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Body height, body mass index, waist-hip ratio, fluctuating asymmetry and second to fourth digit ratio (2D : 4D) in subjects with latent toxoplasmosis. J. FLEGR*, M. HRUSKOVÁ**, Z. HODNY*** and J. HANUSOVÁ*, *Department of Parasitology, Faculty of Science, Charles University, Vinicna 7, 128 43 Prague 2, Czech Republic, **Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Vinicna 7, 128 43 Prague 2, Czech Republic, ***Department of Cellular Ultrastructure and Molecular Biology, Academy Sciences of the Czech Republic.

Between 20% and 60% of the population of most countries are infected with the protozoan *Toxoplasma gondii*. Subjects with clinically asymptomatic life-long latent toxoplasmosis differ from those who are *Toxoplasma* free in several behavioral parameters. Case–control studies cannot decide whether these differences already existed before infection or whether they were induced by the presence of *Toxoplasma* in the brain of infected hosts. Here, we searched for such morphological differences between *Toxoxoplasma*-infected and *Toxoplasma*-free subjects that could be induced by the parasite (body weight, body height, body mass index, waist–hip ratio), or could rather correlate with their natural resistance to parasitic infection (fluctuating asymmetry, 2D : 4D ratio). We found *Toxoplasma*-infected men to be taller and *Toxoplasma*-infected men and women to have lower 2D : 4D ratios previously reported to be associated with higher prenatal testosterone levels. The 2D : 4D ratio negatively correlated with the level of specific anti-*Toxoplasma* antibodies in *Toxoplasma*-free subjects. These results suggest that some of the observed differences between infected and noninfected subjects may have existed before infection and could be caused by the lower natural resistance to *Toxoplasma* infection in subjects with higher prenatal testosterone levels.

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Diagnostics of *Neospora caninum* antibodies of bovine milk by ELISA. L. HURKOVÁ*, D. HARAGÁLOVÁ* and D. MODRY*,**, *Department of Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, 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.

Neospora caninum (Apicomplexa: Sarcocystidae) is a coccidian parasite with a two-host life-cycle. Dogs and coyotes are definitive hosts. A wide spectrum of mammals serves as intermediate hosts (i.a. ruminants, horses, canids). The reproductive failures associated with abortion in cattle have an economic impact. A milk ELISA is a simple serological method for diagnostics of anti-*Neospora caninum* antibodies in dairy herds. We used a commercial *Neospora caninum* iscom ELISA kit (Svanova, Sweden) designed to detect bovine *Neospora*-specific antibodies in blood serum. We examined 332 dairy herds in the Czech Republic. Milk serum was examined at dilution 1:100 according to manufacturer's instructions. The number of milking cows in the examined herds varied, but never exceeded 200 per one bulk sample. Four out of 332 examined herds were significantly positive, the positive bulk milk samples were obtained from herds consisting of 7, 19, 35 and 98 cows. Hitherto, we have studied in detail the smallest herd. The milk and blood samples or only blood samples (in some animals, e.g. calves) were examined individually. We observed the correlation of positivity between milk and blood. Moreover, the mother-descendant positivity/negativity was found. A dog living in the farm was examined, being coprologically negative but serologically positive (IFAT).

This is the next proof of the occurrence of *N. caninum* in cattle in the Czech Republic. In addition, our results refer to an importance of transplacental transmission in cattle.

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The effect of heavy metals on the rumen ciliate *Entodinium caudatum*. K. MIHALIKOVÁ, P. JAVORSKY, Z. VÁRADYOVÁ and S. KISIDAYOVÁ, The Institute of Animal Physiology, Slovak Academy of Sciences, Soltésovej 4–6, 04001 Kosice, Slovak Republic.

The effect of three heavy metals on the growth of the rumen ciliate *Entodinium caudatum* was studied in vitro. The *Entodinium caudatum* (*E.c.*) cells were treated with mercury ($HgCl_2$), copper ($CuCl_2$) and chromium ($K_2Cr_2O_7$) for a period of 4–5 days. The tested concentrations of mercury and copper were 1, 5, 10, 20, 50 mg/l[1] and concentrations of chromium were 2, 10, 20, 40, 100 mg/l. All experiments were performed under two different conditions: either in presence of intact bacterial population in culture or reduced bacterial population, when culture was treated by antibiotics. Concentration of Hg of 5–20 mg/l1 significantly decreased the concentration of *E.c.* cells at the end of experiment. The dose of 50 mg/l was lethal. Copper caused significant decrease in the *E.c.* cell concentration after exposure to 10–50 mg/l. No tested concentrations of copper resulted in culture death. Chromium was the most toxic metal. Concentration of 2–20 mg/l caused significant decrease in cell growth and concentrations of 40 and 100 mg/l were lethal. Cultures treated by antibiotics were more sensitive to tested heavy metals. It could be concluded that all heavy metals decreased the cell growth of *E.c.* at concentration from 5 mg/l.

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Protozoan population and fermentation parameters of rumen contents of sheep from heavy metal contaminated area. Z. VÁRADYOVÁ, K. MIHALIKOVÁ, P. JAVORSKY and S. KISIDAYOVÁ, Institute of Animal Physiology, Slovak Academy of Sciences, Soltésovej 4-6, 040 01Kosice, Slovak Republic.

The rumen contents from Slovak Merino sheep (12 months of age) were used for in vitro study on the influence of area contaminated by heavy metals on rumen fermentation and protozoan activity. Sheep were browsing in the contaminated area of Kal'ava village (Slovakia) and were exposed to 1-year intake of heavy metals. The area of Kal'ava is contaminated by atmospheric pollution from the non-ferrous metal works at Krompachy (Slovakia). Based on the levels of mercury (4.8 mg'kg), copper (232.9 mg/kg), cadmium (1.2 mg/kg), lead (92.5 mg/kg) and arsenic (74.6 mg/[1]) the soil was categorized as profusely contaminated. Grass contamination was below the toxic limits. In the tested materials, copper was present at the highest levels followed by lead and arsenic. Meadow hay was used as a tested substrate of fermentation activity; it was incubated with buffered rumen fluid for 24 h. The significantly decreased values of fermentation parameters (total gas, methane, total VFA, acetate) were associated with a reduced total concentration of protozoans. Significant

decrease was detected in the total rumen ciliate population and population of *c*. No significant differences were observed in the concentration of *Dasytricha ruminantium* and *Ophryoscolex c. tricoronatus*.

The study was supported by funds from Grant Agency for Science of the Slovak Academy of Sciences (2/3058/23, 2/3064/23) and funds from APVT grant (51012602).

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Functional frataxin homologue in hydrogenosomes of *Trichomonas vaginalis*. P. DOLEZAL*, A. DANCIS**, M. EMBLEY*** and J. TACHEZY*, *Department of Parasitology, Faculty of Science, Charles University, Vinicna 7, 128 43 Prague 2, Czech Republic, **School of Medicine, University of Pennsylvania, Philadelphia, PA, USA, ***Department of Zoology, Natural History Museum, London, UK.

The cellular structure of *Trichomonas vaginalis* substantially differs from other eukaryotes. Instead of mitochondria it possesses hydrogenosomes where key metabolic reactions are mediated by FeS proteins. The gene expression and the activity of FeS proteins is fully dependent on the iron availability. Although hydrogenosomes contain a high amount of iron, there is no information about hydrogenosomal iron homeostasis and FeS cluster formation. In mitochondria, frataxin plays an unclear role in iron metabolism. The deficiency of frataxin in human causes Friedreich's ataxia, which is associated with an increased level of mitochondrial iron. It has been suggested that frataxin is involved in various functions such as FeS cluster formation or iron storage. We cloned *T. vaginalis* frataxin homologue (TvFTX) coding for a protein of 121 amino acids. Although lacking transcription initiator element in 5′UTR region, the expression of tvftx was verified by a nuclear run-on assay. Moreover, the gene upregulation in iron deficiency was shown. The N-terminal part of TvFTX contains an extension similar to presequences targeting proteins to hydrogenosomes and mitochondria. To identify its cellular localization we transfected *T. vaginalis* with TvFTX fused to HA tag. The immunodetection of HA tag specifically labeled hydrogenosomes and the corresponding cellular fraction. The hydrogenosomal targeting sequence however does not support the translocation into yeast mitochondria. Thus for the complementation of yeast frataxin homologue (YFH1) a mitochondrial targeting sequence is required. When expressed in mitochondria of δYFH1 cells TvFTX complements the mutant growth defect and restores the activity of aconitase (iron-sulfur protein). Together with recent characterization of *T. vaginalis* cystein desulfurase these data indicate the conserved role of hydrogenosomes and mitochondria in FeS cluster assembly.

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RNA binding proteins MRP1 (gBP21) and MRP2 (gBP25) are essential for editing and stability of a subset of mitochondrial mRNAs in procyclic *Trypanosoma brucei*. E. VONDRUSKOVÁ*, J. BURG**, A. ZÍKOVÁ*, N.L. ERNST***, K. STUART***, R. BENNE** and J. LUKES*, *Institute of Parasitology, Czech Academy of Sciences, and Faculty of Biology, University of South Bohemia, Ceské Budejovice, Czech Republic, **Department of Biochemistry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, ***Seattle Biomedical Research Institute, and Department of Pathobiology, University of Washington, Seattle, WA, USA.

In trypanosomes, MRP1 and MRP2 (previously called gBPx and gBPy, in which x and y indicate the MW of these proteins in a particular species) are guide (g)RNA binding proteins that are part of a large heteromeric complex that may play a role in U-insertion/deletion editing of mitochondrial mRNAs. In order to shed more light on the function of these proteins, we generated procyclic *Trypanosoma brucei* cell lines in which the levels of MRP 1 and/or MRP2 mRNA were downregulated by RNA interference (RNAi). Here we report that the RNAi-mediated knockdown of MRP1 and/or MRP2 resulted in severe growth inhibition and loss of both proteins. This loss occurred even in cells in which only one of the MRPs was targeted by RNAi, indicating a mutual dependence for stability of these proteins. The elimination of the MRPs substantially reduced the levels of edited cytB and RPS12 mRNAs, but resulted in little or no reduction of edited cox2, cox3 and A6 mRNAs, as measured in poisoned primer extension analyses. Surprisingly, we found a five-fold increase in ND7 mRNA editing in MRP1+2 double knockdown cells. In addition, the knockdowns also resulted in reduction in the amounts of mRNAs that do not undergo RNA editing (cox1, ND4 and ND5 mRNAs), but little change was observed for mitoribosomal 12S rRNA. Together, the results indicate that in procyclic *T. brucei*, MRP1 and MRP2 play a role in transcript-specific editing and other RNA processing activities.

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Role of IscS in Fe-S cluster assembly in *Trypanosoma brucei*. O. SMÍD*, E. VONDRUSKOVÁ**, V. VILÍMOVÁ*, R. SUT′ÁK*, J. LUKE** and J. TACHEZY*, *Department of Parasitology, Faculty of Science, Charles University, Vinicna 7, 128 44 Prague 2, Czech Republic, **Institute of Parasitology, Czech Academy of Sciences, and Faculty of Biology, University of South Bohemia, 37005 Ceské Budejovice, Czech Republic.

Despite the significance of proteins containing iron-sulfur cluster (Fe–S proteins), the processes of Fe–S cluster assembly and maturation of Fe–S proteins are poorly understood. However, several key proteins involved in the assembly have been identified, notably IscS, a cystein desulfurase, which provides sulfur for Fe–S cluster and IscU, a metallochaperone acting as a scaffold for cluster assembly. In this work, we studied the process of Fe–S cluster biosynthesis in *Trypanosoma brucei* by identifying the homologue of IscS in the *T. brucei* (TbIscS). To address the function of TbIscS, we inhibited its expression by means of RNA interference (RNAi). After RNAi induction, generation time of the TbIscS knock-down cell line was significantly prolonged. All types of mitochondrial ATP production in the cells were severely affected. Analysis of glucose metabolism end products determined pyruvate as major excreted metabolite of the induced cells, while the uninduced cells produced only small amount of this glycolytic end product. These data demonstrate that mitochondrial metabolism is impaired in cells with TbIscS knocked down. To test whether the observed phenomena were results of Fe–S cluster assembly disruption, we examined the Fe–S cluster-dependent activity of aconitase. This enzyme is localized in its active form in mitochondrion as well as in cytosol of *T. brucei*. After RNAi induction we observed the reduction of aconitase activity in both compartments (approx. 70% reduction in cytosol, approx. 30% in mitochondria). Western blots together with the EPR analysis showed that the reduction in cytosolic activity was due to impaired Fe–S cluster formation, while decrease in aconitase activity in mitochondria corresponded to the reduced level of the protein.

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Parasitic Stramenopila. M. KOSTKA, V. HAMPL, I. CEPICKA and J. FLEGR, Department of Parasitology, Faculty of

Science, Charles University, Vinicna 7, 128 43 Prague 2, Czech Republic.

In the vast and diversified eukaryotic group Stramenopila we can find many different life strategies, one of them being parasitism. There are two groups of stramenopiles containing important parasites of vertebrates: the oomycetes (e.g. *Saprolegnia*) and the wholly parasitic group Slopalinida+its sister taxon—*Blastocystis*. The order Slopalinida comprises families Opalinidae and Proteromonadidae. The two families are considered related based on the structure of basal bodies and their appendages and the presence of subpelicular microtubules. However, a robust recognition of phylogenetic affinities of Opalinidae—the peculiar multinucleated intestine commensals of frogs—has been hindered by the absence of reliable molecular data. Up to now, all attempts to sequence opalinid genes failed, as the obtained sequences labeled as *Protoopalina intestinalis*, *Cepedea virguloidea* and *Opalina ranarum* in GenBank apparently originate from a zygomycete contamination. We present the first molecular data for the family Opalinidae—SSU rRNA gene of *Protoopalina intestinalis*. Our phylogenetic analyses undoubtedly show opalinids as a sister group to *Proteromonas* within the Stramenopila clade, confirming the monophyly of Patterson's order Slopalinida. The enigmatic genus *Blastocystis* is resolved with high statistical support as a sister group to Slopalinida. Our analyses clearly demonstrate that Cavalier–Smith's phylum Bigyra, which comprises Oomycetes and their relatives together with Slopalinida and *Blastocystis*, is not monophyletic. We also show, that *Blastocystis* sp. isolate obtained from the red-footed tortoise (*Geochelone carbonaria*), forms a sister group to all *Blastocystis* isolates from birds and mammals sequenced so far.

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Critical analysis of the topology and rooting of the parabasalian 16S rRNA tree. V. HAMPL, I. CEPICKA, J. FLEGR, J. TACHEZY and J. KULDA, Department of Parasitology, Faculty of Science, Charles University, Vinicna 7, 128 43 Prague 2, Czech Republic.

The morphological classification of the protozoan phylum Parabasala is not in absolute agreement with the 16S rRNA phylogeny. However, there are strong indications that tree-construction artifacts play a considerable role in the shaping of the 16S rRNA tree. We have performed rigorous analyses designed to minimize such artifacts using the slow–fast and taxa-exclusion methods. The analyses, which included new sequences from the genera *Monocercomonas* and *Hexamastix*, in most respects confirmed the previously suggested tree topology and polyphyly of order Hypermastigida and family Monocercomonadidae but detected one artificial cluster of long branches (Trichonymphidae, Pseudotrichonymphidae, *Hexamastix* and *Tricercomitus*). The analyses also indicated that the rooting of the phylum Parabasala on the trichonymphid branch, as widely accepted, is doubtful and that reliable rooting on the basis of current data is likely impossible.

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Secondary endosymbioses and evolution of unicellular eukaryotes. M. OBORNÍK* and B. R. GREEN**, *Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovská 31, 37005 Ceské Budejovice, Czech Republic, **University of British Columbia, Department of Botany, Vancouver BC, Canada V6T1Z4.

Many lines of evidence support the idea that the first chloroplast was the result of an endosymbiotic relationship between a cyanobacterium and a non-photosynthetic eukaryote. Most of the cyanobacterial genes were lost, but a few remained in the chloroplast genome and as many as a thousand were transferred to the host nucleus. Genes encoding functions required by the chloroplast had to acquire presequences to target the products to the chloroplast. The situation gets more complicated when we consider the algae with chlorophyll c. They are the product of secondary endosymbiosis, where a putative red algal ancestor was engulfed by another non-photosynthetic eukaryote, which retained the red algal chloroplast but eventually got rid of the rest of the cell. This left the chloroplast surrounded by two additional membranes: one derived from the red algal plasma membrane and the other from the host's phagocytic vacuole. In order for the endosymbiotic relationship to work, there must have been a substantial amount of gene transfer from the red algal nucleus to the host nucleus to support chloroplast functions. In the cryptophytes we even see an intermediate stage in this process, a relict nucleus (nucleomorph) in the periplastidal space between the outer two membranes and the original chloroplast envelope. Now that the draft genome sequence of the diatom *Thalassiosira pseudonana* (Diatom Genome Consortium) as well as genomes of rhodophyte *Cyanidioschyzon merolae* and green plants are available, it is possible to investigate the evolutionary history of plastid localized metabolic pathways. Phylogenetic analyses of nuclear-encoded putatively plastid-targeted enzymes showed that plastids obviously utilize enzymes not only of expected plastid (cyanobacterial) origin. Within the diatom, apicomplexan, plant and rhodophyte genomes, we have identified several enzymes that originate in α-proteobacteria (mitochondria) or even in eukaryotic nucleus, but possess N-terminal plastid-targeting presequences. Although diatoms are, according to multiprotein phylogeny, related to Alveolates, some plastid-related metabolic pathways show substantially different evolutionary pattern as well as, in silico, predicted localizations of involved enzymes.

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Modulation of murine cellular immune responses and cytokine production by sand fly saliva. I. ROHOUSOVA*,**, M. LIPOLDOVA** and P. VOLF, *Department of Parasitology, Charles university, Vinicna 7, 128 43 Prague 2, Czech Republic, **Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo nam. 2, 166 37 Prague 6, Czech Republic.

Sand flies (*Diptera*: *Phlebotominae*) are hematophagous arthropods and important vectors of *Leishmania* parasites. During blood feeding, both vector saliva and parasites are injected by an infected sand fly. The aim of this study was to characterize how sand fly saliva interferes with host immunity in order to clarify the mechanism underlying enhancing effect of sand fly saliva on *Leishmania* infection. Spleen cells from naive BALB/c mice were incubated in vitro with *Phlebotomus papatasi*, *P. sergenti* or *Lutzomyia longipalpis* salivary gland lysate (SGL). We studied the effect of saliva on cell proliferation and cytokine production in non-stimulated and concanavalin A-stimulated splenocytes. Both spontaneous and mitogen-stimulated lymphocyte proliferation were significantly suppressed with SGLs of all three sand fly species and all SGLs doses tested (equivalent to 1/16, 1/4 and 1 gland per well). The proliferation index (a lysate-treated/lysate-untreated cells ratio) was significantly lower when cells were incubated with SGL and was ranging between 0.52 and 0.30 for non-stimulated splenocytes and 0.69–0.32 for mitogen-stimulated lymphocytes. This result indicates that saliva from different sand fly species is able to suppress host proliferative response even to the potent *Leishmania*-unrelated mitogen. In parallel experiments,

we analysed the effect of SGL (1 gland per well) on cytokine production. The levels of Th1 (IL-2, IFN-γ) and Th2 (IL-4) cytokines were determined in cell culture supernatants by capture ELISA. Exposure to SGL alone did not affect levels of IL-2 or IL-4. The production of IFN-γ was inhibited by *P. papatasi* SGL, whereas the SGLs of other two species were ineffective. In mitogen-stimulated cells both Th1 cytokines were modulated; the level of IFN-γ production was significantly suppressed and IL-2 enhanced by SGL of all three sand fly species. The production of Th2 cytokine IL-4 was inhibited only by *L. longipalpis* SGL; no changes in IL-4 levels were noted after incubation with *Phlebotomus saliva*. This species-specific immunomodulation is in agreement with our previous finding that the composition of sand fly saliva varies among species. Taken together, sand fly saliva modulate cell proliferation as well as cytokine production. These findings further increase our understanding of sand fly–host immune relationship.

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The hyrax (*Procavia capensis*) is reservoir host of *Leishmania tropica* in northern Israel. M. SVOBODOVA*, J. VOTYPKA*, V. KRAVCHENKO**, R. W. ASHFORD*** and A. WARBURG**, *Department of Parasitology, Charles University, Vinicna 7, 128 43 Prague 2, Czech Republic, **Department of Parasitology, Hebrew University, Jerusalem, Israel, ***Liverpool School of Tropical Medicine, Liverpool, UK.

In order to identify a reservoir host of *Leishmania tropica*, we have trapped mammals in a cutaneous leishmaniasis focus in the Galilee region of Northern Israel. During May 2003, racoon traps (in total 275 in 11 nights) and Sherman traps (in total 170 in 8 nights) were put in boulders surrounding four endemic villages. In total, 34 hyraxes (*Procavia capensis*), 32 spiny mice (*Acomys cahirinus*) andone house mouse (*Mus domesticus*) were caught. Biopsies were taken from anaesthesized animals, which were released after recovery. Parasites were isolated from the nose of one hyrax. The strain was typed as *L. tropica* and was identical with previous isolates from patients and sand flies originating from the focus. Biopsies from other hyraxes and mice (ear biopsy) were culture and PCR negative, while PCR from the infected hyrax was positive. Previously, *L. tropica* DNA was detected in two hyraxes trapped in the focus but parasites were not isolated. Thus, we have confirmed the potential of hyraxes to serve as reservoir hosts. After inoculation of the hyrax strain into the ear of spiny mice, 2/4 animals were culture-positive, and 5/5 were PCR positive. Moreover, parasite DNA was detected not only in the inoculation site, but also in the nose and hind foot of one *Acomys*. *L. tropica* is thus able to disseminate in its host. Differences in biology between the two most abundant mammals in the focus are probably the reason why the hyrax, and not the spiny mouse, is a suitable reservoir of *L. tropica*.

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Effects of Time-Variant Extremely-Low-Frequency (ELF) Electromagnetic Fields (EMF) on Cholinesterase Activity in *Dictyostelium discoideum*. ANDREA AMAROLI, FRANCESCA TRIELLI, BRUNO BIANCO*, STEFANO GIORDANO*, ELSA MOGGIA* and MARIA U. DELMONTE CORRADO, Dipartimento per lo Studio del Territorio e delle sue Risorse, Genova, Italy, *Dipartimento di Ingegneria Biofisica ed Elettronica, Università di Genova, I-16100 Genova, Italy.

Recently, we have shown the presence of molecules belonging to the cholinergic signalling system in the ciliate *Paramecium primaurelia* and in the sarcodine *Dictyostelium discoideum*. Propionylcholinesterase (PrChE) activity has been detected in single-cell amoebae of *D. discoideum*, using cytochemical, electrophoretic, and spectrophotometric methods (Falugi et al., *Chemosphere*, 48:407–414, 2002; Amaroli et al., *Europ. J. Protistol*., 39:213–222, 2003). It has been suggested that this enzyme activity is involved in cell–cell and cell–environment interactions, as its inhibition by xenobiotic compounds affects cell migration and aggregation. In this work, we have spectrophotometrically evaluated the effect of an ELF-EMF of 300 T, 50 Hz, on PrChE activity after exposure of single-cell amoebae from 1 h up to 48 h at 22 °C. The enzyme activity was inhibited significantly by 1-h- and 3-h exposures, whereas it was similar to the control value after a 4-h exposure. A significant increase in PrChE activity was found after 5- and 24-h exposures, while a decrease in PrChE activity appeared in 48-h-exposed samples. The increased PrChE activity detected in 24-h-exposed cells returned to the control value 24 h after transferring the amoebae to standard conditions. A delay in both migration and aggregation processes was observed in 3 h-exposed cells, corresponding to a decreased PrChE activity. After a 24-h exposure, a decrease in the fission rate and an increase in the cell size were found.

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An alternative approach for the molecular characterization of uncultivable protists retaining morphological information. ILARIA ANDREOLI, FILIPPO FERRANTINI, LARA MANGINI, GIOVANNI SANTANGELO, GIULIO PETRONI and FRANCO VERNI, Dipartimento di Etologia, Ecologia, Evoluzione, Università di Pisa, I-56126 Pisa, Italy.

Recent culture-independent studies based on small-subunit ribosomal RNA (SSU rRNA) gene analysis revealed the existence of completely new clades of protists. The main problems with this approach are to correlate sequences from environmental rRNA genes with the organisms they belong to and then to detect the ecological role of these organisms in the environment. In order to overcome such problems we chose an alternative approach allowing us both a molecular characterization of uncultivable organisms with a low relative abundance in environmental samples, and a morphological analysis, even if restricted. The experimental protocol consists of two steps: an initial observation and phototaking of the single cell under the DIC (Differential Interferential Contrast) microscope and then PCR amplification and direct sequencing of the 18S rRNA gene of the same cell. The advantages of this method are the possibility to: (1) establish a precise link between morphology and gene sequence; (2) detect the possible occurrence of highly similar species within the studied population; (3) avoid the insertion of *Taq*-polymerase errors in the gene sequence; and (4) detect possible polymorphisms in the gene under examination. Such an approach was used to sequence the 18S rRNA gene of organisms belonging to the class Karyorelictea that comprises several uncultivable ciliates with limited distinctive features. Gene sequences analysis revealed an unexpected genetic variability in trachelocercids and, in particular, the existence of polymorphisms in the SSU rRNA gene of a group of them. Such specimens show a similar morphology and, as a result of phylogenetic analysis, they form a constant clade.