aurelia* complex. The "*P. aurelia*" PFGE profile was a continuous spectrum of different-sized (50-2000 kb) DNA molecules; specific pattern of banding allowed direct comparison of different profiles. Each strain of any species, despite stage of its life cycle, was characterized by constant PFGE profile. Different strains of a certain species mostly shared species-specific banding pattern. Still, for some species noticeable intraspecific differences were registered. Sonneborn (1974) had observed interspecific mating reaction for some species of the *P. aurelia* complex (*P. primaurelia, P. triaurelia, P. tetraurelia, P. pentaurelia, P. septaurelia, P. octaurelia, P. decaurelia, P. dodecaurelia*), though no livable progeny was obtained in such cases. We found that PFGE profiles of strains of all these species were relatively similar, while for the species which are not able to interspecific mating (*P. sexaurelia, P. novaurelia, P. undecaurelia, P. tredecaurelia, P. quadecaurelia, P. sonneborni*) each species was characterized by the highly distinct PFGE profile. The only exception was *P. biaurelia*, which belongs to the second group but did not have highly individual PFGE profile. Interestingly, our data well correspond to the recent phylogenetic tree of *P. aurelia* complex (Hori et al., 2006): on this tree all species (again, with the exception of *P. biaurelia*) in which interspecific mating reaction has not been observed, and which are characterized by well-recognizable PFGE profiles, are diverged from other species of the complex. Thus, changes in macronuclear genome organization pattern coincide with initial stages of speciation in *Paramecium*.

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## THE SYMBIOTIC SYSTEMS OF PROTISTS AND BACTERIA, INHIBITED PROTECTIVE FACTORS OF EUKARYOTIC CELL: MECHANISMS OF FORMATION

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The widespread ability to inhibit lysozyme and histones (antilysozyme and antihistone activities), 97 and 76% respectively, was revealed among bacteria associated with protists in water bodies. It demonstrates the mechanism providing the bacterial resistance to protists' bactericidal cationic peptides (lysozyme, histones). Cationic peptides and their inhibitors were estimated to form functional systems "lysozyme-antilysozyme", "histone-antihistone". We used the model of interaction between the ciliate *Tetrachymena pyriformis* and isogenic strains of bacteria *Escherichia coli*, that differed by the antilysozyme activity. The antilysozyme activity of bacteria was shown to protect them from protozoa and thus to contribute to their survival in protist' cells. At the same time, protists take part in formation of heterogeneity of antilysozyme sign expression in bacterial population. The same data were obtained using the other model: bacteria *Pseudomonas putida* and cyanobacteria *Microcystis* sp. Electron microscopy study showed that the strains with antilysozyme activity induced incomplete phagocytosis in protists accompanied by better bacterial survival in associations. Incomplete phagocytosis of the *Escherichia coli* strain with antihistone activity occurred also during its interaction with *Tetrachymena pyriformis*. Moreover, there was incompact chromatin in cilates' macronucleus. The proportion of protist cells differed by contents of histones was changed. The share of cells with a little amount of histones was increased and one with normal contents of histones was decreased. These results are important for investigation of the mechanisms of symbiotic relations between bacteria and protists at initial stage of symbiogenesis.

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## DIVERSITY OF FREE-LIVING BAIKALIAN CILIATES WITH SOME ECOLOGICAL COMMENTS

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The free-living Baikalian ciliate fauna (300 species) forms several specialized ecological complexes, two of which are of a particular interest. The spring complex develops during the under-ice plankton maximum; it consists of both rare and widespread cold-water and eurythermic ciliates. The temperature optimum for this complex is within the range 0-4°C, and some species are rather associated with the ice algae. The occurrence of endemic ciliates in the Baikal plankton is under discussion. If *Marituja* and *Liliimorpha* may be regarded as post-glacial relicts preserved in some water-bodies of Europe and Asia (Foissner et al., 1999), then the *Bursellopsis* group, consisting of 4-5 closely related species, may be a result of adaptive radiation typical for the Baikal flora and fauna. The psammophilic complex is the most interesting among benthic ciliates. Pronounced morphological adaptations specific for mesopsammon, as well as the diversity (4 genera and 15 species of Colepidae, 6-7 *Frontonia* species, and others) indicate the intensive speciation in this community. Moreover, many eurytopic ciliates, living in fresh-

water sands of Lake Baikal, show adaptive trends to inhabit this biotope only. In spite of predominance of widespread species in the free-living ciliate fauna of this ancient lake, it also includes autochthonous endemic species and even genera.

**EXPERIMENTAL MURINE GRANULOMATOUS AMOEBIC ENCEPHALITIS: IMMUNOHISTOCHEMICAL CHARACTERIZATION OF EARLY STAGES OF THE INFECTION**

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Early stages of experimental murine granulomatous amoebic encephalitis with *Acanthamoeba castellanii*, were immunohistochemically characterized after 48 and 72h post intranasal instillation of $1 \times 10^6$ / 20 µl trophozoites. Mice were sacrificed. Lungs, kidneys, spleens, liver and heads were fixed and processed for their inclusion in paraffin, and sectioned to obtain 5 µm-thick slides and then processed immunohistochemically using IgG of rabbit anti-*Acanthamoeba* and goat anti IgG of rabbit conjugated to peroxidase, revealed with $H_2O_2$ diaminobenzidine and counterstained with Harris hematoxylin. Analysis of all organs in study, revealed the invasion of trophozoites of *Acanthamoeba castellanii*. Heads: some trophozoites were observed in contact with the surface of the layer of mucus of the olfactory epithelium; in some zones, the trophozoites moved through the mucus with disruption of a number of areas; invasion of the olfactory epithelium was observed; cyst-like forms were observed in the brain. Lung, spleen, kidney and liver: trophozoites, as well as cyst, were observed in all the organs; in some cases disorganization but not inflammation of the tissues was evident; amoebae were located near of vessels, frequently migrating through the different tissues. It was believed that *Acanthamoeba* spp. reach the brain by hematogenous dissemination, but we proved that the parasites were able to adhere, migrate and penetrate through olfactory epithelium. In contrast to *Naegleria fowleri, A. castellanii* migrates and penetrates slowly, thus explaining the chronic course of the infection. Immunohistochemistry is a useful technique to identify free-living amoeba since they are difficult to be observed with conventional staining techniques.

**BIOINFORMATIC ANALYSIS OF VARIANT GENETIC CODES IN DIFFERENT PHYLOGENETIC GROUPS OF CILIATES**

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In many ciliates, the universal genetic code is substituted by variant codes in which one or two of the stop codons are reassigned to sense codons. In some Spirotrichea ciliates, UAA and UAG are translated as Gln (for example, in Oxytrichidae), whilst in Euplotida UGA is translated as Cys. These two types of variant codes are also found in phylogenetically distant ciliates, like Oligohymenophorea (UAA and UAG encode Gln) and Karyorelictea (UGA encodes Cys). We examined ciliate species with >100 published protein-coding DNA sequences. Surprisingly, in several cases discrepancies were revealed between our estimation of the genetic code deviation and the type of variant code established earlier. We compared frequencies of in-frame "stop" codons translated as Gln or Cys with frequencies of Gln or Cys encoded by convention codons. We consider the codes characterized by low frequency of in-frame "stop" codons as "young" variant codes, whereas the codes with many accumulated mutations leading to high frequency of in-frame "stop" codons are classified as "old" variant codes. Some closely related ciliate groups exhibit different types of codes. Variant codes of these organisms were in most cases "young". These data allowed us to propose that the initial mutation(s) in stop codon-decoding translation termination factor eRF1 happened in universal code organism leading to lower efficiency of recognition of one or two stop codons. This mutation(s) could be compensated by secondary mutation(s) reversing this eRF1 back to omnipotent type. Alternatively, this codon or codons could be decoded by tRNA which acquired an ability to recognize stop codons after mutation(s). We suppose that even in ciliate protein sequences where stop codons are not reassigned to sense codons a variant code could operate due to restriction of stop codon recognition by eRF1.

**THE EFFECT OF DRYING ON COLONIZATION OF PERIPHYTON COMMUNITIES**

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Surface drying is an important disturbance that shapes biological communities. We tested the effect of drying on the colonization patterns of protozoa and micrometazoa. A total of 192 glass slides were exposed in replicated 1.5 m experimental flumes that were fed by water from a nearby stream. After an initial colonization period of nine days one third of the slides were air-dried for eight days, rewetted, and the recolonization of periphyton was compared with community on the permanently wet slides. During the initial period (i.e.

nine days), we observed distinct colonization pattern starting with single-celled algae, mostly diatoms, followed by flagellates and ciliates. In contrast to the initially slow development of periphyton, recolonization after the dry period was very fast. At the beginning of the re-wetting experiment only desiccated periphyton was detected on glass slides. However, in less than 24 hours flagellates and ciliates appeared in great numbers. Further, drying resulted in a distinctly altered community composition (i.e. first colonizers and dominant species), compared to the composition on the permanently wet slides. The much faster recolonization rate after drying can be explained by the remaining matrix on the glass slides and the encysted forms of microorganisms. In the initial colonization and recolonization experiments species richness increased logarithmically, while ash free dry mass increased exponentially in time. Total biomass and the density of organisms were much higher in the recolonisation experiment.

**MICROSPORIDIA AND GREGARINES OF ALIEN AND NATIVE GAMMARIDS (AMPHIPODA) OCCURRING IN POLAND**

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The goal of our research was to identify microparasites (gregarines and microsporidia) of alien gammarids colonizing recently Polish inland, and coastal waters, from those infecting native species occurring in the same areas. Altogether, over 4000 individuals of 12 gammarid species (5 aliens and 7 natives) were collected from the deltaic system of the Vistula River, Vistula Lagoon, littoral of the Baltic Sea near Debki, and from small rivers draining directly to the sea in this area. All gammarids were identified to the species level, and sectioned under a stereomicroscope. Microparasites were identified with light- and electron microscopy. Gregarines (Apicomplexa, Gregarinidae) were recorded in the digestive tracks of invasive *Pontogammarus robustoides* (*Uradiophora ramosa* Balcescu-Codreanu, 1974 & *Cephaloidophora mucronata* Codreanu-Balcescu, 1995) from the Vistula deltaic system, and in the digestive system of native *Gammarus pulex* (*Cephaloidophora gammari* (Franzius, 1848)) from Stradanka river, an affluent of the Vistula Lagoon. Microsporidia were found only in two gammarid species: *Pleistophora muelleri* (Pfeiffer, 1895) in *G. pulex* from Piasnica river, and *Nosema pontogammari* Ovcharenko & Kurandina, 1987) from the Vistula deltaic system. All the above microparasites are new to Poland. *Pleistophora muelleri* is a widespread species in Europe. Other microparasites have been found earlier only in Ponto-Caspian region, and evidently have been transported to Central Europe with the invasive Ponto-Caspian gammarids. Interestingly, no microparasites were found in an invasive North-American *Gammarus tigrinus*, and in Ponto-Caspian *Obesogammarus crassus*. Also, no transfer of microparasites between native and alien gammarids was observed.

**FIRST RECORD OF MICROSPORIDEAN INFECTION OF *MYXOBOLUS PARVUS* (MYXOZOA) FROM JAPAN SEA GREY MULLET *MUGIL SOIUY***

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An unidentified microsporidium is recorded from myxozoan parasite *Myxobolus parvus* infecting grey mullet, *Mugil soiuy* in Russian coastal part of Japan Sea near Razdol'naja river (46°35'N, 32°16'E). Parasitological material was collected from 63 specimens of grey mullets in period from July to December 2004. Pseudocysts of *M. parvus* were found in gills, gill organ, mesentery, pyloric caeca and intestine of *M. soiuy*. The infected myxosporeans were assembled in intestine of 2 from 33 fish specimens (prevalence: 6 %) catched in October. Total length of hyperinfected hosts was 34,5 - 35,0 cm, and weigth 415 - 435 g. Intensity of myxozoan infection was 1 - 12 plasmodia per intestine. The microsporidean spores were elongated, sometimes slightly bent, measuring $6.4 \pm 0.5$ (5.1 - 7.5) $\times$ $2.9 \pm 0.3$ (2.2 - 3.6) μm on glycerin gelatin slides. Most of the spores were glued to the spore surface of myxozoans. Non infected spores of *M. parvus* measured $7.3 + 0.7 \times 6.3 + 0.6$ μm, their polar capsules were $3.9 + 0.3 \times 2.1 + 0.3$ μm in size. Myxosporean spores from infected plasmodia were $7.4 + 0.8 \times 6.7 + 0.4$ μm in size with polar capsules measuring $3.9 + 0.3 \times 2.2 + 0.4$ μm. Species we found, differs from all documented microsporidians infecting Myxozoa (*Nosema ceratomyxae, N. notabilis, N. marionis, Microsporidium* sp. and two Microsporidia gen. sp.) by shape and dimensions of spores, and by host infected.

**TRANSITION BETWEEN EXTRACELLULARITY AND INTRACELLULARITY IN LOWER SPOROZOANS: A CASE OF *ELEUTHEROSCHIZON DUBOSQUI* BRASIL, 1906 (COCCIDEA, PROTOCOCCIDIIDA) FROM POLYCHAETES *SCOLOPLOS ARMIGER* AND *NAINEREIS QUADRICUSPIDA***

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The order Protococcidiida is a small group of parasites

that develop, extracellularly or intracellularly without schizogony, in marine invertebrates. Protococcidiida are so much different from other coccidia that they need to be investigated in details to infer the early evolutionary history of sporozoans as a whole. We have studied the morphology of *Eleutheroschizon dubosqui* Brasil, 1906. The scanning electron microscopical investigation has demonstrated that these parasites develop on the intestinal epithelium of the White Sea polychaetes *Scoloplos armiger* and *Nainereis quadricuspida*. They are oval in shape and attach to the epithelium layer by a complicated attachment apparatus. Transmission electron microscopy showed that parasitic cells were enclosed in a membranous sac of unclear origin. There are two morphs of *E. dubosqui*: with a large nucleus and with several small nuclei. These morphs are respectively female and male gamonts reasoning from the life cycle description by Chatton and Villeneuve (1936). Lifetime observations showed that macrogamonts and microgamonts were both capable of changing their body shape by alternate shortening and stretching. *E. dubosqui* is a parasite sharing characters of coccidia and gregarines: body polarity, extracytoplasmic intracelullar localization (similar to that of *Cryptosporidium* and *Ditrypanocystis*) in the host intestinal epithelium, cell motility and life cycle without merogony and syzygy. It suggests an evolutionary transition between extracellularity of gregarines and intracellularity of coccidia.

**EVOLUTIONARY TRENDS OF THE LOWER GREGARINES: ULTRASTRUCTURAL AND BIOLOGICAL DATA**

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The aseptate gregarines are the most primitive groups within Gregarinea because they have nonseptated cell body and parasitize in marine invertebrates. In gregarines, the main evolutionary trend is an improvement of the locomotor apparatus and attachment organelle. Here I discuss correlations of the cortical zone ultrastructure with the type of motility and localization in the host of lower gregarines. Mucron of archigregarines is formed from the sporozoite apex and retains elements of apical complex. Mucron of lecudinides has a flattened membraneous surface adhering to host cells as a sucker. In advanced gregarines mucron is replaced by epimerite - an evolutionary new attachment organelle, which has no elements of apical complex and acts as an anchor.Aseptate gregarines demonstrate three distinct modes of motility: bending, gliding, and metaboly (peristalsis), while some of them are motionless. Modes of motility are associated with different structural types of cortical zones of trophozoites. The mode of motility is adaptation of gregarines to parasitizing in a certain hostal biotope. Gregarines inhabiting the intestine usually exhibit bending or gliding. These motility types provoke currents of hostal medium around a parasite cell. Metaboly occurs in protists parasitizing in a medium with increased viscosity and/or in a limited space. In cavities with mobile liquid the motionless gregarines occur. They need no movement because the cavity medium moves by itself around parasites. Motionless gregarines likewise occur in host tissues where tissue pressure prevents them from moving.

**THE TWILIGHT OF SARCODINA**

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Traditional morphology-based system of protozoa grouped all taxa that form so-called pseudopodia in the subphylum of Sarcodina, subdivided into superclass of Rhizopodea and Actinopodea. This system was recently challenged by phylogenetic analysis of DNA sequences, which suggested the polyphyletic origin of amoeboid protists. Based mainly on ribosomal RNA and actin genes, the majority of pseudopodia-bearing protists were placed in two "supergroups": Amoebozoa and Rhizaria. The Amoebozoa include naked and testate lobose amoebae, the mycetozoan slime moulds, amitochondriate entamoebids and pelobionts, as well as some flagellated protists (*Multicilia, Phalansterium*). The Rhizaria comprise protists with filopodia, reticulopodia and actinopodia, as well as some amoeboflagellates. Some amoeboid protists branch within other "supergroups", for example Opisthokonta (*Nuclearia, Ministeria*) or Chromalveolata (Actinophryidae), while others (Centrohelida) remained incertae sedis. Phylogenomic studies confirmed the monophyly of two amoeboid "supergroups", but their position in eukaryotic tree remains unclear. Lack of genomic data for the majority of amoeboid taxa impedes the resolution of phylogenetic relationships within Amoebozoa and Rhizaria. More extensive taxon sampling is also necessary for better assessment of the diversity of amoeboid protists.

**PHYLOGENETIC POSITION OF TRICHOSIDAE**

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The family Trichosidae comprises multinucleate testate marine amoebae with alternation of asexual (schizont) and sexual (gamont) generations. The schizont possesses a supple test covered with mineral spicules ("fuzzy" form) whereas the gamont is devoid of spicules ("smooth" form). The family is represented by a single genus *Trichosphaerium* composed of three described species. In order to establish the phylogenetic position of Tri-

chosidae among amoeboid protists, we obtained the complete small subunit rDNA sequences from three different isolates: *Trichosphaerium sieboldi* (CCAP 1585/2; "smooth" form); *Trichosphaerium* sp. 1 (Madagascar; "fuzzy" form), and *Trichosphaerium* sp. 2 (Mediterranean Sea; "fuzzy"form). Our data show that Trichosidae belong to the "supergroup" of Amoebozoa, but their exact placement is impeded by extremely rapid rate of evolution of their ribosomal genes.

**PHAGOCYTOSIS BY *TRICHOMONAS VAGINALIS*: NEW INSIGHTS**

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The parasitic protozoan *Trichomonas vaginalis* is the causative agent of trichomoniasis. We have studied the *in vitro* capacity of *T. vaginalis* to phagocytose and degrade *Saccharomyces cerevisiae* cells. To analyze the phagocytic ability and capacity, two isolates of *T. vaginalis* presenting different virulence grades were used. Complementary techniques such as fluorescence microscopy, computer based fluorescence analysis, scanning and transmission electron microscopy and the use of drugs that interfere with the actin microfilaments were used in order to follow the actin cytoskeleton behavior during phagocytosis of yeasts by *T. vaginalis*. It was concluded that: (1) *T. vaginalis* changes its shape rapidly and engulfs the yeasts, which are almost as large as the parasite; (2) long-term and fresh cultures are able to phagocytose; (3) T016 strain exhibited an amoeboid morphology during the internalization process; (4) attachment occurred through the whole cell surface, including both anterior and recurrent flagella; (5) both sinking process and the classical phagocytosis where pseudopodia are extended toward the target cell; (6) the internalized *S. cerevisiae* are digested in lysosomes; (7) competitor sugars D-mannose or L-fucose inhibit the phagocytosis; (8) a thick layer of actin microfilaments was present underlying the plasma membrane and especially in the pseudopodia and around the phagocytosed particles; (9) a dramatic change in the distribution pattern of fibrillar actin occurred during phagocytosis; (10) Cytochalasin D depressed the phagocytosis; (11) a non-specific recognition and phagocytosis is mediated by a mannose-receptor present on the parasite surface; (12) the phagocytic process may occur during mitosis of the parasite.

**COMPONENTS OF ALPHA-TUBULIN MINICHROMOSOME PROMOTERS IN THE STICHOTRICHOUS CILIATE *STYLONYCHIA LEMNAE* INVOLVED IN TRANSCRIPTION REGULATION**

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In general, DNA in the transcriptionally active macronucleus of the hypotrichous ciliates is represented by short linear molecules - minichromosomes, encoding a single gene each. Previous studies have not revealed the presence of conventional eukaryotic consensus sequences involved in regulation of transcription initiation and activation in the minichromosomes. In our work we have re-determined transcription start sites in four tubulin genes of *Stylonychia lemnae*. Then we used microinjection of artificial a1 tubulin minichromosomes into the macronucleus of *S. lemnae* as a means to characterize in detail the corresponding promoter. Deletion and block substitution mutations in promoter region of a1 tubulin gene revealed a TATA-like element, an initiator element, three distinct upstream sequence elements (USEs), including repressor element, involved in regulation of transcription initiation. Investigation of a tubulin expression in cells treated with Concanavalin A revealed a capability of a2 tubulin gene for increasing of transcription level. We revealed a promoter region in a2 tubulin gene involved in regulation of transcription activation. Determination of transcription start sites and a sequence alignment indicated that both TATA-like and initiator elements are conserved components of *S. lemnae* tubulin minichromosomes, whereas the USEs appear to be specific for the a1 tubulin minichromosome. On the other hand, the presence of induction promoter element is conserved for the a2 tubulin minichromosome. Supported by grants 07-04-00662 and 03-04-48505-a from the Russian Foundation for Basic Research, German Research Foundation (DFG grants Am 26/31 and Gu371/3) and the German Academic Exchange Service (DAAD grant 325/lin).

**BIODIVERSITY AND ECOLOGY OF PLANKTONIC HETEROTROPHIC FLAGELLATES IN SALINE LAKES**

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Biodiversity and ecology of heterotrophic flagellates was studied in two hypersaline lakes and one brackish lake (Salt-Iletsk, Orenburg region, Russia). Seasonal dynamics and vertical distribution of species richness and occurrence of protists were analyzed. These characteristics of flagellate communities were related with salinity, season, trophic status of lakes and abundance of other hydrobionts. The Bicosoecida and the Metakinetoplastea were mostly common. Wide spread of Heterolobosea was noted in hypersaline environments, that

indicated their good adaptation to extreme salinity. Fifty one species and forms of heterotrophic flagellates have been found. Dominant and rare, stenohaline and euryhaline species were distinguished. Cosmopolitan taxa *Spumella* sp., *Cafeteria roenbergensis, Bodo designis, Paraphysomonas* sp., *Monosiga ovata, Rhynchomonas nasuta* were the most common protists. Two species of heterotrophic flagellates with wide ranges of salinity tolerance, *Cafeteria roenbergensis* and *Cafeteria marsupialis*, were registered in both, hypersaline and brackish lakes. Halophilic flagellates *Pleurostomum salinum, Pendulomonas* sp., *Macropharyngomonas* aff. *halophila* were occured only in hypersaline Razval Lake at salinity varying from 236‰ to saturation. These protists are poorly known; therefore their ecology, salinity toleration, appearance and life cycle were studied in a uniprotist culture. The SSU rRNA gene sequencing allowed to refine the systematic position and phylogeny of *Macropharyngomonas* aff. *halophila*. These extreme halophilic species appeared to fail growing in low salinity conditions. The salinity tolerance thresholds were individual for each flagellate species. Supported by RFBR grants 06-04-96920 and 05-04-49870.

## THE ROLE OF HEAT SHOCK PROTEINS IN THE PROCESS OF SALINITY ADAPTATIONS IN EURIHALINE AND FRESHWATER CILIATES

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The ecological potencies of unicellular organisms might be reflected by the initial level of major inducible heat shock protein of 70kDa family (HSP70), as well as by the behaviour mode of their chaperone system in toto. Freshwater ciliates *Paramecium jenningsi* and *Tetrahymena pyriformis* and euryhaline *P. nephridiatum* acclimated to various salinity media were studied in these terms. The constitutive level of HSP70 in *P. nephridiatum*, acclimated to fresh water (0‰), was higher than in *P. jenningsi* from the same medium. In the cells of *P. nephridiatum*, acclimated to 10‰, the HSP70 level was lower than in 0‰. In *T. pyriformis* from 0, 2 and 10‰ the contents of HSP70 were more or less comparable, but the specters of constitutive HSPs differed. In *P. nephridiatum* salinity shock (transfer from 0 to 10 ‰ for 1 h) did not cause the induction of HSP70 synthesis, to the contrary, some decrease of the protein content was observed, whereas the reciprocal transfer resulted in an increase of HSP70 level. In *T. pyriformis* we could not cause the induction of HSP70 synthesis after shocks in both directions of salinity changes. Euryhaline (*P. nephridiatum*) ciliates appear to be somehow pre-adapted to abrupt environmental changes by the highest extent of constitutive HSP70 level and reactivity of their chaperone system, to compare with that of freshwater *P. jenningsi* and *T. pyriformis*, the latter may be characterized as some intermediate type. Differences in two freshwater ciliates may also be due to their different origin: *P. jenningsi* is a rather new natural isolate and the amicronuclear strain GL of *T. pyriformis* has been traditionally culturing under stable laboratory conditions.

## POLAR TUBE PROTEINS IN MICROSPORIDIA

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Microsporidia are obligate intracellular parasites forming environmentally resistant spores. Members of this phylum possess an invasive apparatus composed of a highly specialised structure, the polar tube that is unique in the eukaryotic world and represents one of the most sophisticated infection mechanisms. The invasion process involves the sudden extrusion of the sporal polar tube (also called spore germination) for initiating the entry of the parasite into a new host cell. Three polar tube proteins the so-called PTP1, PTP2 and PTP3 have been identified by our team in the mammal's microsporidia *Encephalitozoon cuniculi*. Tandemly-arranged genes encoding the major PTP1 and PTP2 have been also reported in the two other *Encephalitozoon* species as well as in *Antonospora locustae* and *Paranosema grylli*, microsporidian parasites of insects. Surprisingly, several genes coding PTP2-like proteins are found in the genome of *A. locustae*. We also recently identified two new polar tube components (PTP4 and PTP5) in *E. cuniculi* and *A. locustae*. In both species, the genes encoding these new PTPs are similarly clustered on one chromosome and present no homology with proteins from databases. The PTP4 in *A. locustae* is of peculiar interest, since this protein seems to be only localized at the end of the extruded polar tubes, suggesting a potential role in host cell adherence. Some preliminary experiments to characterize interactions between PTPs during formation and functioning of the polar tube have been undertaken.

## MOLECULAR BIOLOGICAL IDENTIFICATION OF CULTURED AND NATIVE PROKARYOTIC ENDOCYTOBIONTS OF *ARCELLA* SPP.

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Although in recent years numerous bacterial endosymbionts of free-living amoebae have been reported and characterized by molecular methods and many of them were identified as possible pathogens, the efforts for their cultivation mostly failed. In this study, intracellular bacteria of *Arcella* spp. were not only detected by fluo-

rescence in situ hybridization (FISH), but also successfully cultivated. Single *Arcella* cells were rigorously washed in sterile water and spread on several types of agar plates, designed to imitate the intracellular composition of the host cells. Based on the analysis of their 16S rRNA gene, a phylogenetically diverse group was identified, with members belonging to the α- and β-Proteobacteria, Flavobacteria, Verrucomicrobia and Firmicutes. Closest relatives of some isolates were earlier identified by other researchers as amoebae-resisting bacteria, such as *Candidatus Chryseobacterium massiliae*. Some other isolates represented new taxa or possible human pathogens. The strains were also characterized for ecological tolerance, morphological and basic biochemical properties. Besides culture-based methods, direct PCR-based techniques (molecular cloning) were also used to achieve a complete view on the bacterial spectrum of the Arcella host. The validity of our findings was proved by FISH using specific oligonucleotide probes and by electron microscopy.

## EM AND 3D RECONSTRUCTION OF NUCLEOLAR APPARATUS IN THE MACRONUCLEUS OF THE CILIATE *DIDINIUM NASUTUM*

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Nucleoli are essential structural components of macronuclei of ciliates. The aim of this work was to follow fine structural changes in interphase nucleoli of recently fed and starved *D. nasutum* cells by means of three-dimensional reconstruction on the basis of serial ultrathin sections. To our knowledge, this is the first attempt to study the nucleolar apparatus in ciliates using 3D reconstruction. In both recently fed and starved cells, two types of nucleoli were observed. Some of them looked like compact round structures less than 1 μm in size (mainly 0.2-0.6 μm). Another portion of nucleoli was represented by large structures of irregular shape, exceding 1 μm in size. On the individual sections the number of small (0.2-0.6 μm) nucleoli was about 45% of all nucleoli. 3D models showed that in fact most of them were parts of large nucleoli. Only a few of them made an exception and the total volume of such "free" nucleoli was less than 1.7 % of large nucleoli. Our data showed that large nucleoli, looking on the single sections like individual separate structures, appeared to be parts of the large complicated branchy nucleolar networks. A 30 h starvation did not lead to disintegration of this network, but stimulated formation of numerous vacuoles in the granular component of nucleoli, which became more condensed. Unlike starved *D. nasutum*, in fed ciliates numerous holes appeared in the fibrillar component located at the periphery of nucleoli. These holes may presumably serve as channels for transporting newly synthesized rRNA.

## GROWTH OF *EUPLOTES VANNUS* VAR. *BALTICUS* KAHL, 1932 ON THE MARINE OIL OXIDIZING YEASTS

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Ciliates participate in self-purification processes in marine environment. Flows of pollutants, such as oil, may occur via alimentary chain: oil → oil-oxidizing bacteria → infusoria. It is well known that infusoria feed on bacteria, including oil-oxidizing ones. At the same time, we did not find any reported evidences of ciliates' feeding on marine oil-oxidizing yeasts. To study possibility of using marine yeasts for food, the ciliates *E. vannus* were isolated from the periphyton of the Artillery Bay sea-wall (Black Sea) into laboratory culture. Ten ciliates (cell size 81 μm) were put into Petri dishes with marine sterile water, to which 5 drops of the marine yeasts culture with initial concentration of 13000 cells/ml were added. Necessary concentrations were obtained by 10-, 100- and 1000- times dilution of the yeast culture. Numbers of infusoria and yeast cells were counted with JENAVAL X 500. Active uptake of yeast cells by infusoria was recorded; they were observed intact inside infusoria bodies and were photographed. These results suggest the existence of yeasts → infusoria chain in the process of transformation of the oil pollution flow.

## SIBLING SPECIES CONCEPT IN CILIATES *PARAMECIUM* REVISITED: ZOOGEOGRAPHIC AND GENOMIC ASPECTS OF SYNGEN BIOLOGY

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Importance of two speciation mechanisms, namely, geographic and reproductive isolation, was examined on different levels (genetic species - syngens - strains) within *Paramecium*. Geographic distribution of *Paramecium* species in Northern hemisphere was investigated basing on long-term collecting of paramecia in nature. While no defined areas could be attributed to sibling species of *P. aurelia* complex, some *P. caudatum* syngens appeared to be geographically isolated, and true areas for some *P. bursaria* syngens were confirmed. Molecular phylogeny of five *P. bursaria* syngens inferred from mitochondrial cytochrome c oxidase I (COI) sequences indicated that syngens of this morphospecies already

have evolved separately far enough to be considered young genetic species. In ciliates, which are characterized by drastic genome rearrangement in each cell cycle, one kind of reproductive barrier may occur due to genomic incompatibility of two conjugating partners. So, we checked the level of genomic intra- and interspecific polymorphism of the strains isolated from geographically distant populations of *Paramecium* species. Macronuclear genomes of 15 species belonging to *P. aurelia* complex were analyzed by pulsed-field gel electrophoresis (PFGE). PFGE profile, similar within complex, still may serve as an "identity card" for most of its species, though intraspecific variation of PFGE profiles was observed within some species, sometimes - between geographically isolated strains. *P. jenningsi*, unlike all other *Paramecium* morphospecies, appeared to be very similar by PFGE profile to *P. aurelia* species. According to available in literature and own data, we suggest that this species belongs to *P. aurelia* complex. Supported by RFBR grants 06-04-49504 and 07-04-10073.

**CHARACTERIZATION OF TAXONOMICAL AND FUNCTIONAL GROUPS OF THE CILIATE COMMUNITIES FROM TWO BRACKISH COASTAL LAGOONS OF THE NORTHWEST OF SPAIN: LAGOON OF VIXAN AND LAGOON OF SAN PEDRO DE MURO**

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The structure of the ciliate community that develops in the superficial water of the brackish lagoons of "Vixan" and "San Pedro de Muro" (Galicia, Spain) was analyzed between March 2000 and March 2002. Ciliate communities, which include the main free-living taxonomical categories of the phylum Ciliophora, inhabit periphyton of these lagoons. After we have characterized specific richness of the lagoons, temporal variations, and the "resident component" of both lagoons, it was possible to sort the ciliated community into few groups and to classify the species in each of them according to taxonomic, trophic and size (cellular length) criteria. This simplification of the ciliate community allows us to study its structure in a versatile way, and to obtain a concrete ecological model of the community, that permits revealing the role and activity of each species inside the community, as well as its comparison with other communities. Taxonomic, trophic and size structure of ciliate communities of both lagoons had similar variety and complexity of groups, and it was relatively stable throughout the whole study, suggesting that the succession of species in both communities always took place among species able to carry out similar ecological functions and to occupy similar niches. Supported by the Project PGIDT01PXI20001PR (Xunta de Galicia).

**VARIATION OF THE SAPROBIC POLLUTION DEGREE IN TWO BRACKISH LAGOONS OF THE NORTHWEST OF SPAIN (VIXAN AND SAN PEDRO DE MURO LAGOONS), USING CILIATES (PROTOZOA: CILIOPHORA) AS INDICATORS OF WATER QUALITY**

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Ciliate communities of two brackish lagoons of the Galician coast (northwest of Spain) were studied from March 2000 to March 2002, using the Saprobic System to test saprobity variations in each lagoon throughout the studied period. Presence or absence of certain species is a consequence of environmental water conditions, related to decomposition of organic material. This characteristic differs the Saprobic System from other systems based on the "species deficit", related exclusively to the total species number in the community, without caring of the species composition and without classifying them according to different degrees of pollution. Prevalence of β-mesosaprobic and α-mesosaprobic species in both lagoons indicated a moderate-high level of saprobity, which was quantified by the Pantle and Buck method (1955) to determine the saprobic index. Although San Pedro de Muro lagoon had a higher saprobic index than Vixan lagoon during the whole study, differences in the saprobic pollution of both lagoons were small. "S" values of the index were recorded inside the α-mesosaprobic area, with water quality of class III during most months, according to classification of Friedrich (1990). These results show the progressive eutrophication of the ecosystems marked by invasive growth of the common reed (*Phragmites australis*), and necessity of control of this plant in order to conserve the diversity and richness of these environments. Supported by the Project PGIDT0 1PXI20001PR (Xunta de Galicia).

**THE TERMITE FLAGELLATE *MIXOTRICHA PARADOXA*: ATTACHMENT AND CYST FORMATION OF ECTOBIOTIC SPIROCHETES**

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The symbiotic flagellate *Mixotricha paradoxa* (Parabasalia) lives in the gut of the termite *Mastotermes darwiniensis*. Its association with ectobiotic spirochetes and bacterial rods is investigated with light and electron microscopy. Treatment with different chemicals which disturb molecular interactions and the use of the freeze-

fracture and freeze-etch techniques show that hydrophobic interactions and integral membrane proteins seem to be involved in the firm attachment at the contact sites. There is a complex and regular system of attachment sites for spirochetes and bacterial rods. The adhering and metachronically rowing spirochetes propel the cell. Addition of antibiotics to the food of the termites reduces the number of ectobiots and leads to a disintegration of the cortical attachment systems. As a result, *Mixotricha* becomes spherical and immotile. Most of the cells die after some days. Antibiotics have a further effect: they lead to a transformation of a part of the spirochetes into cystic bodies. Cyst formation of ectobiotic spirochetes is reported here for the first time. Starvation has a similar, though less dramatic, influence than antibiotics. The cysts are rounded (ca. 1 μm) and contain protoplasmic cylinders in their periphery and sometimes larger central bodies as well. The ectobiotic spirochetes of *Deltotrichonympha* sp. living in the same termite form similar cysts when antibiotics are applied. The production of dormant cystic forms may be a mechanism of survival under hostile conditions.

**PRESENCE AND SEASONAL DISTRIBUTION OF FREE-LIVING AMOEBAE IN A GROUNDWATER SYSTEM**

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The presence of eukaryotic microorganisms in groundwater systems has been studied seriously only since recently. The first microbiological investigations of shallow water table aquifers indicated that prokaryotes were the dominant microorganisms present, and that eukaryotic might be absent altogether. However, subsequent studies showed the presence of limited populations of eukaryotes in groundwater systems. So, protozoa were demonstrably present, but in low numbers. While the presence of eukaryotic microorganisms is low in pristine aquifer, there is evidence that, in aquifers contaminated by organic matter, the abundance of the eukaryotic may be much higher. This is, probably, a result of higher growth rates of bacteria which support larger populations of bacterivorous protozoa. The aim of the study was to determine presence and distribution of free-living amoebae (FLA) in an aquifer of Mexico. Groundwater samples from ten wells of the aquifer of Cuernavaca, located in the center of Mexico, were collected monthly between May 2005 and April 2006. Samples were seeded onto non-nutritive agar with *Enterobacter aerogenes*, and free-living amoebae identification was carried out on the basis of morphological features. Free-living amoebae were found in all the sampled wells of the aquifer. The isolated amoebae belonged to thirteen genera: *Hartmannella, Naegleria, Vannella, Vahlkampfia, Rosculus, Platyamoeba, Thecamoeba, Vexillifera, Nuclearia, Saccamoeba, Echinamoeba, Guttillinopsis, Acanthamoeba, Paratetramitus* and *Cochliopodium*. The most frequently encountered was *Hartmannella*. The highest numbers of FLA were in May and June the lowest in October.

**MOLECULAR PHYLOGENY OF *HOLOSPORA* - INTRANUCLEAR SYMBIOTIC BACTERIA OF *PARAMECIUM* SSP.**

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Bacterial genus *Holospora* was described by Hafkin (1890) and redescribed by Gromov and Ossipov (1981). At present genus *Holospora* includes 9 species; each of them is specific to one or another *Paramecium* spp., and to one of two nuclear types: macronucleus or micronucleus. Application of 16S rDNA molecular phylogeny attributed *Holospora* to Alphaproteobacteria and revealed close relation of two *Holospora* spp. studied, namely *H. obtusa* and *H. elegans* (Amann et al., 1991). They both together with distantly related intranuclear bacteria of *Paramecium* - *Caedibacter caryophilus*, and some other endobionts of acantamoeba, constitute an early diverged branch (family Holosporaceae) of the order Rickettsiales (Emelyanov, 2003 and this volume). These bacteria possess complicated life cycle with infectious and reproductive stages. It means that they are able to be transmitted both vertically and horizontally. The mode of transmission is supposed to influence symbiont evolution and co-evolution with the host. Here we investigated 16S rDNA phylogeny inside genus *Holospora* and compared it with *Paramecium* phylogeny (Struder-Kypke et al., 2002). Supported by grants: RFBR 07-04-01755 and RNP 2.2.3.1.4148.

**PECULIARITY OF THE CLINICAL ISOLATES OF *TRICHOMONAS VAGINALIS***

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Human urogenital parasite *Trichomonas vaginalis* exists in different morphological forms: pearlike motile, amoeboid, and round immotile. Biological significance of polymorfism is unclear. Two morphotypes (pearlike and round) were observed in clinical speciments from patients with different forms of urogenital trichomoniasis. Strains of parasite were isolated and were maintainded in TYM Diamonds medium. Morphotype did

not change in the course of cultivation. By use of transmission electron microscopy pecularities of the parasite ultrastructure were investigated. Round forms possessed 5 flagella, but were not motile, and preserved basic features of cell structure. Nucleus, typical for trichomonads, was located anteriorly; pelta and costa extended from kinetosomes. Unlike pearlike forms, in round forms reduction of undulating membrane, axostyle and redistribution of hydrogenosomes were observed. Round morphotype of *T. vaginalis* differed from already described cysts of other parabasalids by its ultrastructural organization. On the basis of data on morphology, as well as on abilities to reproduce in humans, to induce inflammatory process and to grow on the selective medium in laboratory conditions, round form of *T. vaginalis* may be considered as virulent, but not degenerative form. Fagocytosis of different morphotypes were examined by Confocal Laser Scanning Microscope Leica TCS SP 5. The role of phagothrophy in pathogenesis is discussed. Methods of identification of *T. vaginalis* morphotypes in clinical samples are proposed.

### *IN-VITRO* COMPARATIVE EVALUATION OF FOUR PLANT EXTRACTS (KIWI, MULBERRY, POTATO AND MILFOIL) ON HUMAN PARASITIC PROTOZOA

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Human parasitic protozoa affecting the gastrointestinal tract are the major cause of morbidity, and contribute to mortality worldwide. Therefore suitable control and treatment methods with minimum adverse effects, which do not provoke development of drug resistance, are demanded. We tested possibility of application of natural plant extracts as drugs against intestinal parasitic protozoa. Specimens were obtained from diagnostic medical laboratories and were studied using direct microscopic observation. Hemogenation, filtration and supernatant isolation by centrifugation were used to prepare natural kiwi, potato, mulberry or milfoil extracts. In the first series of experiments volumes of parasite suspensions and extracts were added in ratio 1:1, and in the second - 1:3. The observed decrease in parasites numbers registered in 24, 48, and 120 hours was respectively: for potato extract 40%, 46%, 46%; for kiwi 40%, 53%, 54%; for mulberry 57%, 57%, 60%; and for milfoil 60%, 98%, 98%. In the second series decrease rates for potato, kiwi, mulberry and milfoil were 77%, 62%, 88% and 98%, respectively. Results of this study indicate that natural plant and fruit extracts are useful for control and treatment of protozoan infections, and suggest that future studies should be performed in order to investigate effects of natural plant extracts on human intestinal protozoa *in vivo*.

### THE PARASITOPHOROUS VACUOLE MEMBRANE OF *ENCEPHALITOZOON CUNICULI* CONTAINS PORES WHICH SHOULD ALLOW AN EXTENSIVE PARTICIPATION OF THE HOST CELL METABOLITE POOL

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Microsporida are intracellular organisms of increasing importance as pathogens in immunocompromised patients. Since the reduced genome of the model organism *Encephalitozoon cuniculi* displays an extreme loss of biosynthetic pathways, we characterized the amino acid requirements of this pathogen. For accurate quantification of microsporidian replication rate, we developed a cell ELISA, which is based on combination of two stage specific monoclonal antibodies, allowing a simultaneous detection of meronts, sporonts and spores in infected host cells. Cultivation in the medium which is depleted in individual amino acids, resulted for most of the lacking amino acids in a strongly reduced replication rate. This provides experimental evidence for the lack of a de novo amino acid synthesis in *E. cuniculi* and reveals the extreme dependency of this organism on the host cell metabolite pool. Since *E. cuniculi* resides inside a parasitophorous vacuole (PV), nutrients from the host cell need to cross this barrier before entering the pathogen itself. Microinjection of fluorescent dyes into the cytosol of infected cells showed that a 0.5 kDa molecule could rapidly enter the vacuole, while a 10 kDa molecule was stably excluded from the PV lumen. These experiments indicate that the PV membrane possess pores with an exclusion size of 10 kDa or less, while smaller molecules, e.g. amino acids can pass. We recently demonstrated that the parasitophorous vacuole membrane of *E. cuniculi* lacked host cell membrane proteins immediately after invasion. The biogenesis of this membrane is thus still unclear and is currently under investigation in our laboratory.

### NEW DATA ON HYPERPARASITIC MICROSPORIDIANS FROM POLYCHAETES OF THE WHITE SEA

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Five hyperparasitic microsporidian species, *Metchnikovella hovassei* (gregarine *Lecudina pellucida* from polychaete *Nereis virens*), *M. selenidii* (*Selenidium* sp. from *Ophelia limacina*), *M. polydorae* (*Selenidium* sp. from *Pygospio elegans*), *Amphiamblys capitellae* (*Ancora sagittata* from *Capitella capitata*) and one unknown species (*Lecudina* sp. from *Pygospio elegans*), were revealed

during faunistic investigations of the White Sea. Cysts of the unknown microsporidium had the internal light-retracting and external layer with spiral buldges. Cysts resided in the parasitophorous vacuole connected with the gregarine pellicle from the inside. These characters are new to microsporidia and give grounds to establish a new genus *Vivieria* (in honor of E. Vivier) with a species *Vivieria spiralis*. Because of lack of data on fine structure of cysts and spores, as well as on the life cycle of this species, the systematic position of this genus remains unknown. We observed dividing plasmodia with nuclei coupled in diplokarya during our studies of *A. capitellae* life cycle. We suggest these plasmodia to be a result of merogonial reproduction, though merogony seems not to be obligatory. The host gregarines are usually very small, and intensive multiplication of parasites does not seem feasible within such a limited space. A single cyst with 16 falciform spores is regularly formed at the end of sporogony. When gregarine is big enough to hold numerous meronts, merogony takes place. Up to 80 cysts can be produced in this case. The presence of diplokarya in the life cycle of *A. capitellae* supports the view that the genus *Amphiamblys* belongs to the family Amphiacanthidae (Larsson, 2000).

## *HOLOSPORA* TRAFFICKING IN *PARAMECIUM*: THE ROLE OF THE HOST CELL CYTOSKELETON

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Bacteria of the genus *Holospora* are known to be obligate endonucleobionts of paramecia. They belong to α-proteobacteria and show species and nuclear specificity. *Holospora obtusa* invades the macronucleus and *Holospora undulata* - the micronucleus of *Paramecium caudatum*. *Paramecium/Holospora* system has lately become a model one for investigating various aspects of symbiont-host interactions, the way of bacterial transportation in the host cell cytoplasm to the target nucleus being among most crucial for the infection. The bacterium gets into the host cell via phagocytosis but soon escapes the digestive vacuole and reaches the target nucleus. The present study sought to find out whether *Holospora* exploits the host cell cytoskeleton to get to the nucleus. For this purpose the early stages of experimental infection were either subjected to immunocytochemical study with polyclonal antibodies against paramecium actin 1-1 or stained with rhodamine-phalloidin. *H. obtusa* was shown to cause formation of actin "comet tails" in the paramecium cytoplasm at the early stages of infection. To get additional evidence, experimental infection of paramecia transfected with different GFP-fused actin constructs is underway. The analysis of the experimental infection performed under nocodazole treatment demonstrated that disassembly of microtubules blocked the entry of bacteria into the nucleus, without affecting their transportation in the cytoplasm. Taken together our data suggest that host actin may play an important role in propelling *Holospora* to the target nucleus at the early stages of infection, while host microtubules appear to be required only for its proper sluicing into the nucleus.

## MORPHOLOGY, INFRACILIATURE AND PHYLOGENETIC ANALYSIS OF A NEW TINTINNID CILIATE (CILIOPHORA, CHOREOTRICHIA) FROM THE COASTAL LAKE FARO (SICILY, ITALY)

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A new planktonic tintinnid ciliate of the genus *Tintinnopsis*, found in the meromictic coastal Lake Faro (Sicily, Italy), is described from both living cells and quantitative protargol-stained preparations. The sequence of the small subunit rRNA (SSU rRNA) gene is also reported. Loricae of this species are composed of a subcylindrical bowl, featuring a fine mesh of hexagonal structures, with sparse biogenic matter adhering on the chitinous basal layer, and of a branched aboral horn, characterized by an alveolated texture. The lorica length and oral diameter ranges are respectively 152-205 μm and 33-44 μm. Fully extended living cells are elongate obconical, 107 to 144 μm in total length and 28 to 38 μm in maximum width. Two ellipsoidal macronuclei are always present. The somatic ciliary pattern of this species comprises a ventral, a dorsal, and a posterior kinety as well as right, left, and lateral ciliary fields. Loricae of *Tintinnopsis* n. sp. are similar to those of *Tintinnopsis* (*Paratintinnopsis*) *corniger* (Hada, 1964) in their overall shape and in the aboral horn texture. Important differences, however, lay in the presence of 12-15 spiral turns at the oral end and in the scarce agglomeration of foreign particles in *Tintinnopsis* n. sp., the latter character contrasting with the typical traits of the genus. The phylogenetic analysis of this new species, based on the SSU rRNA gene, shows it to group with other *Tintinnopsis* species, but at a basal level in this clade. Supported by Natural and Engineering Science Research Council of Canada.

## GENOTYPE OF OPPORTUNISTIC PROTOZOA IN LATE-STAGE HIV-INFECTED THAI PATIENTS

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We performed a cross-sectional study to determine opportunistic intestinal parastic infections in HIV/AIDS patients attending Wat Prabaht Nam Phu, a hospice AIDS care center in Thailand during February 2004 to January 2005. Of 90 Thai HIV/AIDS patients with diarrhea, 59 (65.6%) had full-blown AIDS. Only 20 patients (22.2%) had a history of antiretroviral treatment. The mortality rate was 43.3% during one year period. The prevalence of intestinal opportunistic parasitic infections was 42.4 %. By nested PCR, *Cryptosporidium* was the most common (34.4%). For species specific diagnosis, RFLP comprised *C. hominis* (27, 87.1%) and *C. meleagridis* (4, 12.9%). *Enterocytozoon bieneusi* genotype D was identified (5, 5.6%) by specific PCR amplification and sequencing of 243 bp ITS region of rRNA gene. *Enterocytozoon intestinalis* was not detected. *Cyclospora ceyatonensis* and *Isospora belli* were each detected in one patient. Multivariate analysis showed that patients who had watery and/or mucous diarrhea and having abnormal stool color were independently associated with opportunistic intestinal protozoa infections.

## TO WHAT EXTENT IS THE DICHOTOMY BETWEEN DYNAMIC PELAGIC AND STABLE BENTHIC OCEANIC ENVIRONMENTS REFLECTED IN THE SPECIES RICHNESS AND LONGEVITY OF DOMINANT FOSSILIZABLE PROTISTS?

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Marine protists live in two clearly separated environments: the water column for the plankton and the sea floor for the benthos. The plankton is subject to rapid changes and has to adapt quickly to new conditions, whereas the benthos lives in a more stable environment and is thought to evolve more slowly. Among the fossilizable marine protists, coccolithophores (Haptophyta) and foraminifers (Foraminifera) have left a rather complete fossil record and have been extensively studied by micropaleontologists. Coccolithophores are planktonic, whereas foraminifers are either planktonic or benthic. In both groups, the shell is the basis of species recognition. Several questions arise with this morphospecies concept: do these paleontological species correspond to biological species? Are there enough criteria on the shell to distinguish species? Are such criteria distinguishing true species or just different ecophenotypes? Because both groups have still extant representatives today, it is possible to compare the information given by the morphology with the one given by the genetics. Molecular phylogenetic studies show frequent cases of cryptic speciation in coccolithophores and planktic foraminifers, indicating that the used morphological criteria are not sufficient to recognize phylogenetic species. For allogromiids (benthic agglutinated forminifers), the genetic variability is also considerably higher than the observed one. However, in rotaliids (benthic calcitic foraminifers), the situation seems to be more complicated. Additionally, the hypothesis that planktic organisms evolve faster than benthic ones seems to be confirmed by molecular phylogenetic studies performed on foraminifers.

## THE ACTIN MULTIGENE FAMILY OF *PARAMECIUM TETRAURELIA*

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*Paramecium tetraurelia* contains an actin multigene family with at least 30 members encoding actin, actin-related and actin-like proteins. They group into twelve subfamilies; a large subfamily with 10 genes, seven pairs and one trio with >82% amino acid identity, as well as three single genes. Analysis of different features on nucleotide and amino acid level (e.g., the number and position of introns; the actin consensus regions; amino acids of the intermonomer interface in filaments, binding sites for ATP, myosin and actin-specific drugs) revealed striking differences in isoforms of actin and actin-related proteins in *P. tetraurelia*, both within the organism and in comparison to other organisms. This diversification suggests unprecedented specification in localization and function within a unicellular eukaryote. To localize the different actin paralogs and to disclose functional implications, we used overexpression as GFP-fusion proteins and antibody labeling, as well as gene silencing. Several isoforms are associated, in different forms, with food vacuoles of different stages. Some paralogs occur in cilia. A set of actins are found in the cell cortex where actin outlines the regular surface pattern. Labeling of defined structures of the oral cavity is due to still other actins, while others are distributed in a pattern suggesting association with the numerous Golgi fields. Silencing of the respective actin genes/gene subfamilies entails inhibitory effects on organelles compatible with localization studies. Knock-down of the actin found in the cleavage furrow abolishes cell division, while silencing of other actin genes alters vitality, cell shape and swimming behavior.

## WHICH PROTISTS WERE THE ANCESTORS OF METAZOA?

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Some researchers consider that the ancestors of the Metazoa were flagellates, some hold that they were ciliates, others nominate for the role of metazoan ancestors amoeboid organisms. The origin of multicellularity is usually explained by non-disjunction of individuals in the series of subsequent cellular divisions, which occurs in some protists (colonial hypotheses). At the same time, temporary or permanent multicellularity is also known to arise in protists by cytotaxis. In this case, conspecific individuals are attracted to each other by special attractants secreted by them. In some protists, e.g. in representatives of the Dictyostelida, metazoan-like individuals formed as the result of contact aggregation of separate mixamoebae (pseudoplasmodia) can move and orient themselves. Analysis of the available data shows that in some Metazoa, especially those from the phyla Placozoa, Spongia and Cnidaria, oocytes and blastomeres of the cleaving embryo are capable of amoeboid movement. However, the cells of the newly formed blastula are flagellated. In the course of gastrulation, some of them may lose undulipodia and revert to amoeboid movement. After experimental dissociation, the cells of lower metazoans (Placozoa, Spongia, Cnidaria) start active movement, flagellar or amoeboid. They gradually move close together by means of cytotaxis, and contacts aggregation occurs; later cells are re-assorted and the original multicellular individuals are reconstructed. The above facts testify in favour of the hypothesis that the ancestors of the Metazoa were some amoeboflagellates, now extinct. Choanoflagellata, which are incapable of either aggregative contact behavior or amoeboid movement, can hardly be considered as possible ancestors of the Metazoa and even of their close relatives.

## *PRODISCOCEPHALUS*: AN EUPLOTID OR STICHOTRICHOUS CILIATE? PHYLOGENETIC ANALYSIS INFERRED FROM ONTOGENETIC AND 18S RRNA GENE INFORMATION (CILIOPHORA: SPIROTRICHEA)

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The *Prodiscocephalus*-like ciliates which are known as cephalized hypotrichs or euplotids, and belong to a unique and distinct group discocephaline are confused considering their phylogenetic position among traditional "hypotrichs" (*s. l.*). The main reasons thereby are that these organisms exhibit many intermediate morphological features and most of them are lacking ontogenetic as well as molecular investigations. As the first one in this group, the complete small subunit rRNA gene of a poorly-known species, *Prodiscocephalus borrori* was sequenced and analyzed, and molecular trees were constructed. Meanwhile, the cortical development during binary division was observed and compared with other related taxa. Based on the data obtained, evolutionary relationships of *P. borrori* within the class Spirotrichea were determined. The results indicate that this taxon is likely an outgroup to the typical hypotrichs (*s. l.*), while the morphogenetic features suggest it might be an intermediate form between hypotrichs and euplotids, yet closer to the former. Hence it might represent an ancestor form for both groups. This understanding disagrees with the suggestion of Lynn and Small (2002), that the suborder Discocephalina might be a sister group to other typical euplotids. Basing on the present work we presume the suborder Discocephalina Wicklow, 1982 should be regarded as a sister clade to other typical hypotrichs, i.e. as an order Discocephalida Wicklow, 1982 nov. grad. in the subclass Stichotrichia Small and Lynn, 1985.

## MULTIPLE TRANSCRIPTION INITIATION SITES IN THE MAXICIRCLE KINETOPLAST DNA OF MONOGENETIC TRYPANOSOMATID *LEPTOMONAS SEYMOURI*

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Transcription of the maxicircle mitochondrial genome of Trypanosomatidae remains poorly studied: little is known about promoters and termination signals, about the number and composition of transcribed RNAs. We constructed a partial transcription map for the maxicircle of insect trypanosomatid *Leptomonas seymouri* using hybridization, RT-PCR, and RACE methods. We identified four polycistronic transcripts (comprising the following genes: *12SrRNA-9SrRNA-ND8, ND7-COIII-Cyb-A6, COII-MURF2, RPS12-ND5*) and three monocistronic primary transcripts (*ND1, COI, ND4*). Mature monocistronic transcripts of the *ND8, COII*, and *MURF2* genes, apparently resulting from cleavage of the primary transcripts, were also identified. The other two polycistronic RNAs remained intact. According to the constructed map some intergenic spacers (before the 12*SrRNA, ND7, ND1, COII, COI, ND4*, and *RPS12* genes) must contain transcription initiation sites. Those spacers were shown to promote transcription of an artificial construct in isolated mitochondria of *Leptomonas seymouri* (*in organello* transcription system) using the following protocol: a fragment comprising the 5'-end region of the primary transcript and the upstream spacer was cloned in a plasmid vector, then a linear construct containing this fragment flanked by a vector sequence was obtained by PCR and introduced into isolated mitochondria;

transcription of this construct was assessed by RT-PCR with transcript- and vector-specific primers. It was also demonstrated that a short sequence (24 bp) upstream of the 12S rRNA transcription start site was essential for transcription initiation *in organello*, shorter sequences being largely ineffective. Thus, maxicircle fragments involved in transcription initiation were directly mapped for the first time.

## ANCIENT SOIL CILIATES (PROTOZOA, CILIOPHORA) ISOLATED FROM PERMAFROST: MORPHOLOGICAL AND MOLECULAR STUDY

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Permafrost is the unique environment which is capable of protecting microorganisms for long-term preservation. The purpose of this study is to detect viable forms of ciliates in permanently frozen sediments of late Pleistocene and Holocene age, isolate and characterize these organisms. A total of 200 samples of Arctic permafrost, buried soils and burrows of fossil rodent ranging from several hundred to 3 Myr were investigated for viable ciliates. To avoid contamination of samples we developed the protocol in which the necessary requirements concerning temperature conditions and sterility, were added. Ciliates' resting cysts were reactivated using the non-flooded Petri dish method (Foissner, 1987), which allowed us to isolate several common soil ciliates species of the genus *Colpoda* from horizons differing in age and origin. Species were identified by various silver impregnation techniques (Foissner, 1997). Thus, four ancient ciliates cultures derived form 30000-year-old paleosol were identify as *Colpoda steinii* (2 cultures), *Colpoda inflata* and *Colpoda* sp., one strain isolated from permafrost samples of late Pleistocene icy complex (depth 7m) - as *Colpoda* sp. and two isolates obtained from Holocene deposits (depth 1.0 and 1.3 m) - as *Colpoda steinii*. Morphological analysis of four clones of *C. steinii* isolated from sediments aging back to 100 - 30000 years was carried out. Results of analysis of variances (ANOVA) applied to morphometric data showed non-significant difference between isolates. Besides, morphometric characteristics of contemporary species were in close correspondence to those of ancient species. Phylogenetic analysis based on partial sequencing of 18S rRNA genes (up to 1200 bp) and using the neighbor-joining method revealed a high similarity between strains of *C. steinii*. All of the ciliates, cultures shared 99% of the 18S rRNA gene sequence identity with previously described *C. steinii* from Ribosomal Data Base.

## DETECTION OF FREE-LIVING SOIL PROTOZOA PRESERVED IN EASTERN ARCTIC PERMAFROST

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Viable ancient microorganisms are known to be present within the permafrost. For the first time the viable protozoa were discovered in frozen layers, and their ability to survive within permafrost during dozen thousands years was shown. A total of 200 samples of Eastern Arctic permafrost and buried soils (including burrows of fossil rodent) were screened for presence of viable protists. Samples of permafrost sediments and buried soils were sterile obtained by sampling from frozen walls of outcrops or from drilling cores down to 25 m depth. The longevity of protozoans cryoconservation corresponds to the permafrost age. Only 28 samples originated from the late Pleistocene and Holocene layers were inhabited by viable representatives of almost all main groups of protists: naked amoebae (Lobosea: Vannellidae, Acanthamoebidae, Leptomyxidae, and Heterolobosea: Vahlkampfiidae), heterotrophic flagellates (Choanomonada, Cercomonadida, Kinetoplastea), and ciliates (Ciliophora: Colpodidae, Platyophryidae, Vorticellidae). The oldest isolated microorganisms dated back to the ~30 thousand years. Isolated paleoprotozoans were represented by relatively small forms (5-50 μm), and most of them were species of soil microfauna commonly distributed worldwide. The life cycle of protists certainly includes a cryptobiotic stage that characterized by formation of resting cysts. The general tendency of increasing the number and diversity of protozoa within buried soils and burrows of fossil rodent was established. This fact may be explained by more favorable conditions of cryopreservation in rich plant debris, and originally rich fauna. Correlations between viable protozoa populations and physicochemical characteristics of sediments (pH, ice content, chemical or textural composition) were not observed.

## DEPENDENCE OF HETEROTROPHIC FLAGELLATES ABUNDANCE ON THE HYDROCHEMICAL PARAMETERS IN DIFFERENT RESERVOIRS IN THE CENTRAL PART OF UKRAINIAN POLISSIA AREA

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It is well known that heterotrophic flagellates are capable of surviving in a broad range of abiotic factors. At the same time the highest numbers of these protists were detected in waters, which have a lot of organic sub-

stances. We studied dependence of heterotrophic flagellates abundance on several hydrochemical parameters (pH, dissolved oxygen content and organic substances) by one-way ANOVA. For species *Allantion tachyploon* Sandon, 1924, *Bodo globosus* Stein, 1878, *Goniomonas truncata* (Fresenius) Stein, 1887, *Phyllomitus apiculatus* Skuja, 1948 and *Rhynchomonas nasuta* (Stokes, 1888) Klebs, 1892 the reliable correlation between flagellates abundance and dissolved oxygen content was determined, whereas for *Ancyromonas sigmoides* Kent, 1880, *Bodo saltans* Ehrenberg, 1832, *Parabodo nitrophilus* Skuja, 1948 and *Protaspis simplex* Vors, 1992 the influence of concentration of soluble organic substances was significant. Furthermore, pH value appreciably affected the abundance of *Parabodo nitrophilus* Skuja, 1948.

## MORPHOLOGY AND GENE SEQUENCE OF AN ENDEMIC, NEW COLEPID (PROTOZOA, CILIOPHORA) FROM THE ANCIENT LAKE BIWA, JAPAN

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Endemism is difficult to prove in micro-organisms. However, the ancient freshwater lakes (Lake Baikal, Lake Tanganyika, Lake Biwa, Lake Ohrid) provide a unique opportunity to look for endemic flagship species. Indeed, some unique protists have been described from all the lakes cited, but mostly algae, while ciliates have been poorly researched. We investigated some samples from Lake Biwa for ciliates and found two undescribed flagship species which are likely endemic to the region or even to the Lake. Here, we report on a new colepid which belongs to a group of ciliates with highly conspicuous cortical scales. We used live observation, silver impregnation, scanning electron microscopy, and molecular biology (SSU rDNA) to characterize the new species. Morphologically, the new colepid differs from most other members of the group by the lack of spines near to the anterior and posterior end of the cell. Genetically, it is far away from the common, likely cosmopolitan *Coleps hirtus* (U97109) and two *Coleps* sp. (DQ 487194 and X 76646) contained in GenBank. Thus, our ciliate likely represents not only a new species but also a new genus. Interestingly, colepids without spines have been described also from Lake Baikal (Obolkina, 1995) and Lake Tanganyika (Dragesco, Dragesco-Kerneis, 1991). Thus, this group of ciliates provides strong support for ciliate endemism. Supported by the Japan Society of Protozoologists, the Lake Biwa Museum Comprehensive Research Project 06-02, and the MEXT, Kakenhi (no. 18760431) (grants to S. Shimano); the Austrian Science Foundation (grants to W. Foissner).

## VIABLE ANCIENT ACANTHAMOEBAE FROM PERMAFROST OF EASTERN SIBERIA: POSSIBLE REASONS FOR HIGH VIABILITY POTENCIES

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Heat shock protein of HSP70 family was revealed for the first time in the cells of *Acanthamoeba* sp. strain Am8 (Acanthamoebidae), which were excysted from cysts, isolated from the permafrost samples (Eastern Siberia) 30000-35000 years old and then cultivated in laboratory at 21 °C. High constitutive level of this protein about 60 kDa MW was demonstrated by the method of immunnoblotting in the unstressed trophozoites. This level was considerably higher, than that in unstressed cells of contemporary freshwater amebae from the genus *Amoeba* (Amoebidae). The comparison of HSP70 levels of those in contemporary representatives of the same genus (*Acanthamoeba* sp., strain 4465, collection of the Institute of Parasitology, Czech Republic) was carried out. The treatment of blot with the same antibodies did not result in revealing any zones. The data on temperature preferences of acanthamoebae strains show that strain Am8 is much more termotolerant than strain 4465. We failed to cause induction of this protein in Am8 cells either by heat (37 °C, 1 h), or by cold ( 8 °C, 1 h) shocks, both treatments resulting in decrease of constitutive HSP level. Paleoamoebae under study belong presumably to an amphizoic species *A. polyphaga*. Levels of HSP in amphizoic and free-living acanthamoebae may reflect the species virulence - low initial level and noticeable induction in free-living species and high initial level and weak anti-stress reaction in pathogenic ones. Unusually high constitutive level of HSP in the cells of paleoacanthamoeba might indicate the high survival potential, even in terms of geochronology.

## ULTRASTRUCTURAL FEATURES OF SPOROGONY OF SOME MICROSPORIDIA PARASITIZING BLOOD-SUCKING MOSQUITOES IN SIBERIA

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The developmental and ultrastructural features of sporogenesis of species of *Amblyospora* (Microsporidia) infecting *Aedes/Ochlerotatus* mosquitoes from Siberia were investigated. Sporogony and sporogenesis occur within a sporophorous vesicle. The vesicle contains

numerous secretory granules of different structure that are involved in formation of the mature spore. During sporogenesis, the episporontal space is cleared and there is a correlation between the structure of the secretory granules and the morphology of the exospore and polaroplast of the spore. A thick complex multi-layered exospore is formed in spores from those species that possess an episporontal space filled with small tubules arranged in large osmiophil lumps, or the space filled with fine-grained secretions. The polaroplast is composed of closely packed lamellae, and the polar filament coils are smooth and frequently arranged in several rows. Thin exospores, by contrast, are formed in those species that possess secretory granules composed of an accumulation of small filaments (fibers), or "tail-like" structures on the surface of envelopes of divided sporonts, sporoblasts and mature spores. Polaroplasts in these species contain closely packed lamellae and large chambers filled with a medium electron dense substance. Polar filament coils are arranged in a single row and inner membranes of the thin coils are stellate and folded on transverse sections. We consider the structure of the secretory granules of the sporophorous vesicle to be a useful taxonomic character in these species. Ultrastructural examination of mature spores further reveals that *Amblyospora, Parathelohania* and *Senoma* species possess polar filaments, which distal ends lie freely in the spore cavity closer to the posterior pole and end with a broad club-like membranous structure that is not connected with the nucleus or posterior vacuole.

## EPICYTE STRUCTURE DIVERSITY IN THE ASEPTATE GREGARINE FAMILY LECUDINIDAE

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Structures of epicytic folds in 16 species of the lecudinid gregarines from White Sea and Far Eastern (Sea of Japan) polychetes, turbellarians, nemerteans, and ascidians were compared with SEM and TEM methods. The high degree of epicytic fold structure diversity was revealed in the species observed. The surface morphology of the trophozoites varied in the degree of fold closeness, and in the way of their undulation: if the folds were located closely to each other, undulation was vertical; and if these were distributed rather rare, undulation was horizontal. The different species were also different in the shape of the folds on cross-sections and in the pattern of the fold internal structures. The most common shape of the folds was ampuliform or finger-like. Such folds had a typical set of internal structures: rippled dense structures (apical arches) and apical filaments in their tops without any supplementary formations. There was a well-developed fibrillar subjacent layer under pellicle with basal bridges separating the inside of the folds from the rest of cytoplasm, so that there used to be neither cell organelles nor inclusions. Some other species had an additional axial electron-dense structure in the fold tops. At last, some species had not the fibrillar bridges in the bases of the folds, so that some cytoplasmic organelles and inclusions were penetrating into the folds. In this case, micropores typically located between the folds, were shifting on the fold sides. High diversity of the cortex structure in the lecudinids could be connected with a low level of their evolution: the final type of the epicyte organization was not yet formed in lower gregarines. The differences among the epicyte structure also might be used as taxonomic markers (generic or specific) in this problem group.

## ORIGIN OF CHROMOSOMES IN PROTISTS

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In spite of the remaining uncertainty in the problem of origin of the contemporary taxa, there is a strong belief that just within the "primitive" unicellular organisms about 2 billion years ago protochromosomes were formed. In different times, various protists were attributed to the most probable direct descendants of the first eukaryotes on the Earth. At present two rather heterogeneous groups can be distinguished among protists in respect to the condensation levels of their chromosomes. Representatives of the first group display condensed chromosomes during the whole life cycle. Among these organisms, the best known are dinoflagellates. A substantial attention was paid to the structure of their chromosomes and the phylogenetic meaning was attributed to this cell characteristic. However, the further investigations clearly showed the secondary simplification of these unicellular organisms during evolution. On the contrary, members of the second group possess weakly condensed chromosomes even in mitosis. For these "invisible" chromosomes a special term was coined - "weakly condensed chromosomes" (WCCs, Skarlato, 2003). WCCs are common for bodonids, trypanosomatids, diplomonads, trichomonads, several groups of other free-living and parasitic flagellates and amoebae, haemosporidia, microsporidia, etc. It is tempting to speculate that such a characteristic as "weak condensation of chromosomes" has a phylogenetic meaning. Most probably the chromosome apparatus of protists has developed from a prokaryote-type genetic apparatus, undergone evolution within the ancient Protista (including the stage of weakly condensed protochromosomes), and finally achieved the eukaryotic state in contemporary unicellular and multicellular organisms. It is likely that

some of now existing eukaryotic microbes possess WCCs that have a number of protochromosome characters of their protistan ancestors. It can be assumed that it were the WCCs but not the permanently condensed chromosomes of Protista (as accepted earlier) that have served the starting platform for the evolution of genetic system of all eukaryotes. Supported by grant No. 07-04-00662 from the Russian Foundation for Basic Research.

## MOLECULAR EVOLUTION OF CHLORARACHNIOPHYTE NUCLEOMORPHS

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Chlorarachniophytes are a relatively small group of cercozoan protists that acquired photosynthesis through secondary endosymbiosis by engulfing a green alga. These amoebae are interesting because they are one of the two known groups of algae that have retained a vestigial nucleus of the endosymbiont, usually called the nucleomorph. The nucleomorph of *Bigelowiella natans* has been recently sequenced and showed an extremely compact structure. One of the striking features is a high density of very short spliceosomal introns, with a very narrow size range: 18 to 21 base pairs. Aiming to shed light on why these introns are retained in spite of their extreme size reduction, and how they are removed, we carried out comparative analyses by generating EST and genomic sequences from *Gymnochlora stellata*, a species from a lineage that diverged from *B. natans* at the base of the chlorarachniophyte tree. Our data from about 50 nucleomorph genes and 130 introns show that intron size is generally similar to *B. natans* (18 to 21 bp) with a slightly higher representation of 20 bp introns, consistent with the larger size of *G. stellata* nucleomorph (ccc versus 373Kbp in *B. natans*). We also found that intron loss occurred in the *B. natans* lineages and that introns larger than 21 bp can be processed by the spliceosome. We discuss the significance of these findings in the context of the current hypotheses about the evolution of eukaryotic introns.

## PHYLOGENY OF VANNELLID AMOEBAE - SSU HETEROGENEITY IN CLONAL CULTURES AND THE PROBLEM OF "MOLECULAR ECOLOGY" OF GYMNAMOEBAE

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The family Vannellidae Bovee 1979 contains 40 species and is one of the largest and commonest groups of naked lobose amoebae, isolated from all kinds of environment. In traditional view it contained five genera - *Vannella, Platyamoeba, Pessonella, Lingulamoeba* and *Clydonella*, differing in cell surface structure, locomotive morphology and floating form. Comprehensive phylogenetic analysis performed for 57 Vannellidae sequences indicated that species of two key genera of this family - *Vannella* and *Platyamoeba*, together encompassing about 85% of the diversity of vannellid amoebae, are completely mixed and do not form distinct clades. Several very closely related species pairs exist, each with a *Vannella* and a *Platyamoeba* differing in only a few nucleotides. Therefore, presence (*Vannella*) or absence (*Platyamoeba*) of glycostyles in the cell surface coat is an invalid generic distinction; we therefore merged the two genera (suppressing *Platyamoeba*) in a forthcoming paper in Protist. Molecular trees revealed a separate clade of vannellids with specific and rather short SSU gene (ca 1800 bp instead of the usual ca 2000). We established a new genus *Ripella* for the only named species of this clade - *R. platypodia*. Sequencing molecular clones obtained from the same DNA sample indicated that vannellid amoebae show unusual microheterogeneity of SSU sequences in clonal cultures. Similar data exist for the genus *Neoparamoeba*; there are a few more examples of such SSU heterogeneity. This finding contradicts the paradigm "species = rRNA sequence" and makes the "molecular ecology" of these naked amoebae based on 18S rRNA sequences from total DNA isolated from the environment very difficult, if ever possible, because species cannot be identified by complete sequence identity. During our study of vannellids that included named species and all type cultures plus many unnamed and environmental sequences we could identify none of the environmental sequences and found no identical sequences from different strains or DNA samples, suggesting undersampling of vannellid diversity and/or intraspecific heterogeneity. Molecular signatures combined with LM morphology of isolates may help sorting out the species problem among gymnamoebae, but a study of single copy protein gene sequences not prone to microheterogeneity may be essential to identify better molecular markers for amoeba clones. Supported by a NERC grant to TC-S and RFBR grants (03-04-48718 and 06-04-49387) to AS.

## THE SPECIES DIVERSITY OF THE FRESHWATER TESTATE AMOEBAE OF AZERBAIJAN

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The current study of testate amoebas from inland waters of Azerbaijan started in the 1990s. Prior to our research, only 41 species were recorded from Azerbaijan inland waters as a result of general hydrobiological investi-

gations in 1930-1940. Some areas of inland waters described in those early investigations do not exist at the present time. In total, 200 species of testate amoebas have been identified during our survey covering inland waters of West and Northern-East Azerbaijan, as well as some freshwaters of Apsheron peninsula. The fauna of testate amoebas of these regions appeared to be extremely interesting and contained many taxa, new for science. In our opinion, the study of testate amoebas of inland waters is still far from being completed. We believe that regions of Nakhichevan and Southern-East of Azerbaijan (Lenkoran) are most interesting for further faunistic research, because they are considered as centers of Caucasian formation of species, and are expected to have many endemic species.

## GREGARINE SYZYGY AS A MODEL FOR MORPHO-FUNCTIONAL STUDY OF SEPTATE JUNCTIONS

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An enormous contribution of specialized intercellular junctions (SIJs) into intercellular communications of Metazoa is now established. It is generally accepted that septate junctions (SJs) are typical for epithelial tissues of invertebrates. Always SJs are accompanied by other SIJs (desmosomes, tight, intermediate, and gap junctions). This fact causes difficulties in the interpretation of functional role of each contact. During the study of *Gregarina polymorpha* syzygy from *Tenebrio molitor* by electron microscopy we revealed presence of SJs between syzygy individuals: primite and satellite. The structure of the SJ was analogous to SJ from invertebrate tissues. It should be noticed that the gregarine syzygy is one of rare examples of two-cellular stage in the life cycle of Protozoa possessing specialized intercellular contacts. Absence of some other SIJs makes this system very convenient for morpho-functional study of SJ. Scanning and transmission electron microscopy (SEM and TEM) demonstrated the complicated structure of the gregarine pellicle forming longitudinal ridges on the cell surface. In the preparations for SEM it was well seen that ridges form different regions with various directions along the longitudinal cell axis. It seems that during sliding movements the cell is being turned around. On ultrathin sections with application of lanthanum chloride during fixation, the typical SJs were observed in sites of contact of primite and satellite ridges. The gap in this region was measured 13-14 nm. It was intersected by periodically located septa. Thickness of each septum was 5-6 nm, the distance between them - 10 nm. On the tangential sections septa looked as parallel zigzag lines. For understanding a functional role of SJs, measurements of electrical resistance in the contact zone were carried out. Besides, experiments with fluorescent dye were performed. Nor the electrical connection, neither transfer of dye from one cell to another was registered, thus we could not reveal existence of any connection between primite and satellite. The results obtained in this work suggest that SJs are not the sites of ion and molecule exchange between both cells. We presume that gregarine SJs play a role in recognition and subsequent mechanical maintenance of connection between the cells of syzygy in the period of time preceding to their sexual reproduction. Some evolutionary aspects of the presence of SJs in the different groups of animals are discussed.

## *AMOEBA PROTEUS* MYOSIN VI-IMMUNO-ANALOG IS INVOLVED IN CELL MIGRATION, PINOCYTOSIS AND PHAGOCYTOSIS

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Actin cytoskeleton plays a crucial role in panoply of key cellular functions, such as maintenance of the cell shape, intracellular trafficking and migration. Myosin VI is a molecular motor walking to the "-" end of actin filaments (opposite direction to other known myosins) that is involved in intracellular transport, translocation and adhesion. *Amoeba proteus* has been widely used as a model to study cell motility, but molecular mechanisms underlying its actin-based-only locomotion and intracellular trafficking remain poorly understood. We detected a 130-kDa protein interacting with several antibodies against different regions of myosin VI and immunoprecipitating with anti-myosin VI antibodies. This protein possessed biochemical features characteristic for myosins, and peptides derived during mass spectrometry analysis, revealed significant identity to different human and invertebrate myosins VI. In migrating amoebae, myosin VI immunoanalog (mVIi) localized to membranous vesicular structures, particularly within the perinuclear and sub-plasma membrane areas. Moreover, mVIi colocalized with dynamin II in regions enriched in actin filaments; both proteins were also detected on the same isolated vesicles. In TEM, mVIi was found on actin filaments bundles within the cell. In pinocytotic cells, mVIi concentrated within pinocytotic pseudopodia and strongly colocalized with dynamin, while in phagocytotic cells mVIi localized to phagosomes. Blocking endogenous mVIi with anti-myosin VI antibodies caused changes in cell morphology, inhibited the rate of cell locomotion, impaired pinocytosis as fewer pinocytotic channels were formed,

and caused severe defects in phagocytosis. These results indicate that this novel myosin VI isoform is involved in cell migration, pinocytosis and phagocytosis.

### INTERACTION OF PHOSDUCIN WITH THE G-PROTEIN BETA SUBUNIT IN THE CILIATE PROTOZOAN *BLEPHARISMA JAPONICUM* UPON ILLUMINATION

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Immunological methods (immunoblotting, coimmunoprecipitation, immunocytochemistry, confocal microscopy) and high resolution FRET technique have been used to study the in vivo interaction of phosducin (Pdc) with the β-subunit of G-protein (Gβ) in the ciliate *Blepharisma japonicum*. The dephosphorylation of Pdc observed in response to cell illumination was found to be followed by translocation of Pdc from the cell cytoplasm to the vicinity of the plasma membrane, where it colocalized with Gβ. Coimmunoprecipitation analysis showed that Pdc and Gβ proteins that appear colocalized in an immunocytochemical assay, interact with one another in tested cells. Formation of Pdc-Gβ complex in cells exposed to light, was evidenced also by FRET between these proteins. The FRET efficiency values for light-stimulated ciliates were several times higher, compared to control dark-adapted cells. Similar Pdc dephosphorylation and its colocalization with Gβ were observed in dark-adapted cells pretreated with H-89 or KT-5823, kinase G and kinase A activity inhibitors respectively, whereas no changes in these FRET parameters were estimated in ciliates under the same experimental conditions. The results of these experiments suggest that the observed Pdc-Gβ interaction in the ciliate *Blepharisma japonicum* is determined not only by Pdc dephosphorylation upon cell illumination, but it is evident that for this molecular process the light activation of cell photoreceptor followed by Gα dissociation from G-protein (Gαβγ) is necessary. Supported by grants 2P 04C 014 27 and 2P 04C 013 30 from the Ministry of Science and Higher Education.

### SOCIAL PARASITISM IN MICROSPORIDIA: *THELOHANIA SOLENOPSAE* DEVELOPMENT IN COLONIES OF THE FIRE ANT, *SOLENOPSIS INVICTA*

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*Thelohania solenopsae* is a unique microsporidium whith a life cycle finely tuned to parasitizing fire ant colonies. Unlike other microsporidia of social hymenopterans, *T. solenopsae* infects all castes and stages of the host. Each of its four developmental sequences is specialized to a certain insect caste or stage and plays a particular role in the *T. solenopsae* life cycle to promote maximum success in parasite multiplication and in vertical, horizontal, intra-, and inter-colony transmission. Four distinctive spore types are produced: diplokaryotic spores, which develop only in brood; octets of octospores within sporophorous vesicles, the most prominent spore type in adults but never occurring in brood; *Nosema*-like diplokaryotic spores developing in adults; and megaspores, which occur occasionally in larvae-4 and adults of all castes but predominantly infect gonads of alates and germinate in inseminated ovaries of queens. *Nosema*-like spores function in autoinfection of adipocytes. Increased proliferation of diplokaryotic meronts in some cells is followed by karyogamy of diplokaria counterparts and meiosis, thereby switching the diplokaryotic sequence to octospore or megaspore development. Megaspores transmit the pathogen transovarially to the next generation. From the egg to larvae-4, infection is unapparent and can be detected only by PCR. Juvenile and megaspore sequences are abruptly triggered in larvae-4, which is the key stage in intra-colony food distribution via tropholaxis. Larvae-4 lack buccal filters, can consume solid food, and participate in horizontal transmission of spores, presumably via cannibalism and/or meconium utilization. Molecular, morphological and life-cycle data indicate *T. solenopsae* must be assigned to a new genus, work in progress.

### EFFECT OF "MICOBACTOVIR", A NEW BROAD-SPECTRUM FUNGICIDE, ON *ENCEPHALITOZOON CUNICULI* REPLICATION *IN VITRO*

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"Micobactovir" (MBV), a newly synthesized derivate of arilidenine-1-3-pyrimidine, suppressed growth of multicellular fungi and yeasts *in vivo* and *in vitro*; was effective against gram+ and gram- bacteria; and inhibited reproduction of Herpes virus. MBV is considered as a drug for systemic and topical use, effective against fungal, fungi-bacterial and fungi-viral infections. The goal of this study was to assess effect of MBV on *Encephalitozoon cuniculi* and *E. intestinalis* in cultured rabbit kidney cells (RK-13). *E. cuniculi* or *E. intestinalis* spores were added to confluent monolayers of RK-13 cultivated in 6- and 24-well plates. Simultaneously 1 mg/ml water solution of MBV was added to test the concentrations 500, 250, 125, 61, 30 and 15 μg/ml. Statistically significant cytotoxicity against RK13 cells

was observed at 500 and 250 μg/ml. Visual observation of monolayers did not show any differences in morphology of parasitophorous vacuoles (PVs), or timing of spore maturation between treated and untreated monolayers. No statistically significant variations were registered in number of PVs per cell, or PV volume, or number of host cells with PVs. Incorporation of BrdU, followed by application of anti-BrdU antibodies conjugated with Alexa-488, was observed in treated and untreated parasites, suggesting the parasite replication was not blocked by the drug. Non-toxic concentrations of MBV that suppressed the parasites proliferation by 40-80%, were 60-125 μg/ml. For comparison, Albendazole and Fumagillin caused 100% inhibition at 0.01 and 0.1 μg/ml correspondingly. Microsporidia occurred to be more resistant to MBV than *Candida* spp., inhibited by 1 μg/ml. Supported by RFBR grant 03-04-49629.

## CILIATE DIVERSITY, ADAPTATIONS, AND NICHE OCCUPATION IN TWO CONTRASTING LAKES LOCATED AT DIFFERENT ALTITUDES

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In Piburger See (913 m a.s.l.), the planktonic ciliate assemblage was highly diverse with >100 species found over the course of the year. The succession of the species was closely linked to changes in abiotic parameters, e.g., oxygen concentration. For example, along the water column, we found common euplanktonic ciliates (e.g., prostomatids, oligotrichs) in the upper meters and more anaerobic species (e.g., heterotrichs) in the deepest zone. In summer, we found around 20 *Chlorella*-bearing species that dominated in the upper meters during highest levels of ambient solar radiation. In several of those mixotrophs, we identified UV-absorbing mycosporine-like amino acids (MAAs). Moreover, we identified two possible sources of the compounds: the *Chlorella*-symbionts and the ciliates' diet. In the highly UV-transparent alpine lake Gossenkollesee (2,417 m a.s.l.), we found by contrast only three predominant species during the ice-free period with only one bearing *Chlorella*, i.e., *Askenasia chlorelligera*. In this species, we observed higher concentrations of MAAs per cell than in the mixotrophs from Piburger See. Thus, we argue that the accumulation of MAAs in *Chlorella*-bearing ciliates represents an additional benefit in this symbiotic partnership and an adaptation to elevated UV-radiation. In Gossenkollesee, the heterotrophic prostomatid *Balanion planctonicum* numerically dominated over the other species and occupied the deepest water layers. This distribution pattern was in agreement their sensitivity to UV-B radiation.

## CYTOPLASMIC MICROTUBULES ARE REQUIRED FOR EGESTION OF A FOOD VACUOLE, WHILE ACTIN FILAMENTS ARE INVOLVED IN MEMBRANE RECYCLING IN A CYTOPROCT IN *TETRAHYMENA THERMOPHILA*

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Ciliates including *Tetrahymena thermophila* take nutrient through a food vacuole (FV). Undigested contents in an FV are egested through membrane fusion in a cytoproct. This cellular event is attractive for studying control of membrane dynamics via cytoskeletons. We prepared antiserum against one of myosins in *T. thermophila*, Myo13, and found that it specifically labeled a cytoproct. Interestingly, it was found that localization pattern of Myo13 in a cytoproct was changed from a linear shape to a ring by a treatment of cells with an inhibitor for actin polymerization, Latrunculin B. Moreover, SEM observation revealed that the cytoproct was dramatically opened, and that membrane was protruded from there in these cells. Optical microscopic video images showed that the protrusion was probably derived from egested FVs. Inhibition of actin polymerization is likely to suppress a membrane recycling of an FV in a cytoproct. Immunofluorescence microscopy revealed that strong accumulation of actin surrounded an FV when it was egested. However, undigested contents in an old FV were defecated even in the absence of F-actin. Therefore, the actin cytoskeleton seems to be required for membrane recycling but not for egestion of an FV. On the other hand, cytoplasmic microtubules localized as dense network surrounding FVs. Moreover, egestion of an FV was rarely seen in cells treated with microtubule depolymerizing reagent, nocodazole. It seemed that cytoplasmic microtubules were required for the egestion of old FVs in *T. thermophila*.

## *CHLORELLA*-BEARING CILIATES AND THEIR UV-PROTECTION STRATEGIES

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Mutualistic associations between ciliates and unicellular green algae of the genus *Chlorella* is a common phenomenon. We investigated *Chlorella*-bearing ciliates in terms of their strategies to resist the negative effects of UV radiation. Therefore, we carried out UV experiments, which demonstrated a much higher UV tolerance of symbiotic *P. bursaria* compared to their aposymbiotic counterpart. HPLC-analyses revealed the presence of colourless UV-absorbing mycosporine-like

amino acids (MAAs) in several *Chlorella*-bearing ciliate species, but not in *P. bursaria*. To test, if *Chlorella* symbionts of the investigated ciliate taxa were unrelated to each other, we sequenced part of the nuclear 18S rDNA, the ITS-1 region and the partial chloroplast 16S rDNA of the algae, and detected a pronounced symbiont variability within the investigated host species, but also indications for species specificity. An ultrastructural characterisation of the *P. bursaria* symbiosis and an examination of the distribution of the symbionts within the host cell revealed distinct distribution patterns under different UV and light intensities. Results from UV-experiments and HPLC-analyses of sunscreen-compounds in combination with the morphological analyses suggest at least two different UV-protection strategies of the ciliate-*Chlorella* symbiosis: (1) an internal (self-)shading of sensitive cell targets (ie. the nuclei) provided by a specific distribution of the symbionts, depending on the incident radiation as investigated in *Paramecium bursaria*, and in addition, (2) protection through MAAs in other *Chlorella*-bearing ciliate species.

## COMPARATIVE PROTEOMICS OF *TRICHOMONAS VAGINALIS* TROPHOZOITE AND PSEUDOCYST HYDROGENOSOME

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Hydrogenosomes are mitochondrion organelles which contain hydrogenase and produce hydrogen and ATP by glycolysis. These organelles have been identified in unicellular eukaryotes such as *Trichomonas vaginalis*, *Tritrichomonas foetus, Neocallimastix* and *Nyctotherus ovalis*. The present study elucidated the complete hydrogenosome proteome of *T. vaginalis* by using 2-dimensional gel electrophoresis (2DGE) and strong cation exchange liquid-chromatography coupled with a mass detection system (SCX-LC/MS). A total of 121 unique proteins were identified by both techniques. However, only 37 of the identified proteins were also predicted by The Institute of Genome Research (TIGR) from the recently published *T. vaginalis* genome. The remaining 84 proteins were newly identified hydrogenosomal proteins. We also compared the hydrogenosome proteome of the trophozoite and pseudocyst stages. Fructose-1,6-bisphosphate aldolase (F1BP), Glyceraldehyde-3-phosphate dehydrogenase (G3PD), heat shock protein 70 (HSP70) and thiol peroxidase (Tpx) were expressed at a higher level in pseudocyst. One interesting observation is the expression of specific isoforms within a protein family at different stages. This may explain why *T. vaginalis* contain the largest number of genes among all unicellular organisms. The protein expression levels of all the identified hydrogenosomal proteins were also associated with the gene expression level by using the *T. vaginalis* expressed sequence tag database to study the turnover rate of mRNA and proteins. The newly identified proteins are being integrated into existing pathways to decipherate the hydrogenosomal interactome.

## INTRASPECIFIC DIFFERENTIATION WITHIN *PARAMECIUM NOVAURELIA* (CILIOPHORA, OLIGOHYMENOPHOREA) USING RDNA AND MTDNA ANALYSIS

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*Paramecium novaurelia* is one of 15 genetic species of *Paramecium aurelia* species complex, characterized by moderate inbreeding. Differentiation within *Paramecium novaurelia* was previously studied by strain crosses and molecular methods like RAPD-PCR (Random Amplified Polymophic DNA). *P. novaurelia* was regarded to be restricted to Europe, but later the dominant species was also recorded in Asia (Turkey, Anatolian Upland). The first American strain of *P. novaurelia* was recently identified in a sample collected in Boston, USA. We present molecular comparisons of 25 *P. novaurelia* strains, including the American strain and the strain from Asia. The intra-specific differentiation of *P. novaurelia* was studied using sequencing of two DNA fragments, i.e. a fragment of rDNA: 18S-ITS1-5.8S-ITS2-28S (1200 bp) and a fragment of mtDNA - CO I (660bp). Obtained results were analyzed by NJ, MP, ML and Bayesian analysis. Intra-specific differentiation within *P. novaurelia* discriminates four groups of the studied strains with different genotypes. P. caudatum strain was used as an outgroup. Geographical correlation between genotypes was not observed. The distance among *P. novaurelia* strains in ribosomal fragment was at the level of 2.4%, and COI mtDNA fragment revealed much higher divergence equal to 24.8%.

## MICROZOOPLANKTON DIVERSITY IN THE BALTIC COASTAL WATERS

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Baltic coastal waters are characterized by a variety of ecosystems - from large exposed shallow gulfs and estuaries to lagoons and fjords. This variability of

biotopes along with other specific external abiotic factors provides high biological diversity of mainly freshwater and brackish aquatic flora and fauna. The on-going eutrophication of the Baltic coastal ecosystems influences the species diversity, population structure and productivity of aquatic communities which can serve as indicators of ecosystem alterations. Results of long-term investigations of zooplankton in the Neva Estuary, one of the largest urbanized coastal ecosystems of the Baltic Sea, Darss-Zingst Bodden Chain and other Baltic coastal areas revealed the necessity of a profound research into biodiversity of pelagic communities and the need of a regional illustrated zooplankton guide with a revised species checklist. The paper provides data on the zooplankton diversity in the Baltic coastal waters, with special emphasis on planktonic protists and rotifers, in relation to the problems of biodiversity loss, biological invasions, trophic interactions in the pelagic communities, and ecosystem functioning. Principles and mechanisms regulating the microzooplankton diversity in estuaries are discussed. Supported by the Program "Biodiversity" and RFBR grants 04-04-49207, 05-04-90588.

**CILIATES: A BIOTECHNOLOGICAL APPROACH TO REDUCE ANTIBIOTIC-RESISTANT BACTERIA FROM THE OUTLET OF WASTEWATER TREATMENT PLANTS**

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Sewage treatment plants, especially the biological purification steps, are hotspots for antibiotic-resistant bacteria, which are released in relatively high numbers into the receiving waters. The further entry of antibiotic resistances into the environment - a major threat to human health - should be prevented. We developed therefore a novel approach to reduce the cell-numbers of suspended bacteria by filter-feeding ciliates, the natural predators of bacteria. The capability of two ciliates *Tetrahymena pyriformis* wt and *Colpidium campylum* to decrease three representative bacteria species (*Escherichia coli, Bacillus subtilis* and *Pseudomonas putida*) was analysed at five different temperatures in a 100 ml-scale under batch conditions over 8 h. These results stimulated upscaling of the experiments to volumes of 2 l and 25 l. In addition, the time period was increased to 100 h under continuous conditions. Both, *T. pyriformis* wt and *C. campylum* have the potential to reduce efficiently and economically the number of suspended bacteria released into the receiving waters.

**STRUCTURE OF FREE-LIVING HETEROTROPHIC FLAGELLATE COMMUNITIES IN DIFFERENT TYPES OF FRESHWATER AND MARINE ECOSYSTEMS**

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Species diversity, cell morphology, community structure and distribution of free-living heterotrophic flagellate were investigated. Material from different freshwater and marine biotopes was collected during 2002-2006. Samples from several microbiotopes with different substratum were taken within each of the studied ecosystems. 304 species and forms of heterotrophic flagellates from all known higher level groups of eukaryotes were observed. The main factors of community differentiation in different types of ecosystems were revealed. Heterotrophic flagellate communities are highly heterogenous systems; community differentiation does not depend on geographic remoteness of biotopes; species distribution is caused by local factors, such as type of microbiotope, hydrochemical and trophic conditions. The most complex communities with specific set of species are formed in biotopes rich in organic matter with reduced (or acidic) medium, and are characterized by the highest values of integral community characteristics. Eurybiont species with a great tolerance range to environmental factors define major patterns of heterotrophic flagellate communities. Ratio of alpha vs. beta components of species diversity depends on the scale of investigation. At the microscale (partial ecosystems elements) species richness of heterotrophic flagellates is determined mainly by ability of community to differentiation due to intercommunity mechanisms. At the meso- and macroscales (ecosystems and regions) species richness depends mainly on peculiarities of the ecosystem, the region type and evolutionary history of taxonomic groups.

**DIAGNOSIS OF MICROSPORIDIAN INFECTIONS IN ARTHROPOD HOSTS**

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Various methods were exploited to reveal microsporidian infection in a broad range of arthropod hosts: crickets and locusts (Tokarev et al., 2005, 2007), beet webworm (Malysh et al., 2006); hard ticks (Tokarev, Movila, 2004; Tokarev et al., in press), leafrolling

weevils, cotton bollworm, flour beetles etc. For mass-scale screening of alive and dead material we found staining with DAPI the most useful technique. This DNA-specific dye is known also to stain membranes (Favilla et al., 1993) and proteins (Mazzini et al., 1994). In *Nosema*-like microsporidian spores, DAPI intensively stains diplokaryon and feebly stains exospore and cytoplasm (allowing to counterstain the endospore), extruded polar filament and other intracellular structures. This "non-specificity" prominently helped to discriminate even single spores found on smears, especially when diplokaryon staining was unclear. In lepidopterans, invaded with *Thelohania*-like spores, DAPI did not stain nuclear apparatus of spores and bound only to the exospore. The usability of DAPI for detection of single *Nosema*-like spores of microsporidia on smears makes this method more helpful as compared to PCR with SSU rDNA primers, as the threshold sensitivity of the latter is reported to be 100 spores ml-1 for human microsporidia (Weiss, Vossbrink, 1999) and even 1000 spores ml-1 for insect microsporidia (Sokolova et al., 2004). On the other hand, PCR detection of microsporidia was a reliable tool to localize latent infection in locusts when other methods were not helpful.
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## LIGHT AND ELECTRON MICROSCOPY STUDIES ON MORPHOLOGICAL POLYMORPHISM IN CLONAL CULTURES OF *ARCELLA* SPECIES WITH SPECIAL REGARD TO THEIR ENDOCYTOBIONTS

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To date our understanding of the size polymorphism range in testate amoebae is very limited. Clonal cultures of various *Arcella* species were established in order to study both inter- and intraspecific polymorphism. A further aim of the study was to find bacterial endosymbionts in this important lobose testate amoeba genus and investigate their potential impact on the clonal growth capacity. We present the morphological and biometric characterization of active and encysted stages of the cultivated *Arcella* species completed with the growth rates in different culture conditions. We demonstrate the first findings on different symbiotic bacteria in the testate amoeba revealed by fluorescent *in situ* hybridization (FISH) applying general both, eubacteria and more specific probes, designed according to 16S rRNA gene sequence results. Endocytobiont nature, morphology and localization of symbiotic bacteria were confirmed by transmission electron microscopy, which results were compared with the published data.

## FIRST EVIDENCE ON BACTERIAL ENDOCYTOBIONTS IN THE TESTATE AMOEBAE OF THE GENUS *ARCELLA*

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While prokaryotic symbionts in ciliates are extensively searched out, medically harmless species of free living amoebae have been widely neglected in symbiont studies. The present study gives the first evidence on bacterial endocytobionts in the genus *Arcella*. Clonal cultures of variety of *Arcella* species were set up and investigated for presence of endocytobionts with bacterial rDNA sequencing, fluorescent *in situ* hybridization (FISH), and transmission electron microscopy. *Arcella* species were kept axenically for eukaryotes and fed with *Enterobacter aerogenes*. Rich diversity of eubacterial sequences, like *Acidovorax* and *Verrucomicrobium* among others, have been identified by PCR either by direct isolation of rDNA from the testacean cells or by culturing individual *Arcella* cells on different types of media. FISH with eubacterial probes demonstrated presence of single bacterial cells scattered throughout the cytoplasm, clearly different from those clumped together in food vacuoles. First transmission electron microscopical surveys revealed single rod-like bacteria located in different parts of the cytoplasm. In symbiont-bearing *Arcella* clones, lysis of host cells was not detected; instead cells in older cultures occasionally started cyst formation. This phenomenon might indicate that so far detected endocytobionts are not harmful to host cells, but their beneficial role is still to be proved. Bacteria from the same clone appeared to belong to phylogenetically distant groups, some were related to symbionts of other eukaryotic organisms, others - to human pathogens, suggesting a potential role of arcellas as natural reservoirs.

## PATTERNS OF SPATIAL AND TEMPORAL VARIABILITY OF TESTATE AMOEBAE COMMUNITY IN SPHAGNUM BOG

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Testate amoebae are common and important component of *Sphagnum*-dominated ecosystems and are increasingly used in peatland monitoring and palaeoecological research. However data on the spatial and seasonal structure of communities are completely lacking. This is an important aspect since quantitative models used for paleoecological reconstruction and monitoring are based on species assemblages. We investigated spatial and temporal distribution patterns of testate amoebae in sphagnum sward of the raised bog Bezimyanoe. The study revealed 63 taxa belonging to 21 genera. Analysis of horizontal microdistribution patterns in scales from 1 cm to 2 m showed that species formed feebly marked aggregations with unclear bounds. These aggregations probably resembled more or less expressed patches of different sizes smoothly passing from one to another, than a distinct spatially constrained group. Size of these patches was species-specific and in some cases (*Assulina muscorum* and *A. seminulum*) positively correlated with the amoebae shell sizes. Vertical distribution pattern of testate amoebae assemblages depended on moisture of the biotope. Communities in drier conditions were the most heterogeneous. In upper sphagnum layer (0-3 cm) species number and species diversity were minimal, whereas abundance was maximal. The opposite tendencies in distribution of species *Hyalosphenia papilio-H. elegans* were marked, that reflected separation of the ecological niches along the sphagnum stem. From May to September, it was revealed that species number increased, while species diversity and evenness remained at the same level with insignificant fluctuations. During this period, species abundance in different assemblages of testate amoebae could increase, decrease, or vary without well-defined or directed tendencies.

## CYTOLOGY AND MORPHOLOGY OF MYXOSPORIDIA (MYXOZOA)

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The analysis of organic and non-organic substances in the valves of long stored in water *Heneguya oviperda* (Cohn, 1895) and *Myxobolus pseudodispar* (Gorbunova, 1936) myxospores has been made using the cytochemical methods and atomic-absorptional spectroscopy. The high content of Si (up to 94%) and the presence of Ca, Mg, Fe, Mn cations (Cu, Al, Ni, Cr in fewer amounts) have been detected. The investigation of plasmodium and spore nuclei, f-actin, α- and γ-tubulin of three Myxosporidia species: *Zschokkela nova* (Klokacewa 1914), *Myxidium gasterostei* (Noble 1943) and *Myxobolus pseudodispar*, was performed using confocal microscopy and indirect cytofluorimetry. Features of a structure of nucleus and changes of their structure are found out during division and formation of myxospore. For a long period of time two factors have impeded investigations of myxosporidia cytology: the small size of their nuclei and the absence of data about existence of condensed chromosomes in these organisms. Significant progress in biological research methods provides new approaches to investigations of Myxozoa chromatin and chromosomal apparatus. In this work for the first time the chromosomes in generative cell nuclei of *Zschokkela nova* was found out and described using confocal scanning microscopy. It was shown that its karyotype consists of six chromosomes (three pairs). Two pairs of chromosomes have the oblong rod-shaped form (metacentric chromosomes). Third pair of chromosomes has the boomerangs form (submetacentric chromosomes). Lengths of chromosomes: 5 microns (1 pair), 4.8 microns (2 pair) and 3 microns (3 pair).

## A GENOME SURVEY OF *ENTEROCYTOZOON BIENEUSI*

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*Enterocytozoon bieneusi* is clinically the most significant pathogen among the Microsporidia that infect humans. Until recently, investigations on *E. bieneusi* have been hindered by the inability to propagate these organisms in the laboratory. Recent developments, which include methods for spore purification and animal propagation, have made it possible to undertake a genomic survey of *E. bieneusi*. *E. bieneusi* spores were purified and concentrated from the stool of a human patient. Genomic DNA was extracted and a small insert (1-2 kb) plasmid library was constructed. The first draft assembly of the genome consists of 1,743 contigs, ranging in size up to 131,000 bases, covering ~3.8 Mb of unique sequence. This represents ~55% of the genome, which is estimated to be 6-8 Mb by pulsed field gel electrophoresis. Preliminary analyses of the genomic sequence suggest a high AT content (>65%) and an absence of introns. Approximately 2,300 open reading frames (ORFs) have been identified in *E. bieneusi*. 40% of the *E. bieneusi* ORFs have been assigned a putative function based on homology to known genes. Of these, 70% were homologues to genes identified in *Encephalitozoon cuniculi*. Assignment of identified proteins to pathways and syntenic analyses with the genomes of *E. cuniculi* and *Antonospora locustae* are underway. In addition, the putative *E. bieneusi* methionine aminopeptidase-2 and polar tube proteins have been identified and polyclonal antibodies against the polar tube proteins have been produced.

## APPLICATION OF POLYMERASE CHAIN REACTION IN THE DIAGNOSTICS OF AVIAN CRYPTOSPORIDIOSIS

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Studies of the animal disease cryptosporidiosis induced by the Cryptosporidiidae family of protozoa and manifested in gastro-intestinal, respiratory and immune system diseases, have been for long under way all over the world. In Russia, the pathogenic agent of cryptosporadiosis was for the first time revealed in 25-year old calves (Nikitin, Pavlasek, 1983), and at present cryptosporidia are treated as harmful pathogens for not only animals but for humans as well. This invasion started being frequently diagnosed in acquired immune deficiency patients. According to the data reported by the WHO, over 500 thousand persons annually caught the disease in the 1990s, with 2 to 3% of them suffering from it chronically. Cryptosporidiosis in AIDS-affected patients is manifested by profuse dropsy diarrhea and is regarded as a serious complication. Under the disguise of various contagious diseases, cryptosporidiosis makes it difficult to carry out timely diagnostics and adequate therapeutic and preventive treatments. Cryptosporidiosis in birds has been less sufficiently described than in mammals. It is exhibited in a number of clinical manifestations, e.g. diarrhea, suppression, depression and respiratory conditions such as cough, nasal running and huskiness. Clinicall manifestations of the disease are dependent on a number of factors, with the bird's kind, age and immune status being the most important ones. Although the disease in an adult bird proceeds with no symptoms, this one is yet a parasite-carrier. Cryptosporidiosis is not recurrent in birds that have survived it. The amount of isolated oocysts in adult hens is considerably smaller as compared with chickens, owing to the higher immune status of the former ones. Invasion extensiveness of 95% can be observed in 30-35 day-old broilers. Poultry-raising farms continue suffering considerable economic losses. Alabama State (USA), for instance, loses weekly 25 thousand cryptospirosis broilers, while the survived birds lose 100-150 g of weight. In a Japanese poultry-breeding farm, the high number of morbid cases could not be decreased for over two years and a half. Infected poultry's body mass growth decreases 9 to 20% during the disease period even under most beneficial growth conditions. Monitoring of the epizootic situation in West Scotland's poultry-breeding farms showed the infection of the poultry to be 71.7%. The presence of cryptospiridia is supported by complex examination methods. Laboratories make wide use of the microscopy of Cyl-Nilsen stains as well as of immunological reactions (direct and indirect fluorescence, latex agglutination reaction, the immune enzymatic method). At the present time, the Laboratory of Protozoology in cooperation with the Laboratory of Molecular Diagnostics, Institute of Molecular Genetics, RAS, has developed a highly sensitive testing system of PCR for detection of cryptosporidia in avian genetic material. The assessment of results acquired during our examination of an ailing bird's pathological material by several methods showed that microscopic testing for kryptospirosis of 363 samples taken from 10 to 40 days-old birds revealed 7.4% positive results, microscopy with flotation produced 38% positive cases and the PCR testing system gave 73% positive results. Microscopy methods with or without flotation, widely applied at present for the kryptosporidiosis diagnostics, have proved to be insufficiently effective. In our opinion, polymerase chain reaction (PCR) seems more promising for detection of *Cryptosporidium baileyi*.

## COMPARATIVE EXTERNAL MORPHOLOGY OF DEVELOPMENTAL STAGES OF GASTRIC *CRYPTOSPORIDIUM* SPP. FROM ENDOTHERMIC AND POIKILOTHERMIC HOSTS

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*Cryptosporidium* is a vertebrate pathogen that has gained much attention in the last years due to its phylogenetic affinities within the phylum Apicomplexa. In *Cryptosporidium* spp., the invasive stage (zoite) is finally enveloped by parasitophorous vacuole, the inner membrane of which originates from plasma membrane of the apical region of the affected gastric cells. Together with the zoite envelopment, a unique structure (feeder organelle) is formed at the zoite-host cell interference zone. Scanning electron microscopic examination of the developmental stages of *Cryptosporidium muris* Tyzzer, 1910 from the stomach of experimentally infected multimammate rats (*Mastomys natalensis*) and gastric *Cryptosporidium* sp. from naturally infected toads (*Bufo* sp.) showed differences in the attachment strategy of the cryptosporidians depending on density of *Cryptosporidium* developmental stages and character of the microvillous border of gastric cells. According to our transmission electron microscopic observations on *C. muris* and *Cryptosporidium* sp., zoites attach to the host microvillous surface, being apparently epicellular and not intracellular-extracytoplasmic as it is traditionally referred to. The enveloped zoites obviously do not come into close contact with the host cell cytoplasm, except

for the region of a feeder organelle. Supported by MSM 0021622416, grant no. 524/03/H133 of the Grant Agency of the Czech Republic, and by Research Centre "Ichthyoparasitology" (LC522).

## PROTECTION FROM OXIDATIVE STRESS BY METHIONINE SULFOXIDE REDUCTASES IN *EUPLOTES*

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Reactive oxygen species are physiologically synthesized by every cell and oxidize a vast array of cellular constituents. If the oxidative damages are not repaired by anti-oxidative enzymes, oxidized macromolecules are no longer active and start accumulating within cells, thus affecting the organism lifespan. Elective targets of protein oxidation are methionine residues, in particular those which are carried exposed on the molecular surface. Their modification into hydrophilic sulfoxides may result into effective changes in the protein polarity, with dramatic effects on its bioactivity and stability. In *E. raikovi*, protein oxidation appears to commonly affect the water-borne signalling pheromones that control growth and mating of this ciliate. Uninterrupted rhythms of cell division lead systematically to the production of pheromone molecules carrying methionines modified by oxidation and showing mitogenic and mating-inducing activities appreciably lower than non-oxidized molecules. In experiments of macronuclear DNA amplification, we identified and cloned two genes whose nucleotide sequences predict the synthesis of two different methionine sulfoxide reductases (Msr). One of these enzymes, responsible for the cell protection against oxidative damages, is of the type A (MsrA) specific for the reduction of the oxidized methionine-S form; the other one is of the type B (MsrB) specific for the reduction of the oxidized methionine-R form. Both MsrA and MsrB are expressed although to different extents, and their expression appears to be an inducible phenomenon.

## COMPLEX DISTRIBUTION PATTERN OF PRIMARY AND SECONDARY SYMBIONTS IN *EUPLOTES*

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*Polynucleobacter necessarius* (Betaproteobacteria) was first described as endosymbiont of strain E24 of the ciliate *Euplotes aediculatus*. Further studies showed that the *Polynucleobacter-Euplotes* association is an obligatory symbiotic system between a monophyletic group of *Euplotes* species and bacteria belonging to the genus *Polynucleobacter*. Recent studies revealed the existence of obligatory free-living populations of *Polynucleobacter necessarius*-like bacteria that are phylogenetically closely related to the endosymbiotic ones. Moreover, *Polynucleobacter*-harboring *Euplotes* often harbor also secondary symbionts. A detailed characterization of primary and secondary endosymbionts was carried out by the 16S rRNA full cycle approach in several strains of these *Euplotes* species. Phylogenetic analyses on the obtained 16S rRNA sequence data were performed to clarify the position of these bacterial symbionts. Surprisingly, in two of the *Euplotes* strains we found, as primary endosymbionts, bacteria which do not belong to the *Polynucleobacter necessarius* cluster. These bacteria were representative of a new phylogenetic lineage branching in a basal position with respect to genera *Polynucleobacter* and *Ralstonia*. Likely they represent a new genus for which no free-living relatives have been described so far. The characterization of the secondary symbionts showed the presence of many bacterial species either completely new or formerly described as endosymbionts of different ciliates. The obtained results suggest that, in the investigated group of *Euplotes* species, a replacement of primary endosymbionts occurred; whether they fulfill the same host demand is still under investigation, as well as the analysis of the functional meaning of the secondary symbionts so far characterized.

## MICROSPORIDIA IN GENOMICS AND POST-GENOMICS AGE

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The microsporidian world includes more than 1,200 species of unicellular eukaryotes, all obligate intracellular parasites, that infest some protozoa, many invertebrates such as insects and crustaceans, and vertebrates such as fishes and humans. The microsporidian genomic era started with the sequencing of the tiny genome of the human opportunistic parasite *Encephalitozoon cuniculi* (2.9-megabase, 2,000 potential protein coding genes on 11 chromosomes). Phylogenetic analysis of this complete genome sequence supported the fungal origin of microsporidia and revealed a high frequency of fast-evolving genes. Genome compaction was reflected by reduced intergenic spacers related to a high frequency of overlapping gene expression, and by the shortness of most putative proteins relative to their eukaryote orthologues. This protein compaction permitted to analyse the structure and mechanism of mRNA cap (Guanine-N7) methyl-

transferase. Gene shortening may reflect a lowered diversity of protein-protein interactions as a result of the loss of numerous genes during the evolution of highly host-dependent parasites. This strong host dependence was moreover illustrated by the lack of genes for some biosynthetic pathways. Despite being very distantly related, *Antonospora locustae* partially sequenced genome confirmed these main results as well as an unexpected degree of synteny which has been successfully exploited to identify ptp1 and ptp2 genes in two insect-infecting species assigned to the *Antonospora* clade. In *E. cuniculi* genome, most repeated CDSs are of unknown function and distributed in subterminal regions that mark the transitions between subtelomeric rDNA units and chromosome cores. In particular, a potential multigenic family (*InterB* family) was described. This thirty-member protein-encoding family is present in *Encephalitozoon, Vittaforma* and *Brachiola* genera which differ in their host ranges but are all able to invade humans. Genomic studies have also focused on chromosomal composition analyses as exemplified by the complexity of *Paranosema grylli* (insect parasite) molecular karyotype. This was related to the pronounced length polymorphism of homologous chromosomes, which may be a consequence of ectopic recombination at the chromosome extremities. An important area of proteomics research implies the use of mass spectrometry as a powerful tool to identify small amounts of proteins from complex mixtures, frequently after separation by 2-D gel electrophoresis procedures. So, analysis of the *E. cuniculi* spore proteome has led to the identification of about 177 different proteins, one-quarter of these having no clearly predicted function. In a next future, it is necessary to evaluate microsporidian protein subsets subjected to major post-translational modifications such as phosphorylation and glycosylation ("phosphoproteome" and "glycoproteome"), as well as to specifically study proteins required for cell cycle progression or for spore wall and polar tube organisation.

## CHRYSOPHYTE FLORA FROM GLACIAL LAKES IN THE BASIN OF KARA RIVER (POLAR URAL)

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The Polar Ural region represents one of the last true wildness areas in Europe (Heal et al., 1998). Future effects of global climate changes and local anthropogenic disturbances (e.g. coal-mining and gas pipeline building) can be especially significant in the Arctic region. The purpose of our study was: (i) to examine by means of electron microscopy a well-recognized group of bioindicator organisms from a pristine, but ecologically sensitive area of the world; (ii) to detect possible distributional limits of individual species; (iii) to form a baseline from which future effects of anthropogenic disturbances can be estimated. The flora of chrysophytes was documented from 35 sites of oligotrophic glacial lakes with low content of dissolved salts, and range in pH from 6.5 to 6.8. First investigations of chrysophytes in the region showed a relatively diverse and abundant flora, including 43 different taxa from the genera *Chrysosphaerella* (2), *Dinobryon* (5), *Mallomonas* (16), *Paraphysomonas* (3), *Spiniferomonas* (6) and *Synura* (8). All taxa are new records for the region and two species are new for Russia: *Mallomonas multisetigera* and *Synura petersenii* f. *praefracta*. The most frequently distributed species were *Chrysosphaerella brevispina, C. longispina, Mallomonas striata, M. alpina, M. crassisquama, Paraphysomonas gladiata, Spiniferomonas cornutus, S. bilacunosa* and *Synura spinosa*. The majority of taxa are cosmopolites of the temperate rather than arctic zone. Our findings extend the known geographical distribution of many chrysophycean taxa into the cold northern region. Issues of occurrence and distribution of the chrysophyte flora in relation to some ecological data are discussed.

## ULTRASTRUCTURE OF *BOTHRIOPSIDES HISTRIO* (APICOMPLEXA: EUGREGARINIDA: ACTINOCEPHALIDAE) FROM *ACILIUS SULICATUS* (INSECTA: COLEOPTERA: DYTISCIDAE)

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The intestine of the *A. sulicatus* beetle is infected with attached and non-attached trophozoites of the eugregarine *B. histrio*. Trophozoites are able to change the outline of their bodies from spindle-like to barrel-like shapes due to contractions. Trophozoites attach to the intestinal epithelium by the highly depressed frontal edge of the protomerite, which is also kept in non-attached parasites. The surface of the parasite, except of its attaching site, is covered with numerous epicytic folds of typical structure varying in form and size in different parts of the cell. On the protomerite folds are smaller, than on the deutomerite, and have round profiles. On the deutomerite they have club-shaped profiles. At the tip of the folds there are apical arcs and apical filaments. Internal lamina underlies the pellicle. Structure and location of micropores are typical for gregarines. Bacteria are accumulated between the epicytic folds. When protomerite borders deutomerite, several annular collars are formed by the ectocyte and endocyte. Longitudinal superfolds can also be observed on the deutomerite. They are extended from the anterior to posterior end of the deutomerite, consist of an ecto- and endocyte, and bear epicytic folds. There are numerous circular microtubules underlying the surface of

trophozoites. Presence of attached and non-attached trophozoites is suggested to represent two different physiological states of the parasite. Formation of superfolds facilitates changes in the trophozoite body shape.

## SYMBIOTIC AND NON-SYMBIOTIC ALGAE DIVERSITY IN SINGLE CELLS OF *PARAMECIUM BURSARIA*

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It was shown earlier by 18S RNA gene sequence analysis, that the algae *Chlorella* sp., the endosymbionts of *P. bursaria* of both northern and southern ecotypes, are closely related to *C. vulgaris, C. sorokiniana* and *C. lobophora*. The distinctive property of 18S rRNA genes of symbiotic *Chlorella* species is the presence of one and three introns in algae from northern and southern *P. bursaria* ecotypes correspondently. Nested PCR with primers flanking the first intron, was developed for identification of Chlorella species in the single cell of P. bursaria. This method was applied to six P. bursaria cultures of different origin. As a result, at least two fragments were amplified from each cell. According to the fragment sizes we presumed, that the ~1000 bp amplicon belonged to symbiotic chlorella, bearing the intron, while the ~700 bp fragment corresponded to non-symbiotic chlorella, lacking the intron. For more precise analysis, DNA fragments amplified from two selected paramecia cells were cloned. The RFLP analysis of the cloned DNA inserts was performed. Several cloned fragments representing particular RFLP types were sequenced. As expected, specific types of symbiotic chlorella were detected in ciliates of northern vs. southern ecotypes. The endosymbionts of both ecotypes possessed one intron of the same size, which was located at different sites of the gene. Several non-symbiotic species similar to *C. sorokiniana, C. vulgaris* and other *Chlorella* spp., were found in each ciliate.

## MICROSPORIDIA OF CLADOCERANS: DISTRIBUTION AND LIFE CYCLES

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Freshwater microcrustaceans are frequently hosting microsporidia. More than 40 species of microsporidia have been reported from Cladocera, most of them from pond populations of *Daphnia pulex* (10 species) and *D. magna* (8 species). The study of microsporidians from Cladocera was performed only in several European countries and Canada, and therefore investigation of microsporidia of this host group is insufficient. The analysis of hosts of 18 microsporidian species showed that these parasites are highly host-specific. The finding of *Agglomerata sidae* in the cladocerans of two different families (Sididae and Holopediidae) seems to be an exception. Presporal development and a spore structure of many microsporidia of cladoceran hosts have unique features and on this basis 7 new genera (*Agglomerata, Baculea, Berwaldia, Glugoides, Gurleyides, Norlevinea* and *Ordospora*) were created. On the other hand the taxonomic position of many species is unclear. For example, according to the author observations, all life cycle stages of *Larssonia obtusa* have isolated nuclei and a validity of genus *Larssonia* must be re-examined. Life cycles of microsporidia of these hosts are investigated insufficiently. There are 5 positive results of horizontal transmission for *Glugoides intestinalis, Ordospora colligate, Microsporidium* sp. (Refardt et al., 2002), *Gurleya daphniae* (Friedrich et al., 1996) and *Gurleya* sp. (Voronin, Makrushin, 2006). Two species (*Flabelliforma magnivora* and *Gurleya* sp. Vavra, 1964) are transmitted with nearly 100% fidelity from mother to offspring. The attempts of artificial infection of cladocerans with 5 microsporidia species were unsuccessful, which indicates to possible occurrence of complex life cycles among microsporidia of Cladocera.

## MORPHOLOGY AND DIVERSITY OF MARINE FREE-LIVING PERITRICH CILIATES

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Peritrichs are among the oldest-known groups of ciliates having first been observed by Antony van Leeuwenhoek in 1676. Some are free-swimming while others are sessile, being attached to a substrate via a stalk, scopula or lorica. They have colonised a wide range of habitats, marine, freshwater and terrestrial, and may either be free-living or ectocommensal. They form one of the most specious groups of ciliates with around 1,000 species representing 12 families and over 50 genera. Although genus identification among peritrichs is relatively straightforward, species identification is often difficult mainly due to inadequate species descriptions and morphological variation. Historically, species descriptions were based only on *in vivo* observations. In recent years, however, modern methods such a silverstaining have been routinely applied revealing taxonomically informative characters such as the pellicular silverlines and oral infraciliature. Using such techniques, extensive surveys of peritrichs have been carried out in NE coastal regions of China. These have revealed over 100 species which represents more

than10% of the global peritrich species diversity. This talk provides a brief overview of the morphology and diversity of marine free-living peritrichs based on studies carried out in coastal waters near Qingdao, China. Supported by the Darwin Initiative Programme (project no. 14-015) which is funded by the UK Department for Environment, Food and Rural Affairs.

## EFFECT OF COFILIN-LIKE PROTEIN ON MIGRATION AND ENDOCYTOSIS OF *AMOEBA PROTEUS*

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Similarly to tissue cells, organization of the contractile network in amoebae is highly regulated by activity of actin binding proteins. Capping, severing and other proteins keeping polymerization-depolymerization balance, as well as motor proteins, namely myosins, keep together proper structure and tension of the cytoskeleton. The cofilin homologue from *A. proteus* has mass of about 19 kDa, similar to the mass of cofilin from Jurkat cells. The distribution of this protein and its co-localization with actin filaments was investigated in migrating, pinocytotic and fagocytotic amoebae. In migrating amoebae cofilin-like protein co-localized with filamentous actin in the cortical layer of the middle-anterior region of the cell, perinuclear cytoskeleton and in the area of cellular adhesion. The examined protein is also randomly distributed in the endoplasmic streaming. We suggest that it is involved in actin dynamic reorganization of the contractile layer and cytoskeleton network depolymerization of the endoplasm. In the pinocytotic amoebae cofilin homologue and actin filaments co-localized strongly beneath the whole cell membrane that may be interpreted as facilitating of actin reorganization related to pinocytotic channels formation. In the fagocytotic cells cofilin-like-protein seems to be absent in the newly formed fagocytic cup, but co-localized with F-actin on the surface of food vacuoles. The *in vivo* role of cofilin-like protein in amoebae migration and endocytosis was assessed by blocking endogenous cofilin with polyclonal antibody against human cofilin. It evoked significant decrease of motile activity. We revealed that actin dynamics induced by cofilin-like protein are crucial for normal morphology and motility of *Amoeba proteus*.

## SIGNIFICANCE OF pH AS ENVIRONMENTAL FACTOR LIMITING THE DISTRIBUTION OF FRESHWATER PROTISTS

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pH is a major environmental factor of aquatic ecosystems at the interface of physico-chemical and biological processes. It is regulated by carbonate equilibrium, both in the ocean and in most inland waters, and is impacted by biological processes such as photosynthesis and respiration. While pH is relatively constant in the ocean, it varies between <2 to 12 in fresh water. The general reduction of species diversity with decreasing pH and the tolerance limits towards low pH are known for major freshwater taxa such as fish, zooplankton and algae, but little is known on pH tolerance of aquatic protists. We investigated the pH reaction norm, i.e. population growth rate (μ) vs. pH and the response of cell size to changing pH, of 6 freshwater ciliate and one cryptophyte species in the laboratory. The protists can be broadly classified into pH tolerant and pH sensitive species; the former showed positive μ over more than 4 pH units, the latter over 2 pH units or even less under standard laboratory conditions. Using the oligotrich ciliate *Meseres corlissi*, we also studied intraspecific differences, investigating 5 clones from geographically distant environments. The results obtained suggest local adaptation in the pH response of this species to specific habitat conditions. The significance of pH impact on growth and survival of the protist species was evaluated relative to the effect of other environmental factors such as temperature, food, and predators.

## MICROSPORIDIAN DIVERSITY IN LAKE BAIKAL AND SURROUNDING WATERS

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Lake Baikal in Siberia hosts a large diversity of endemic amphipod crustaceans. There is virtually no mixing of the endemic species and the species that inhabit the surrounding tributaries and other water bodies. Microsporidia are a common parasite of amphipods and are well studied within European amphipod populations. It should, therefore, be possible to investigate the distribution of microsporidia throughout the populations, and infer whether or not the immiscibility of Baikalian amphipod populations has effected or been affected by microsporidian infection. Amphipods were screened from the lake, its surrounding waters, and from other lakes, for microsporidian infection. This was done by polymerase chain reaction used to amplify the 16s rRNA gene. Early results have indicated little or no microsporidian infection among Baikalian amphipods, an interesting point given that the majority of European amphipod have screened positive for microsporidian infection. Within Lake Baikal, microsporidia of the genus *Dictyocoela* have been found in a restricted number of amphipod species

and occur at very low prevalence. In contrast, high prevalence of microsporidian infection were found in *Gammarus lacustris*, an amphipod species inhabiting water bodies surrounding Lake Baikal but absent from the main lake. Sequencing of 16S rRNA has revealed unusually high genetic variability of *Dictyocoela* within these populations when compared with *G. lacustris* populations in European fresh water habitats.

## DIVERSITY OF MITOCHONDRIAL PROCESSES IN MICROSPORIDIA

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Microsporidia are important human parasites and are one of the most common causes of diarrhoea in people with AIDS. They are also important models of extreme eukaryotic genome compaction and cellular adaptation, and appear to be highly reduced and simplified relative to other eukaryotic cells. One such example of reduction is that of the mitochondrion or mitosome, which is a recently described but poorly, characterised organelle. An idea of the processes occurring within the organelle has been pieced together from proteins encoded in the complete *E. cuniculi* genome sequence. This suggests as a highly streamlined mitochondrion that appears to have lost both the genome and the electron transport chain. We have studied the insect infecting microsporidian *Antonospora locustae* and shown that the function of the mitochondrion may differ in this species. The mitochondrion of *A. locustae* appears to more complex in terms of how it imports proteins, the types of metabolites it can import, and pathways housed within the organelle relative to *E. cuniculi*. We have begun to characterize some of these additional proteins in order to understand how and why mitochondrial processes differ form species to species. The data suggest that mitochondrial function across microsporidia may be quite variable and show different levels of reduction across the phylum.

## ENDOCYTIC ROUTES IN *PARAMECIUM*: CONSERVATIVE ELEMENTS

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We identified conservative elements of endocytic pathways in *Paramecium* at the gene, protein and cellular level. Cloning, immunoblotting, real time PCR and immunolocalization in confocal and electron microscopy revealed presence of dynamin and Rab 7, the proteins indispensable for early and late stages of endocytic pathways, respectively, displaying a very high homology to mammalian counterparts and performing the functions similar to those in multicellular species. Dynamin was detected in the coated pits/vesicles during receptor-mediated endocytosis (RME) where it colocalized with clathrin (Wiejak et al. *Biochem Cell Biol.* 82, 2004; *J Exp Biol.* 20, 2004) and also in the membrane pool indispensable for phagosome formation (Wiejak et al. *Eur. J. Protistol.* 39, 2003). Dynamin expression quantitated in immunoblots with a specific antipeptide antibody, was elevated during internalization of transferrin, the marker of RME, and it was diminished when this process was inhibited. Trafficking from the early to late endocytic compartment is regulated by Rab7. We cloned two closely related genes encoding this protein in *Paramecium* displaying 81.6-82.1% homology to human Rab7 (Surmacz et al. *Acta Biochim. Polon.* 53, 2006). In *Paramecium* Rab 7 was detected in endosomes and in phagolysosomal compartment (Surmacz et al. *Biol. Cell* 95, 2003). Expression of two *Paramecium* Rab7 isotypes increased when cells phagocytosed latex as shown by real time PCR. Distinct pattern of localization of Rab7a and Rab7b was observed during phagocytosis with the usage of specific antibodies raised against their C-termini that differ in four amino acids residues out of 206 in total.

## MOLECULAR EPIDEMIOLOGIC STUDIES OF *ENTEROCYTOZOON BIENEUSI*: THE PERU EXPERIENCE

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*Enterocytozoon bieneusi* is a common human pathogen, responsible for more than 90% of overall human microsporidian infections. Few epidemiologic studies, however, have been conducted to characterize the infection patterns, risk factors and transmission routes of human *E. bieneusi* infections. In recent years, we have characterized the transmission of *E. bieneusi* in a case control study in AIDS patients and longitudinal cohort study in children in Lima, Peru. Seventy five of 2,652 HIV+ patients (3%) studied during September 2000 to December 2003 had microsporidiosis detected on stool microscopy at the time of enrollment, while 30 additional patients had microsporidiosis detected on a subsequent visit. Of these 105 patients, 89 had microsporidia amplified and genotyped; all were *E. bieneusi* and 11 genotypes were differentiated. Infections with *E. bieneusi* were associated with chronic diarrhea in logistic regression models adjusted for CD4+ cell count and infections with other enteric protozoa. The evaluation of associated clinical mani-

festations showed that genotypes Peru-3 to Peru-11 were significantly associated with a 4-fold increased risk in chronic diarrhea compared to patients without these parasites. The two most common genotypes, Peru-1 and Peru-2, were not associated with significant increases in chronic diarrhea. Risk factors for *E. bieneusi* infection segregated by genotype: contact with duck or chicken droppings and lack of running water, flush toilet, or garbage collection with genotype Peru-1 and watermelon consumption with other genotypes. In the longitudinal cohort study of infection in children during March 2002 to March 2006, 71 episodes of microsporidian infections were identified in 70 of 656 children studied, of which 51 were genotyped, with 14 *E. bieneusi* genotypes found. The most common genotypes found were Peru-2 and Peru-11, with Peru-1 in only one child. Most infections occurred in children under 3 years of age, and 29% of infected children had transient diarrhea lasting an average of 2.1 days. Risk factor analysis identified significant association between *E. bieneusi* infection and poor sanitary and socioeconomic conditions. Even though a direct transmission of *E. bieneusi* (Peru-16) was found between a child and guinea pigs in a household, contact with animals or animal feces was not identified as risk factors for *E. bieneusi* infection in children. Thus, molecular epidemiologic tools are very useful in the characterization of microsporidiosis transmission and the transmission of *E. bieneusi* might be very different between children and HIV+ persons living in the same area.

## MONOGRAPH OF THE SPATHIDIIDA (CILIOPHORA, HAPTORIDA)

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The spathidiids belong to the ciliate subclass Haptoria (Protozoa, Ciliophora), that is, they are predators using toxicysts to overwhelm the prey. Spathidiid ciliates prefer terrestrial and semiterrestrial habitats, but many occur also in freshwater, and some are marine. Over 200 nominal spathidiid species have been described, sometimes based on seemingly minute differences. Thus, many protozoologists considered them as indeterminable and claimed for a detailed revision. The present monograph carefully revises the taxonomy, nomenclature, and ecology of all nominal species and shows that spathidiid diversity has been greatly underestimated. Based on reinvestigation of the described species with modern methods (silver impregnation, scanning electron microscopy) and the first description of over 50 new species, the family Spathidiidae is split into four families and 20 genera. Each species is described and figured in detail, making it unnecessary to go back to the original literature often difficult to obtain. Two identification keys are provided, viz., one for taxonomists and another, simple key for users not specifically trained in ciliate taxonomy. The first part of the monograph that contains the families Protospathidiidae, Arcuospathidiidae, and Apertospathulidae, has been published (Foissner, W. & Xu, K., 2006. Springer, Monographiae Biologicae, 487 pp). The second part will come out soon and contain the family Spathidiidae and a new family, Pharyngospathidiidae. This monograph is part of our attempt to revise the free-living ciliates. Supported by the National Natural Science Foundation of China (No. 40576072), the '100 Talents Project' of CAS, and the FWF, Project P-15017.

## A NEW MICROCOSM AND ITS USE IN BIOMONITORING OF MARINE POLLUTION STATUS USING PFU PROTOZOAN COMMUNITIES

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A laboratory PFU (polyurethane foam unit) microcosm was developed to enable the biomonitoring of marine pollution status using protozoan communities. This method consists in soaking several PFU blocks to target water and incubating in laboratory. The marine pollution status is evaluated through the structural parameters of protozoan communities colonizing the PFU. We test the efficiency of the microcosm on two occasions by using sea water samples collected from six stations with different pollution levels in the Kyeonggi Bay, Korea. The data obtained suggested that neither single species richness nor abundance of protozoans could be used as an effective indicator. However, the Margalef diversity index which combines the two parameters worked well and could clearly distinguish the different classes of water quality. The highest diversity index value coincided well with the best water quality. By contrast, poor water quality corresponded with the distinctly low index values. Furthermore, the diversity index values not only generally correlated with the temporal marine pollution intensity, as inferred from the physico-chemical parameters, but also indicated the effects of long-term stress of various pollutants. Practically, the PFU microcosm can be used to carry out a comprehensive evaluation of marine water condition by using both surface and benthos water samples. It overcomes the sampling difficulties in assessing the long-term effects of marine pollution in open waters. Supported by the National Natural Science Foundation of China (No. 40576072) and the '100 Talents Project' of CAS.

### COORDINATED DISTRIBUTION OF *PARAMECIUM BURSARIA* SYNGENS AND VIRUSES OF THEIR ENDOSYMBIOTIC *CHLORELLA*

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Symbiotic chlorellae from *P. bursaria* are known to be a specific host for the giant viruses - *Paramecium bursaria Chlorella* viruses. Based upon physiology, DNA hybridization and virus infection, two types of *Chlorella*, called "American" (or "Southern") and "European" (or "Northern"), have been reported to date. Correspondingly, two types of viruses: "Southern" and "Northern", specific to one or another type of *Chlorella*, were described. Distribution of two types of *Chlorella* and viruses was supposed to depend either on latitude, or altitude, that is why two types of classification were proposed earlier. We supposed that distribution of different types of *Chlorella* and viruses could be related to the distribution of *P. bursaria* syngens. Geographical distribution of *P. bursaria* syngens was examined. 400 strains collected from the distant geographical regions were analyzed, five syngens were found. Syngens 1 and 2 were found in Europe and Asia. Syngen 1 was registered from latitude 40° to 60° North, whereas syngen 2 was spread from latitude 40° North up to and across the polar circle (>63°). Syngen 3 was found in Eastern Asia, and in North America. Syngen 4 was found in USA. Syngen 5 single strains were found in Astrakhan (Russia). A hundred of strains belonging to five syngens were examined on the presence of virus, type of virus was determined. For each syngen both virus-free and virus-containing strains were detected. 45% of examined *P. bursaria* populations contained viruses. Viruses of the "Northern" type were detected in the strains of syngens 1 and 2, whereas viruses of the "Southern" type in the strains of syngens 3 and 4. There was only one case of "Southern" virus in the strain of syngen 1 from Tajikistan. Such virus distribution indicates that different *P. bursaria* syngens might contain different types of *Chlorella*. Supported by RFBR grants 06-04-49504 and 07-04-10073.

### NUCLEAR DIFFERENTIATION OF MATING TYPES IN THE LOWER CILIATE *DILEPTUS ANSER*

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*Dileptus anser* (= *D. margaritifer*) is one of the ciliates which excrete specific inducers of conjugation (called mating pheromones or gamones) into the surrounding medium. Accordingly, mating type (MT) of a cell is determined by the kinds of mating pheromones and their receptors the cell synthesizes. So far, only three MTs have been found in the species. These three MTs are stably inherited during vegetative reproduction and usually demonstrate typical Mendelian behaviour in crosses (a single mat locus with three alleles showing peck-order dominance). However, in some crosses we observed MT instability in young, just matured exconjugant clones. In a clone of this kind, the states of maturity and immaturity (or adolescence) often alternate and/or one MT changes to another, sometimes repeatedly, during the period of several weeks after the clone's maturation. On occasion, all three MTs can be expressed consecutively. These observations suggest that the mat locus in dilepti is a compound integral one; it is inherited as a whole and can specify expression of any one of possible MTs (much as it occurs in *Tetrahymena thermophila*). Some other mechanisms, supposedly epigenetic ones among them, control what MT will be expressed in a given exconjugant clone in particular. Steady functioning of these mechanisms in the micronucleus provides stable, unambiguous differentiation of the compound mat locus to one and only one MT and subsequent Mendelian behavior of the character over sexual generations. If, for some still unknown reasons the control of such differentiation is disturbed, MT expressed in a given exconjugant clone becomes unstable and its Mendelian behavior can be violated.

### MORPHOLOGICAL VARIABILITY OF CILIATES FROM THE GENUS *CONCHOPHTHIRUS* (OLIGOHYMENOPHOREA: PLEURONEMATIDA)

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Investigation of abiotic and biotic factors influence on organisms, populations and communities of symbiotic ciliates is relevant in view of constant anthropogenic changes of environment. Some peculiarities of different-level response of ciliates could be used as indicative for detection of such environmental changes. Ciliates of the genus *Conchophthirus* are common mesobiontes of different species of fresh-water bivalve mollusks. Our investigation included only some species of these habitants of the mantle cavity, living in unionides (Unionidae) and dreissenes (Dreissenidae): *Conchophthirus acuminatus* Clap. Lachm., *C. unionis* Raabe, *C. curtus* (Engelmann), *C. anodontae* (Ehrbg.). Values of 4 meristic characters of ciliate body and 4 indices have been used for statistical analysis. Samples from the waters, heated by the power plant (Konin lakes), and samples from not extremely disturbed biotopes (Mazurian Lakes, Dnipro River, Dnister River) were chosen for comparison. As a result, dependence of some studied parameters upon water temperature has been revealed. Also our data confirmed a significant role of

relative isolation of host individuals for formation of parasitic ciliates variability, in particular, for representatives of a genus *Conchophthirus*. Existence of such variability of *Conchopththirus* sp. ciliates dependent on environmental factors, was tested and confirmed in experiments.

## SPECIES DIVERSITY AND ECOLOGY OF CILIATES FROM SMALL WATER BODIES IN THE AREA OF SARATOV RESERVOIR (THE LOWER VOLGA RIVER)

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Data on the ciliate fauna of small water bodies (lakes and ponds) adjacent to large reservoirs of the Volga River are lacking. By this study we tested the hypothesis that the "alien" ciliate species recorded occasionally in the Volga reservoirs are native for these small lakes and ponds. Survey of the fauna of ciliates in small water bodies was carried out in the region of Samarskaya Luka (the Lower Volga). Ciliate diversity increased in the direction: reservoir - flood lands - super flood lands - elevated lakes. In total, 178 and 234 species were identified in Saratov reservoir and the adjacent ponds respectively, among which 76 species were first records for the Volga basin. Twenty five species were observed exclusively in the Saratov reservoir, 72 - in elevated lakes of karstic and non-karstic origin. As many as 93 species (53% of the total) were common for all investigated water bodies. Zones of "optimal development" of the ciliate populations varied in different water bodies and fluctuated from upper to lower threshold tolerance limits. Ciliates inhabiting only large reservoirs or only small drainless lakes displayed the narrowest limits of tolerance and physiological optimum. Changes in spectrum of dominant species, as well as alterations of the ciliate community structure and dynamics varied in lakes located at different distances from the reservoir. Penetration of many "new species" into the reservoir is limited by its hydrology. We infer that changes of the hydrological mode of the reservoir benefits emergence of non-indigenous species. Thus, ciliate fauna of small ponds serves as a potential source for maintenance of ciliate diversity in large reservoirs. Findings of *Folliculina boltoni* Kent, 1881 in some lakes connected with large reservoirs of the Central and Lower Volga basin support this hypothesis.

## TINY GIANTS OF THE GREAT SEAS: FORAMINIFERA

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Foraminifera, shelled marine protozoa usually less than 1 mm in size, well known for their important role as bioindicators of past and present marine environments used in many scientific disciplines, have been dubbed as "Tiny Giants of the Great Seas". Their extremely diversified and exquisite shell morphology are virtually art forms in Nature. To popularize their scientific and aesthetic application, Zheng Shouyi, Senior Scientist of the Institute of Oceanology, Chinese Academy of Sciences, personally sculpted the proportionately enlarged 200 original foraminiferal models, which are used as popular science and educational tools, tourist souvenirs, jewelry, lamps, garment decorative designs, etc. A Foraminiferal Popularization Base in Qingdao City, China, displays foraminifera from different geological ages, as well as foraminifera, indicative of different marine environments including the entire China seas, Pacific, Atlantic, Indian, Arctic and Antarctic oceans. Foraminiferal Sculpture Park consisting of more than a hundred foraminiferal sculptures enlarged thousands of times their original size, was created in Sanxiang Township, Zhongshan City in Guangdong Province, China, being the first of the kind in the world. A new vista of microscopic foraminifera integrating science, marine culture, and art, is unfolded for the public to appreciate and enjoy the beautiful artistry of Mother Nature endowed on foraminifera, to inspire artistic creations and innovations, and to promote the cause of popular science.

## PREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES IN SERA OF STRAY CATS FROM SARI CITY, NORTHERN IRAN, 2004

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Infection by the protozoan parasite *Toxoplasma gondii* is widely prevalent in humans and animals throughout the world. The incidence of Toxoplasmosis in urban areas can be related to environmental contamination with oocyst. An interesting alternative for measuring *T. gondii* urban spreading is seroprevalence in free-living urban animals, such as stray cats. With this aim, we tested serum samples from 100 stray cats in five urban regions from Sari city, northern Iran for antibodies to *T. gondii* by latex agglutination test (LAT). Antibodies to *T. gondii* were found in 40 (40%) of 100 stray cats, with LAT titers of 1:1 (4 IU/ml) in 4, 1:2 (8 IU/ml) in 2, 1:4 (16 IU/ml) in 4, 1:8 (32 IU/ml) in 4, 1:16 (64 IU/ml) in 6, 1:32 (128 IU/ml) in 4, 1:64 (256 IU/ml) in 2, 1:128 (512 IU/ml) in 6, 1:256 (1024 IU/ml) in 7,

1:512 (2048 IU/ml) in 1. Seropositivity (LAT 1:1 or more) was significantly higher in adult (= 26 teeth, 54.54% of 66) than in non-adult (>26 teeth, 11.76% of 34) cats (P = 0.000), in weighting group 1.5-2.9 kg (56% of 25) than in weighting group 0-1.4 kg (11.76% of 34) and weighting group > or = 3 kg (53.66% of 41) cats (P = 0.000). There were no significant differences among female (44.44% of 72) and male (28.57% of 28) cats (P > 0.05), or among cats from western (50% of 20), northern (35% of 20), southern (35% of 20), eastern (35% of 20), or central regions (45% of 20) (P > 0.05). These seropositive cats are likely to have already shed *T. gondii* oocysts in the environment around Sari city. The high seroprevalence of *T. gondii* antibodies found in the present study suggested a widespread exposure of stray cats to *T. gondii*.

## THE ROLE OF PROTOZOANS IN PERIPHYTON COMMUNITIES

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Studies of microperiphyton were conducted in the lakes of Karelia, the Rybinsk reservoir, Lake Baikal, Lake Ladoga, Lake Windermere, the acidic lakes of Darwin National Park, the White Sea, the River Amur and in experimental microcosms/mesocosms. Protozoans are an important component of the periphyton communities at the early stages of colonization. Abundances of unicellular organisms in most natural and artificial periphyton assemblages range from 100 to 100 000 cells per sq.cm, and the number of species is usually more than 150. Microbial species are important members of attached communities in all ecosystems and play major roles in energy flow. The diversity and abundance of protozoan species provide information about bacterial activity, hence protozoa are integral members of the decomposer community. Periphyton communities that are formed on artificial substrates, can be defined as "periphyton model communities" (PMC). These communities are easily manipulated in the laboratory, and member species span the range of sensitivities of more familiar species. Periphyton biodiversity 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 the indicator of the trophic status of water-bodies was developed. Several advantages of utilizing PMC and new indices for pollutional stress assessment are discussed (http://biomonitoring.narod.ru). Since biota may differ essentially from one ecoregion to another, a universal biological monitoring system should use species with cosmopolitan distribution.

## THE ULTRASTRUCTURE OF MARINE CARNIVOROUS FLAGELLATE *METROMONAS SIMPLEX* (CERCOZOA INCERTAE SEDIS)

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The ultrathin structure of carnivorous marine flagellate *Metromonas simplex* has been investigated. This predator captures the whole cell of the prey, normally bodonids, or chrysomonads. A cytostome as a cell pocket has not been found. The cell surface is composed of the plasma membrane covered with two layers of fibrous material. Long flagellum bears very thin hairs of 0.8 - 1.0 μm in length, short flagellum is naked and reduced in length. The transitional zone does not contain the spiral or any other additional elements. The transversal plate is located upper the cell surface. The flagellar root system is very simple and has at least one microtubular band of two microtubules, which originates near the kinetosomes. The latter are approximately parallel to each other and interconnected with the bridges. The vesicular nucleus, Golgi apparatus and endoplasmic reticulum are of usual structure. Oval mitochondria of 0.6 x 2.5 μm contain lamellar cristae. The rod-like extrusomes (trichocysts) 1.0-1.4 μm in length and 0.12 - 0.08 μm in diameter derive from the Golgi apparatus. Trichocysts have wheel-shaped structure with 13 spokes visible in cross sections. Contractile vacuole is absent. *M. simplex* is similar to *Metopion fluens* and cryothecomonads. Supported by the Russian Foundation for Basic Research grants 05-04-48180 and 06-04-49288.

## HOW CHOANOFLAGELLATES CONQUERED THE WORLD: A SYNTHESIS BASED ON MORPHOLOGY, ECOLOGY AND EVOLUTION

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How could a group of humble filter-feeding unicells, now considered to be the nearest living protistan relatives of the animal lineage, become one of the most successful assemblages of free-living protozoa in the world? The answer would appear to lie in the most remarkable versatility of their cell coverings. This is splendidly illustrated by the evolution of a basket-like lorica which surrounds the cells of many marine species. Subtle variations in this structure have allowed the choanoflagellates to colonise a myriad of micro-niches within the oceans. Recent evidence for the evolutionary closeness of the choanoflagellates to the animals will be presented as well as the most detailed illustrated information yet available on the evolution and the remarkable life-style of these flagellates in their natural environment. Structural complexity blends seamlessly with functional significance and ecological importance.