Halteria, is not a sister taxon in the molecular analysis. Instead, *Strobilidium* and *Strombidium* each form separate branches, although both are still more closely related to the stichotrichs than to the hypotrich *Euplotes.* These phylogenetic relationships will be discussed in the context of morphological and morphogenetical data.

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De novo synthesis of Neutral Glycoglycerolipids and Phosphoglycolipids by *Leishmania donovani* Promastigotes. M. A. WYDER, D. SUL and E. S. KANESHIRO, Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio 45221.

Complex lipoconjugated antigens of *Leishmania* have now been studied by several investigators, but the less complex glycolipids are not as well characterized. Thus, in the present study, we analyzed these lipids in *L. donovani* promastigotes to determine whether some of them could be precursors for the complex surface antigens, such as the lipophosphoglycans (LPG). In some studies on LPG, when organisms were grown in lipid-containing culture medium, it is difficult to determine whether the lipid moiety or their precursors were scavenged from the medium or synthesized de novo by the parasite. Thus, promastigotes were cultured axenically in a chemically defined, lipid-free medium. At least 3 neutral and 3 phosphorylated glycolipids were detected. Using nuclear magnetic resonance (NMR) analyses, ether linkages were not detected in the 6 glycolipids, although it was not ruled out that 2 of the phosphorylated glycolipids isolated and purified by thin-layer chromatography (TLC) could have contained some ether-containing molecules. Sphingolipid long chain bases were not detected by gas chromatographic (GLC) and mass spectrometric (MS) analyses. Thus, most of these glycolipids appear to be end products of lipid biosynthesis, and are not precursors of the LPG molecules characterized by several groups. The fatty acid and monosaccharide compositions of the 6 glycolipids were characterized by GLC and GLC-MS. All fatty acids detected were released by alkaline hydrolysis, indicating that they were ester-linked. Myristate (14:0), palmitate (16:0), palmitoleate (16:1); stearate (18:0), oleate (18:1) and linoleate (18:2) were the major fatty acids present. The fatty acid profiles of two of the neutral glycolipids were similar to those of two phosphoglycolipids, suggesting a precursor-product relationship between these two pairs of glycolipids. Monosaccharides were analyzed by GLC as their alditol acetate, as well as their trimethylsilyl, derivatives. Arabinose, mannose, glucose, and galactose were detected in the glycolipids. Since these lipids were found in cells grown in a lipid-free medium, it was concluded that they were not taken up from the medium, but were synthesized de novo by *L. donovani.*

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Paramecium duboscqui. E. C. BOVEE, EMERITUS, Biol. Sci., Univ. of Kansas, Lawrence, KS, USA.

The recent excellent article by Shi et al. (JEM, 44(2):134–41) on *Paramecium duboscqui* Chatton & Brachon, 1933, causes me to report from old notes the presence of *P. duboscqui* in Florida. Two water samples were taken March 16, 1958 at the boat landing of the Suwannee River at White Springs, FL, one at surface at 19 C and a second from bottom at 9.5 C. They were taken to the laboratory and placed in a refrigerator at 10 C. On March 25, 1958 a number of *P. duboscqui* were found in the bottom sample. They were pipetted into Sonneborn's medium and the culture placed on a laboratory table overnight at room temperature. The next morning, none were recovered. Since Wichterman's book (1953) then considered *P. duboscqui* to be *inquirenda,* no formal report of *P. duboscqui* in Florida was then made. Shi et al. report it to be a cold water species, and it has also been reported from Russia (Fokin and Goertz, 1993).

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Paramecium caudatum Has How Many Cilia? E. C. BOVEE, EMERITUS, Biol. Sci., Univ. of Kansas, Lawrence, KS, USA.

To make a scale model (1000x) of *Paramecium caudatum* I needed to know the numbers and distributions of its cilia. However, I could not find in the literature an accurate count of them. By examining published electron micrographs, I determined the number, orientation and probable length of rows of cilia, the spacing of cilia along the rows, the probable number of cilia per row, and the numbers of cilia in the peniculus and along the rows of the preoral groove. I made the model of wood of the length indicated by the length of rows and size of *P. caudatum* in the micrographs. I then drew each row on the model with pencil, marked the position of each cilium on each row, drilled one hole per cilium marked, and inserted and glued a cilium (made of nylon weed-trimmer cord) into each hole. The completed model has 2,429 cilia.

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The Use of Non-Invasive Micromanipulation in Protozoology. Z. MORAVCÍK, R. JANISCH and P. ZEMÁNEK*, Department of Biology, Faculty of Medicine, Masaryk University, Brno; *Institute of Scientific Instruments of the Academy of Sciences of the Czech Republic, Brno.

The laser tweezers developed at the Institute of Scientific Instruments in Brno were tested using a variety of biological specimens. These experiments were video recorded and, after digitalisation of the record, evaluated on a Pentium PC. This novel laser device provides new opportunities for non-invasive micromanipulation in cell and molecular biology. The optical trap produced by the laser beam is capable of catching and holding both static and motile cells, as demonstrated by our experiments with *Saccharomyces cerevisiae* and *Euglena* cells, respectively. The cells trapped by mechanical forces of the tweezers could be moved in the microscope viewing field two- and three-dimensionally. These forces, however, were not sufficient to hold the cells of *Paramecium caudatum* in place and the cells had to be immobilised beforehand with the use of the agar drop method. In paramecium cells, intracellular manipulations with several cytoplasmic organelles were carried out. Free mitochondria were transferred across the whole cell diameter. Cytoplasmic crystals could be moved in relation to the cytoskeleton structure and to the optical characteristics of the cytoplasm, which are factors limiting the tweezers' power of mechanical forces. Paramecium cells were fed with an aqueous suspension of polystyrene spheres, 5 μm in diameter. The spheres were engulfed by food vacuoles, which were subsequently trapped by the optical tweezers, and an attempt was made to move these away from their original position. It appeared that food vacuoles were firmly fixed in their location and we could move them only to a limited extent. Non-invasive micromanipulations were also carried out in the cells of *Amoeba verrucosa.* Many of the cytoplasmic vesicles inside the cell are easy to transfer over the whole length of the pseudopodium. The optical tweezers proved to be a suitable tool for the study of phagocytosis in this species. As shown by video records, phagocytosis could be induced by moving a minute object trapped by the tweezers close to the amoeba. The use of optical tweezers can be extended, usually in combination with pulse laser, to facilitate microsurgical intervention in individual cells. Light energy focused on the plasma membrane in *Amoeba verrucosa* cells resulted in membrane optoporation. This local destruction of the plasma membrane is a self-repair process. In the cells of *Blepharisma undulans americanum,* neither repeated optoporation of the plasma membrane nor the subsequent eflux of the cytoplasm into the surrounding medium produced any detectable damage to the cell. This research was supported by grant no. 202/96/1077 "Use of the laser beam for manipulation with micro-objects" provided by the Grant Agency of the Czech Republic.

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Growth Kinetics of Cyclidium sp. in the culture fed with *Vibrio cholerae.* M. MACEK*,** and M. E. MARTINEZ PEREZ**, *Hydrobiological Institute, Academy of Sciences of the Czech Republic; 370 05 Ceske Budejovice, Czech Republic; **National Autonomous University of Mexico campus Iztacala, Tlalnepantla 54090, Edo. Mex., Mexico.

Data on feeding rates of *Cyclidium* cf. *glaucoma* upon *Vibrio cholerae* in different microcosms were analysed for the ciliate growth kinetics. In the experiments, waters of two Mexican natural saline lakes and a brackish lagoon were used and vibrios were added as the major organic—matter source at a concentration of 106 to 107 ml-1. The ciliate feeding rates were evaluated using fluorescently labelled *Vibrio cholerae* (FLB). Uptake rates were calculated based both on total direct bacterial counts (DC; DAPI staining) and on *V. cholerae* counts (indirect fluorescent antibody method and colony forming units, CFU on a selective medium). Growth rate response either to DC or to CFU was not observed. Moreover, calculated uptake rates showed that the ciliate did not ingest *V. cholerae* FLB proportionally to the growth rate (if feeding typically 1,000 to 1,400 bacteria ciliate-1 h-1). The highest clearance rates on *V. cholerae* (up to 200 nl cell-1 h-1) were reached consequently to a significant decrease in *V. cholerae* CFU (although the maximum calculated CFU uptake was observed before). A toxicity effect of *V. cholerae* on ciliates was suggested the most important mechanism leading to a supposed selective feeding on other bacteria than *Vibrio cholerae.*

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Influence of Methanogenic Inhibitors on Methanogenesis of Rumen Ciliates *Entodinium caudatum* and *Epidinium ecaudatum.* S. KISIDAYOVÁ, Z. VÁRADYOVÁ, P. SIROKA and I. ZELENÁK, Institute of Animal Physiology, SAS, Soltésová 4-6, 040 01 Kosice, Slovak Republic.

The methanogenic activity of *Entodinium caudatum* (*E.c.*) culture was investigated and compared to the culture of *Epidinium ecaudatum* f. *caudatum et ecaudatum* (*Epi.e.*). The production of methane, volatile fatty acids and digestibility of the substrates were measured. The effects of penicillin G, streptomycin, chloramphenicol, 2-bromoethanesulfonic acid and pyromellitic diimide on the methanogenesis of ciliate protozoan cultures were tested. The methanogenic activity of both *E.c.* and *Epi.e.* was well-preserved after long-term cultivation. Microscopic observation revealed that methane production by *E.c.* was probably caused by their intracellular methanogenic activity, while methane production by *Epi.e.* could be accounted to both the methanogenic bacterial fraction of their external surface and their intracellular activity. Decrease of digestibility and differences in the fermentation end products accompanied the inhibition of methanogenesis in both cultures. *E.c.* appeared to be more sensitive than *Epi.e.* to the compounds tested.

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Myxozoa Infecting Green Scat, *Scatophagus argus.* I. FIALA, Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovská 31, 370 05 Ceské Budejovice, Czech Republic.

Five myxosporean species were found in *Scatophagus argus* (Linnaeus, 1766) (Actinopterygii: Perciformes) imported from Southeast Asia. *Myxobolus* sp. found in the gills had spores averaging 8 × 8 μm with a massive suture line and anteriorly crossed polar capsules. In the gall bladder, disporic plasmodia of a *Ceratomyxa* sp. occurred; the crescent-shaped spores averaged 6 × 17 μm. The gall bladder harboured a *Myxidium* sp. producing fusiform spores with sharply pointed ends, averaging 10 × 8 μm. The urinary bladder was infected by a *Chloromyxum* sp. Disporic plasmodia produced spherical spores averaging 8 × 8 μm; the spores had two caudal projections. Ellipsoidal spores of *Ortholinea* sp. with wider anterior end (average size 7 × 6 μm) were found in the urinary tract. Extrasporogonic stages inducing xenoma formation appeared in its developmental cycle. These stages induce hypertrophy of cells of glomerulus, which reveals a hypertrophic nucleus and cytoplasm replete with myxosporean stages.

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Mammalian Microsporidia, Rare or Frequent Parasites? J. VÁVRA and B. KOUDELA, Department of Parasitology, Charles University, Prague, Czech Republic and the Institute of Parasitology, Academy of Science, Ceské Budejovice, Czech Republic.

The microsporidian fauna of mammals (including man) is presently limited to 11 species of 7 genera. Only the representatives of 2 genera (*Enterocytozoon*—1 species, *Encephalitozoon*—3 species) occur frequently, the species of the remaining genera are reported as sporadical cases. Most reports on microsporidia-infected mammalian hosts concern man and a few other hosts only (e.g. rabbit, laboratory and wild rodents, some carnivores). It is believed that the low prevalence of microsporidia in mammals is a kind of artefact, as some factors (large body size, effective immune system, low pathogenicity of microsporidia) are making the detection of microsporidia in a mammalian body more difficult as compared with the situation in invertebrates. Recent reports on the occurrence of microsporidia in additional mammalian host (e.g. *Enterocytozoon* in pigs and rabbits, *Encephalitozoon intestinalis* in ruminants), obtained thanks to more sensitive diagnostic methods, favour the assumption that microsporidian infections in mammals are more frequent than hitherto supposed. The idea is proposed that the 11 species of mammalian microsporidia represent two groups, the dividing character being the ability to withstand the

mammalian body temperature of 37 C. The "deep organ" parasites (*Enterocytozoon bieneusi, Encephalitozoon spp., Vittaforma corneae, Trachipleistophora spp., Brachiola spp., Thelohania apodemi*) probably represent the "true" mammalian microsporidia, while the species causing ocular infections only (*Nosema algerae, N. ocularum, Microsporidium ceylonensis, M. africanum, M. buyukmihcii*) represent "accidental" infections by non-mammalian microsporidia able to grow at temperatures below 37 C in an immunoprivileged site of the mammalian cornea.

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Results of COWP Gene Analysis of Human Isolates of *Cryptosporidium parvum.* O. HAJDUSEK* and O. DITRICH**, Faculty of Biological Sciences, University of South Bohemia, Branisovská 31, 370 05 Ceské Budejovice, Czech Republic; **Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovská 31, 370 05 Ceské Budejovice, Czech Republic.

Cryptosporidium parvum Tyzzer, 1912 is an intracellular, extracytoplasmatic parasite in the enterocytes of mammals including humans. Genetic analyses of oocysts from human and animal stools have shown 2 different genotypes of *C. parvum.* The first genotype (human) has been found only in humans, the second genotype (bovine) both in humans and animals. These two genotypes can be differentiated both at the biochemical and molecular level. They have a variable infectivity and virulence for different host species. In water outbreaks genotype 1 has been commonly found while in sporadic cases both genotypes occurred. For typing of human samples from South Bohemia, Czech Republic, PCR-RFLP (RsaI restriction enzyme) was used with the COWP (*Cryptosporidium* oocyst wall protein) gene as a marker. Samples were from children of age 1 to 5 years. Since we observed only genotype 2 (bovine), we suppose that children in this region could be infected mostly by close contact with calves, directly or through other infected persons. Since we did not observed genotype 1, we suggest that this genotype is typical for water outbreaks, never registered in the Czech Republic. However, *Cryptosporidium* sp. is present in water supplies in the Czech Republic. We have done this PCR-RFLP analysis with *Cryptosporidium muris* and *C. baileyi* too. By using this method we were not able distinguish these two species from *C. parvum* genotype 2.

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Cryptosporidium Oocysts and *Giardia* Cysts in Surface Waters in the Czech Republic. G. PUZOVÁ,* O. DITRICH,** P. DOLEJS*** and T. MACHULA***, *Biological Faculty, South Bohemian University, Branisovská 31, 37005 Ceské Budejovice; **Institute of Parasitology, AS CR, Branisovská 31, 37005 Ceské Budejovice, ***WE&T Team, Box 27, Písecká 2, 37011 Ceské Budejovice.

This study was carried out to estimate the levels of contamination of water sources by *Cryptosporidium* oocysts and *Giardia* cysts in the Czech Republic. The SUPER MICRO-WYND filters and standard EPA procedure were used, however the method of it's processing was slightly modificated (different regime of filter washing, different way of counting, use of citric acid for dissolving of iron flakes if present). The efficiency of recovery for this method ranged from 10.1%–26%. The tested ELISA method was found rather inapplicable for water samples because of it's low recovery of oocysts and the fact it doesn't distinguish between normal and broken oocysts or cysts. Of the 27 raw water samples examined, 85% contained *Cryptosporidium* oocysts (maximum 32140 oocysts/100 l) and 30% *Giardia* cysts (maximum 485 cyst/100 l). 22 drinking water samples originating from contaminated raw water were also examined, oocysts were found in 73% (maximum 3 130/100 l) and cysts in 9% of all indicating low removal rates of especially oocysts in the waterworks. In one selected reservoir significant increase of oocysts after the floods in 1997 and the slow decrease in the following year were documented.

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FreeTree—Freeware Program for Construction of Phylogenetic Trees on the Basis of Distance Data and Bootstrap/Jackknife Analysis of the Tree Robusticity. Application in the RAPD Analysis of Genus *Frenkelia.* A. PAVLÍCEK,* S. HRDÁ** and J. FLEGR, *Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague; **Department of Parasitology, Faculty of Science, Charles University, Prague, 128 44 Czech Republic.

The program FreeTree was primarily intended for the analysis of results of DNA fingerprinting methods (RFLP, RAPD, AP-PCR) or other methods which provide binary data (presence/absence of the characters). For such data the program computes the distance matrix, constructs the phylogenetic or phenetic tree and computes bootstrapping or jackknifing values for internal branches of the tree. The program can be used also for the construction of trees on the basis of frequency data (e.g. results of isoenzyme analysis). We used the program for an analysis of RAPD data from 22 strains of *Frenkelia,* the coccidian parasite of small rodents. The results suggest that *F. glareoli* and *F. microti* are two distinct species despite the fact that cysts of some strains included into the analysis have a slightly intermediate phenotype. The program is available at http://www.natur.cuni.cz/~flegr/programs/freetree.

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Discovery of the Life Cycle of *Sarcocystis lacertae* Babudieri, 1932 (Apicomplexa: Sarcocystidae). J. VOLF,* D. MODRY,*,** B. KOUDELA** and J. R. SLAPETA*, *Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 612 42 Brno, Czech Republic; **Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovská 31, 370 05 Ceské Budejovice, Czech Republic.

Oocysts/sporocysts of *Sarcocystis* sp. were found in the intestinal contents of the smooth snake, *Coronella austriaca.* Common voles *Microtus arvalis,* bank voles *Clethrionomys glareolus,* green lizards *Lacerta viridis,* and common wall lizards *Podarcis muralis* were experimentally inoculated as potential intermediate hosts. Only common wall lizards were found to be susceptible intermediate hosts. Transparent, macroscopically hardly visible sarcocysts found in tail striated muscles of the lizards were 480 (390–640) × 210 (190–230) μm in size at 72 days post-infection. Using the light microscopy, the sarcocyst wall was about 1μm thick with apparent layer of villi approx. 2 μm thick. Ultrastructurally, the primary cyst wall was characterised by spine-like villar protrusions, up to 2.5 μm in length and 0.5 μ m in diameter. Based on sarcocyst morphology and experimental data, the discovered *Sarcocystis* species is suggested to be conspecific with *Sarcocystis lacertae* Babudieri, 1932.

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Sarcocystis Muris Possesses Both Diheteroxenous and Dihomoxenous Characters of Its Life Cycle. B. KOUDELA* and D. MODRY**, *Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovská 31, 370 05 Ceské Budejovice, Czech Republic; **Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 612 42 Brno, Czech Republic.

The cystozoites of *Sarcocystis muris* were infective to other mice after oral inoculation. They transformed into gamonts and after fertilisation performed sporulation with the production of infectious oocysts/sporocysts in lamina propria of the small intestine. This study demonstrated that *S. muris* possesses both diheteroxenous and dihomoxenous characters of life cycle and can be transmitted by the cannibalism among mice.

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Finding of Haemogregarines in Marine Snakes of the Genus *Laticauda.* D. MODRY,** M. VESELY,*** J. VOLF,* and B. KOUDELA**, *Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého 1-3, 612 42 Brno, Czech Republic; **Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovská 31, 370 05 Ceské Budejovice, Czech Republic; ***Department of Zoology, Faculty of Natural Sciences, Palacky University, tr. Svobody 26, 771 46 Olomouc, Czech Republic.

Parasitological examination of 3 *Laticauda colubrina* and 4 *L. laticaudata* (Serpentes: Elapidae) freshly imported from New Caledonia revealed numerous intraerythrocytic gamonts of haemogregarines in 2 *L. colubrina* and 2 *L. laticaudata.* Observed gamonts, measuring ~20.7 × 4.3 μm, were localised eccentrically in erythrocytes, which were enlarged up to 130% of normal size. Intraerythrocytic meronts were not observed. Postmortal examination of two snakes revealed meronts, ~20–30 × 18–22 μm, localised in endothelial cells of blood capillaries of liver, kidneys, myocardium, intestine submucosa and mainly in lungs. 20–30 vermiform merozoites, 15–18 × 2–3 μm were observed within fully developed meronts. No signs of inflammation were observed in infected tissues. Although final generic determination is impossible without knowledge on the data on morphology of oocysts/sporocysts from final host/vector, found haemogregarines can be tentatively placed into the genus *Hepatozoon* Miller 1908.

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Blood Parasites and Haematophagous Insects of Raptors (Falconiformes) in the Czech Republic. J. VOTYPKA, M. SVOBODOVÁ, P. VORÍSEK,** L. PESKE, D. LACINA,* and P. VOLF, Dept. Parasitology, Charles University, 128 44 Prague, *Czech Society for Ornithology, Prague, **Laboratory of Ornithology, Palacky University, Olomouc, Czech Republic.

Blood parasites of raptors were investigated during the breeding seasons to find vectors of blood protists of raptors in Czech Republic. In 1996–1998 blood was collected from nestlings and adults of the common buzzard (*Buteo buteo,* n = 184), kestrel (*Falco tinnunculus,* n = 185) and sparrowhawk (*Accipiter nisus,* n = 505). Blood films and cultivation on blood agar were done. A high prevalence of *Leucocytozoon* (80% resp. 90%), *Trypanosoma* (70% resp. 80%) and *Haemoproteus* (80% resp. 40%) was found in adult buzzards and sparrowhawks. All these parasites were found in young buzzards and sparrowhawks, too, but the prevalence was markedly lower. No blood parasites were found in the nestling kestrels. *Plasmodium* was not found at all. Haematophagous insects feeding on nesting birds were collected using the sticky bands (during 1996–1998), and the CDC traps (in 1998 and 1999). In total, we captured 4 species of blackflies (90% *Eusimulium angustipes*), 12 species of biting midges (mainly *Culicoides pictipennis, C. festivipennis* and *C. cubitalis*) 2 species of mosquitoes (mainly *Culex pipiens*), and the hippoboscid fly *Ornithomyia avicularia.* In guts of insects caught using CDC traps, we found trypanosomatid parasites in hippoboscid flies (prevalence 6%), mosquitoes (4%), blackflies (1.5%), and ceratopogonids (1%). Our results show, that an important part of populations of raptors (family Accipitridae) from different localities is infected with blood parasites. Vectors of different blood parasites in respective hosts are being sought for.

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Naegleria Strains Isolated from Organs of Freshwater Fishes. I. DYKOVÁ, I. KYSELOVÁ, B. MACHÁCKOVÁ and H. PECKOVA, Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovská 31, 370 05 Ceské Budejovice, Czech Republic.

Twenty strains of amoeboflagellates were isolated from organs of seven species of fish. Morphometric analysis assigned 18 clones into the genus *Naegleria.* The corresponding strains were isolated from the gills of 11 specimens of *Oncorhynchus mykiss* and of one specimen of *Salvelinus fontinalis.* Six strains were isolated from various organs of the body cavity of *Perca fluviatilis* (3), *Blica bjoerkna* (1), *Salmo trutta* (1) and hybrid of *Clarias gariepinus* and *macrocephalus* (1). Amoebic trophozoites transformed into flagellated stages within one or two hours, after the agar plate cultures have been overlaid with water. All clones produced cysts. Statistical analysis supplied a basis for the selection of *Naegleria* species to be used for species determination by means of molecular analysis. Three clones derived from two strains isolated from the gills of *Cyprinus carpio* could not be allotted either to the genus *Naegleria* or to any of the genera of the family Vahlkampfiidae. They differed in having mitochondria with branched tubular cristae and cyst walls without spores. In addition one of the three clones differed from the other two in having binucleate cysts and a single flagellum in the flagellated stage. This assemblage of clones is quite unique in respect to its origin. The results obtained thus far suggest the need of further study of isolated strains and indicate that fish hosts may yield important material for the research of *Naegleria* spp. and amoeboflagellates in general.

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Cutaneous leishmaniasis in Two Refugees Camp in Northern Afghanistan. M. L. AMIRI,* M. S. JALILI,** R. HOLKOVA and N. JALILI, Institute of Parasitology, Faculty of Medicine, Comenius University, Bratislava, Slovakia; *Institute for Malaria and Parasitic Diseases, Mazar-e-Sharif, Afghanistan; **Army Hospital, Mazar-e-Sharif, Afghanistan.

Parasitic flagellates from the genus of *Leishmania* are responsible for several clinically distinctive diseases characterised by chronic inflammatory infiltration, focal necrosis and fibrosis. Worldwide, some 12 million people are estimated to be infected and over 2 million new cases occur each year. From April 1995–March 1997 we diagnosed 1258 samples (taken from the periphery of patients' ulcer), from suspected patients for cutaneous leishmaniasis in two refugees camp in and around the town of Mazar-e-Sharif in Balkh province—northern Afghanistan. From the diagnosed number 1161 samples found to be positive. Of which, 1071 cases (92%) were

detected as *Leishmania major* (rural form), 90 cases (8%) as *Leishmania tropica* (urban form). In our results we recorded the high incidence of positive cases (mainly rural form) during the autumn that can be explained by the incubation period of leishmaniasis and the activity of vectors especially in summer months.

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Phlebotomine Sandflies and Rodents in Endemic Focus of *Leishmania tropica* in Urfa, Turkey. M. SVOBODOVÁ, J. SÁDLOVÁ, J. VOTYPKA, P. VOLF and K. P. CHANG*, Department of Parasitology, Charles University, 128 44 Prague, Czech Republic; *Department of Microbiology/Immunology, Chicago Medical School, IL 60064 USA.

Sandflies (Diptera: Psychodidae) and small mammals were investigated as possible vectors and reservoirs of *Leishmania tropica* in Urfa focus. Sandflies were caught using CDC light traps in houses, basements and stables in endemic quarters. From 3573 sandflies trapped, 80% were *Phlebotomus sergenti,* and 20% *P. papatasi,* the other species representing less than 1%. All 541 dissected females were *Leishmania*-negative. Small rodents (*Rattus rattus, Mus musculus, Meriones* cf. *tristrami, Crocidura suaveolens,* N = 59) were caught in snap traps and live traps; isolation attempts from lymph nodes, spleen and liver were negative. Laboratory colonies of *P. sergenti* and *P. papatasi* were established with 205 and 97 initial females, respectively. *P. papatasi* was growing well while teneral adults of *P. sergenti* were suffering mortality, probably due to *Ascogregarina* sp. infection. Golden hamsters inoculated with *L. tropica* MHOM/TR/98/SU23 (from Urfa) developed lesions. Two unnatural vectors were used to test the infectivity of *L. tropica*-positive hamsters for sandflies. Sandflies fed on lesions were dissected 7–11 days later. Out of 100 *Lutzomyia longipalpis,* 8 were positive. On the other hand, all 100 *P. duboscqi* females were negative.

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Effect of Aphidicolin on the Growth and Encystation of *Giardia intestinalis* in vitro. S. PEICHL and E. NOHYNKOVÁ, Department of Tropical Medicine, 1st Faculty of Medicine, Charles University, Studnickova 7, 128 00 Prague, Czech Republic.

Giardia intestinalis is a cause of diarrheal infections in humans and animals worldwide. The cell cycle and checkpoint control systems of this common parasite, however, are mostly unknown. We used aphidicolin, a specific inhibitor of eukaryotic DNA polymerases which arrests cell cycle of the most eukaryotic cells at the G1/S border, to investigate effect of the drug on growth, DNA synthesis and encystment of *Giardia* in vitro. Aphidicolin, 0.5μ g, 1μ g and 3μ g/ml, inhibited 50, 75 and 100% of control *Giardia* growth, respectively. The inhibitory effect was reversible up to 5μ g/ml of aphidicolin. Treatment of trophozoites with concentrations of the drug which blocked the growth completely (3–5μ g/ml for 24h), yielded a morphologically uniform population of broad, non-dividing cells with well developed median bodies. In contrast, the median body was present in only 45% of proliferating control *Giardia.* As revealed by pulse-labeling with 5-bromodeoxyuridine (BrdU) followed by indirect immunofluorescence, DNA synthesis was completely inhibited in both nuclei after exposure of the cells to 5μ g/ml aphidicolin. When the trophozoites of the human isolate (HHR) which readily encysts in vitro were exposed to 3μ g/ml aphidicolin for 24 h prior to transfer to encystation medium, the encystation was almost completely inhibited. Our results indicate that (1) aphidicolin arrests *Giardia* cells either in the G1/S or in early S-phase of the cell cycle, (2) assembly of the median body takes place during the G1-phase and (3) DNA synthesis is a prerequisite of the process of encystation of *Giardia.*

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Cell-Cycle Dependent Localization of Gamma-Tubulin in a Unicellular Eukaryote, *Giardia intestinalis.* E. NOHYNKOVÁ,* P. DRÁBER,** J. REISCHIG,*** and J. KULDA****, *Department of Tropical Medicine, 1st Faculty of Medicine, Charles University, Studnickova 7, Prague, **Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Vídenská 1083, Prague, ***Department of Biology, Faculty of Medicine, Charles University, Karlovarská 48, Pilsen, ****Department of Parasitology, Faculty of Science, Charles University, Vinicná 7, 128 44 Prague 2, Czech Republic.

Giardia intestinalis, a bi-nucleated amitochondrial flagellate representing an ancient lineage of protists, possesses a complex cytoskeleton based on several microtubular systems (flagella, adhesive disk, median body, funis, mitotic spindles). MTOCs of the individual systems have not been fully defined. By using monoclonal antibodies against a conserved synthetic peptide from the C-terminus of human γ-tubulin we investigated occurrence and distribution of γ-tubulin during *Giardia* cell cycle. On the immunoblots of *Giardia* cytoskeletal extracts the antibodies bound to a single polypeptide of approximately 50 kDa. Immunostaining of the interphase cell demonstrated γ-tubulin as four bright spots at the basis of four out of eight flagella. γ-Tubulin was associated with perikinetosomal areas of the ventral and posterolateral pairs of flagella which are formed de novo during cell division. Basal body regions of the anterolateral and caudal pairs of flagella which persist during the division and are integrated into the flagellar systems of the daughter cells did not show γ-tubulin staining. At early mitosis, γ-tubulin spots disappeared reappearing again at late mitosis in accord with reorganization of flagellar apparatus. Antibody-detectable γ-tubulin was absent at the poles of both mitotic spindles. Albendazole treated *Giardia,* in which spindle assembly was completely inhibited, showed the same γ-tubulin staining pattern thus confirming that the fluorescence label is exclusively located in the basal body regions. Our results point to a role of γ-tubulin in nucleation of microtubules of newly formed flagella and indicate unusual mitotic spindle assembly in absence of γ-tubulin. Moreover, the demonstration of γ-tubulin in *Giardia* shows ubiquity of this protein through the evolutionary history of eukaryotes.

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RAPD Analysis of Trichomonads. V. HAMPL, A. PAVLÍCEK and J. FLEGR, Department of Parasitology, Faculty of Science, Charles University, Vinicná 7, 128 44 Prague 2, Czech Republic.

DNA of 47 strains of 12 trichomonad species was analysed by RAPD using 18 random primers. Freetree program was used to construct the phylogenetic tree and to carry out bootstrap analysis of the tree robustness. The position of 8 strains of cattle pathogen *Tritrichomonas foetus* and 6 strains of parasite of pig *Tritrichomonas suis* suggests that all of them belong to the same species. On the other hand large genetic distance between two strains of *Tritrichomonas mobilensis* indicates that these strains can be in fact two distinct species. Our results suggest the existence of concordance between the genetic relationship of strains of *T. foetus-suis* and their geographic origin. A concordance was also found between the genetic relationship of 18 strains of *T. vaginalis* and their resistance to metronidazole. Such concordance was not found with respect to the geographic origin and presence of dsRNA virus in strains of *T. vaginalis.* The intraspecies genetic variability of *T. vaginalis* is remarkably higher than the variability of *T. foetus-suis.* This could be either a result of different evolution rate in these species or of different history of host populations. The history of domestication of cattle or pig was more then one order of magnitude shorter than history of *Homo sapiens.* Therefore the strains of *T. foetus-suis* had less time to diverge than the strains of human parasite *T. vaginalis.*

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Isolation, Morphology and ITS-5.8S rRNA Sequence Analysis of *Trichomonas Canistomae.* J. TACHEZY, K. KUTISOVA, B. KOUDELA and J. KULDA, Department of Parasitology, Faculty of Science, Charles University, Vinicná 7, 128 44 Prague 2, Czech Republic.

Trichomonas canistomae has been described from the mouth of dog by Hegner and Ratcliffe in 1927. Levine (1973) transferred the species to the genus *Tetratrichomonas.* However, electron micrographs published in thesis by Breuker (1995) indicate *Trichomonas* characters. To verify taxonomic position of the organism we have isolated 2 strains of the oral trichomonad in polyxenic culture on Diamond's TYSGM medium. Transmission electron microscopy showed typical ultrastructure of trichomonadinae subfamily: undulating membrane consisting of a cytoplasmic fold adjacent to unmodified recurrent flagellum, B-type costa attached laterally to the kinetosome of the recurrent flagellum. Four anterior flagella and absence of the trailing part of the recurrent flagellum, clearly indicated pertinence to the genus *Trichomonas.* The taxonomic position of the trichomonad was further verified by a sequence analysis of the 5.8S rRNA gene and flanking internal transcribed spacer regions ITS1 and ITS2. The regions were amplified by PCR, the amplified DNA fragments (about 370 bp) were subcloned and two clones were analyzed. For comparative reasons, the set included 4 other trichomonad species sequenced by us (*Tetratrichomonas gallinarum, Tetratrichomonas prowazeki, Tetratrichomonas* sp., *Tritrichomonas foetus*), as well as sequences of 5 species present in GenBank (*Trichomonas tenax, Trichomonas vaginalis, Pentatrichomonas hominis, Tritrichomonas suis, Tritrichomonas mobilensis*). The sequences were analyzed using Clustal method with weighted residua weight table. *Trichomonas canistomae* consistently grouped together with other *Trichomonas* species, while separate groups were formed by tetratichomonad and tritrichomonad species. Both morphology and sequence data show that the trichomonad of the dog oral cavity belong to the genus *Trichomonas.*

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Phylogeny of the Subclass Peniculia (Ciliophora, Oligohymenophorea) Inferred from SSU rDNA Sequences. M. C. STRUEDER-KYPKE*, A.-D. G. WRIGHT**, S. I. FOKIN*** and D.H. LYNN*, *Dept. of Zoology, University of Guelph, Ontario, Canada; CSIRO Animal Production WA 6014, Australia; Biological Institute of St. Petersburg State University, Russia.

Peniculines are distinguished from other ciliate taxa by well-defined characters like the ultrastructure of their somatic mono- and dikinetids, the occurrence of trichocysts, a large vestibulum, and the ophryobuccokinetal pattern of their stomatogenesis. Different models exist regarding the classification of the peniculine ciliates, which differ in particular in the position of *Urocentrum turbo.* We sequenced the SSU rRNA genes of *Lembadion bullinum, Urocentrum turbo,* and 11 different *Paramecium* species from PCR-amplified products of the SSU rDNA, aligned the sequences, and compared them by distance matrix and parsimony methods. The genus *Paramecium* is monophyletic and *P. bursaria* branches basal to the other *Paramecium* species. The morphologically defined subgroups "bursaria" and "woodruffi" must be regarded as paraphyletic, while the "aurelia" subgroup is monophyletic. The molecular analysis is consistent with assignment of the peniculines to a separate subclass in the class Oligohymenophorea. Moreover, sequence analysis of representative species of three subgroups of the peniculines shows monophyly for the order Peniculida sensu de Puytorac (1994). The position of *Urocentrum turbo* has still to be resolved: morphological characters propose a placement within the subclass Peniculia while the SSU rDNA sequence analyses favor assignment as a separate clade within the subclass Hymenostomatia.

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Trachelocercid Karyorelictids (Protozoa, Ciliophora) have a Parakinetal Stomatogenesis. W. FOISSNER and K. AL-RASHEID, Universität Salzburg Institut für Zoologie, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria and King Saud University, Zoology Department, Riyadh, Saudi Arabia.

Ontogenesis in *Sultanophrys arabica* Foissner & AL-Rasheid, 1999, a trachelocercid karyorelictid ciliate, was investigated using live observation, silver impregnation, and scanning electron microscopy. Division is homothetogenic and occurs in freely motile (non-encysted) condition. The parental oral apparatus does not reorganise and cell shape is maintained. Stomatogenesis is parakinetal, that is, the anlage for the opisthe oral apparatus is derived directly from the first ordinary somatic ciliary row right of the glabrous stripe and has no connection with parental mouth structures. The oral primordium appears slightly subequatorially and consists of an anarchic field of basal bodies, from which many short dikinetidal kinetofragments differentiate. The kinetofragments migrate centrifugally and assemble to a circumoral kinety and three minute adoral organelles (brosse kineties). The somatic kineties, the bristle kinety, and the lateral kinety divide without anlagen formation. Thus, morphogenesis of trachelocercid karyorelictids is simple and distinctly different from that of loxodid karyorelictids, which develop the oral primordium buccokinetally. This shows that different stomatogenic modes developed very early