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80A

Protist genomes: a strange brew of broken rules. P. KEELING, Department of Botany, University of British Columbia, Vancouver, BC, Canada.

By nearly all criteria, protists make up the great majority of eukaryotic diversity. This is well illustrated at the level of the genome, where protists have broken just about every rule we think we know about genome form, content, and function. I will examine some “extreme” genomes studied in my lab, and what these deviants can tell us about genome evolution. Some of the most unusual nuclear genomes we know about are found in microsporidian intracellular parasites and the algal endosymbionts called nucleomorphs in cryptomonads and chlorarachniophytes. In both cases genomes have been highly reduced by gene loss and by compacting the remaining genes into a tighter space. Both processes have had significant impacts, most obviously on form but also on function. These processes are frequently studied in the most extreme of these genomes (i.e. the smallest ones), but the nature of their less compacted relatives is also of interest as they can tell us how these genomes got to be the way they are and whether this trajectory is reversible. In contrast to the highly compacted genomes, other groups (notably dinoflagellates) have extremely large genomes, and once again these appear to be linked to massive but poorly understood changes in how the genome works. In terms of genome content, one of the most radical affects on a genome can come about through endosymbiosis. The merger of two cells involves the merger of many genomes: in some cases as many as six genomes and in some dinoflagellates the historical footprint of as many as 10, come together to make up one cell. While endosymbiosis is a catastrophic example of changes in genome content, the gradualist process of lateral gene transfer may be no less important. Indeed, as the gene complement of protist genomes is studied in more detail, they are emerging as models for how genes can flow between distantly related species one (or a few) at a time. The impacts of many small transfers may even be greater than the impacts of massive transfers associated with endosymbiosis.

81A

Studies of protistan diversity in Antarctica. R. J. GAST,^a D. M. MORAN,^a M. R. DENNETT,^a D. J. BEAUDOIN^a and D. A. CARON^b, ^aWoods Hole Oceanographic Institution, Woods Hole, Massachusetts, ^bUniversity of Southern California, Los Angeles, California.

Traditional microscopical studies of protistan assemblages in the Ross Sea, Antarctica have contributed greatly to our understanding of species biogeography and microbial food web structure in extreme cold-water environments. However, these investigations have still not clearly characterized the species diversity of the communities, nor determined the abundances or trophic contribution of the dominant organisms. Therefore, we sought to determine the genetic diversity of microbial eukaryotes in ice and water samples from the Ross Sea, and establish both morphologic and physiologic links between enrichment cultures and the genetic data. DGGE and small subunit ribosomal DNA

(srDNA) clone library analyses indicate that the protistan communities are very diverse, but that those present in similar habitats are more alike than those in different habitats at the same site. These analyses also indicated that a single srDNA sequence type dominated clone libraries from seawater and slush samples taken within the pack ice. This dominant clone was derived from a dinoflagellate, and phylogenetic analyses supported its placement as a sister group to *Karenia* and *Karlodinium*. Quantitative PCR analyses of environmental samples documented that this dinoflagellate occurred at bloom levels in Antarctic protistan assemblages. The organism was successfully recovered in culture, and preliminary morphological analyses have shown that it contains chloroplasts, is gymnodinoid, appears not to have thecal plates, and has an apical groove and sulcal structure that confirm its placement not only as a relative of the *Karenia/Karlodinium* group, but as a new genus. Plastid srDNA sequences from the dinoflagellate indicated that they were the same as those of *Phaeocystis antarctica*, which led us to hypothesize and subsequently confirm that the organism is kleptoplastidic. The kleptoplasty is unusual in its longevity (about 8 mo), and suggests that this new genus of dinoflagellate may be in the process of swapping its plastid endosymbionts.

82A

Antigenic variation in ciliates. M. C. SIMON and H. J. SCHMIDT, University of Kaiserslautern, Kaiserslautern, Germany.

Protists are capable to express variant surface proteins. In the past decades, the major focus of antigen variation research was on parasitic protists with the aim to enable an intelligent vaccine design. However, antigenic variation occurs also in free-living protists: antigenic systems of the ciliates *Paramecium* and *Tetrahymena* have been studied for more than 100 yr. In spite of different life strategies and distant relationship the antigenic systems of free-living ciliates and parasitic protists have a lot in common. This is for example the presence of repeated structures; membrane anchoring by GPI and the exclusive expression of multigene families. The function of antigens in free-living ciliates is still unknown and it becomes more of a puzzle regarding the parallels to parasites. Up to now no detailed monitoring of antigen expression of free-living ciliates in natural habitats has been performed. Unlike stochastic switching in parasites, antigen expression in ciliates can be directed, e.g. by temperature, which holds great advantages for research on the expression mechanism. In any way, exclusive expression occurs and the responsible mechanism is complex and interferes on transcriptional and post-transcriptional levels. The involvement of homology dependent effects has been proposed several times but was not proved yet.

83A

Structure, evolution, and expression of *Pneumocystis* gene families that encode variable surface proteins. J. R. STRINGER and S. P. KEELY, University of Cincinnati, Cincinnati, Ohio.

All of the four *Pneumocystis* species tested to date have a family of genes encoding various forms of a major surface glycoprotein, which is generally called MSG. *P. pneumocystis carinii* (rat parasite), which has served as the model species in the regard, has approximately 90 MSG gene family members in its genome. PRT1 and MSR genes also repeated in *P. carinii*, and are about one half as numerous as MSG genes. PRT1 is not repeated in either *P. jirovecii* (human parasite) or *P. murina* (mouse parasite). It is not known if MSR gene families occur outside of

P. carinii. In *P. carinii*, gene families cluster together at chromosome ends and the three-gene set PRT1-MSR-MSG is common. These structures suggest that all three families have expanded in concert via unequal homologous crossing over. Major surface glycoprotein genes show evidence that positive selection for protein variation has shaped evolution of the family. A single locus called UCS is associated with expressed MSG genes. The MSG genes that are not adjacent to the UCS locus appear to be transcriptionally silent, but can donate genetic information to the UCS-linked MSG gene. Comparison of donor MSG genes (i.e. those not at the UCS locus) to expressed MSG genes (i.e. those at the UCS locus) showed that the donor gene pool does not account for most of the genes found at the UCS. It appears that expressed genes can be formed by copying segments of one or more donor gene into the MSG gene adjacent to the UCS. This process provides a very large number of different MSG proteins. Unlike MSG genes, multiple MSR genes seem to be transcribed in one *P. carinii* cell. However, cell to cell variation in MSR proteins expressed may still occur via random mutation because some MSR genes have long runs of G:C basepairs, which are prone to change in size thereby causing a frameshift mutation.

84A

Trypanosome antigenic variation—generation of diversity at different levels. L. MARCELLO and J. D. BARRY, Wellcome Centre for Molecular Parasitology, Glasgow, United Kingdom.

Antigenic variation in *Trypanosoma brucei*—the switching between alternative isoforms of the variant surface glycoprotein (VSG)—relies on expression of distinct VSG genes, of which there are ~2,000 in the genome of the sequenced strain. Surprisingly, >95% of the silent genes are pseudogenes or fragments. Switching is achieved through the duplication of silent VSGs, creating an extra, expressed copy in a transcriptionally active locus. Although most silent VSGs are incomplete, they can contribute to switching by donating intact fragments to the active locus. Diversification occurs also within the archive of silent VSGs, where there is a very high rate of DNA rearrangement. Using a combination of experimental analysis and bioinformatics, we have characterized switching events and VSG archive structure in the genome sequencing strain. This reveals how the archive contributes to switching events and how it evolves. It is becoming apparent that the trypanosome has enormous potential for antigenic variation.

85A

Position effects at telomeres of *Plasmodium falciparum* that control *var* gene expression. A. SCHERF,^a L. MANCIO DA SILVA,^a A. P. ROJAS-MEZA^{a,b} and R. HERNANDEZ-RIVAS^b, ^aInstitut Pasteur, Paris, France, ^bInstituto Politecnico Nacional, Mexico D.F., Mexico.

Plasmodium falciparum parasites use antigenic variation to avoid immune clearance and increase chronic infection in the human host. Variation at the surface of parasitized red blood cells is mediated by a family of surface antigens encoded by *var* genes. Mono-allelic activation of a single member of the *var* gene family is controlled by a number of different epigenetic factors such as reversible chromatin changes at promoter regions and nuclear relocation of the active *var* gene. *Var* genes are silenced at the nuclear periphery, a zone generally characterized by heterochromatin. Activation of a *var* gene, however, occurs in a particular perinuclear area, which remains elusive. *Plasmodium falciparum* chromosome ends play a particular role in reversible *var* gene

silencing. Telomeres anchor chromosome ends to the nuclear periphery and recruit proteins that apparently facilitate spreading of compact chromatin into telomeric *var* genes leading to gene repression (called “telomere position effect”, [TPE]). A search for candidate genes involved in perinuclear heterochromatin formation and TPE has identified several proteins that accumulate in the nuclear periphery and co-localize with chromosome ends. Gel shift and chromatin immunoprecipitation (ChIP) analysis demonstrated the specific interaction of PfSir2 and two other proteins named PfTelBP2 and PfTelBP3 with telomere repeats and adjacent telomere associated repetitive elements (TARE). At truncated chromosomes (complete deletion of TAREs), PfSir2 does not spread into the telomere adjacent coding region, demonstrating that TAREs (in particular Rep20) act as cis-acting sequences required for TPE in *P. falciparum*.

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African trypanosomes: intracellular trafficking of host defense molecules. S. L. HAJDUK,^a A. M. SHIFLETT,^a S. D. FAULKNER^a and L. F. COTLIN^b, ^aMarine Biological Laboratory, Woods Hole, Massachusetts, ^bUniversity of Alabama at Birmingham, Birmingham, Alabama.

Trypanosoma brucei brucei is the causative agent of nagana in cattle and can infect a wide range of mammals but is unable to infect humans because it is susceptible to the innate cytotoxic activity of normal human serum. A minor subfraction of human high-density lipoprotein (HDL), containing apolipoprotein A-I (apoA-I), apolipoprotein L-I (apoL-I) and haptoglobin-related protein (Hpr) provides this innate protection against *T. b. brucei* infection. Both Hpr and apoL-I are cytotoxic to *T. b. brucei* but their specific activities for killing are low. Once assembled into the native HDL particle activities increase several 100-fold. This HDL subfraction, called trypanosome lytic factor (TLF), kills *T. b. brucei* following receptor binding, endocytosis and lysosomal localization. Trypanosome lytic factor is activated in the acidic lysosome of *T. b. brucei* and facilitates lysosomal membrane disruption. Lysosomal localization is absolutely necessary for *T. b. brucei* killing by TLF. *Trypanosoma brucei rhodesiense*, which is morphologically and physiologically indistinguishable from *T. b. brucei*, is resistant to TLF-mediated killing and causes human African sleeping sickness. Human infectivity by *T. b. rhodesiense* correlates with the evolution of a human serum resistance-associated protein (SRA) that is able to ablate TLF killing. When *T. b. brucei* was transfected with the SRA gene it became highly resistant to TLF and normal human serum. In the SRA transfected cells, intracellular trafficking of TLF was altered and TLF was mainly localized to a subset of SRA containing cytoplasmic vesicles but not to the lysosome. These results indicate that the cellular distribution of TLF is influenced by SRA expression and may directly determine susceptibility.

87A

Regulation of GPI valence by conditional dimerization in African trypanosomes. K. J. SCHWARTZ, J. D. BANGS, Department of Medical Microbiology and Immunology, University of Wisconsin Medical School, Madison, Wisconsin.

The fate of GPI-anchored membrane proteins in bloodstream stage African trypanosomes correlates with GPI valence. At steady state, VSG (GPI²) is stably incorporated into the surface coat, while transferrin receptor (GPI¹) resides in the flagellar pocket and endosomes, and is slowly turned over in the lysosome. Recombinant GPI-minus VSG (GPI⁰) is rapidly delivered to the lysosome and degraded. Engineered GPI¹ reporters behave in a

manner consistent with this correlation, parsing between delivery to the cell surface and/or degradation in the lysosome (Schwartz et al. (2005) *J. Cell Sci.* 188:5499). Surprisingly, delivery to the cell surface results in extracellular shedding due to the relatively low hydrophobicity of bloodstream trypanosome GPI anchors. We now exploit a conditional homodimerization domain (F36V, ARIAD Pharmaceuticals) to modulate between the monomeric and dimeric GPI states. When expressed in bloodstream parasites the EPFv1 reporter (F36V dimerization domain fused to EP procyclin) receives tomato lectin-reactive poly-*N*-acetyllactosamine modification to both its single *N*-glycan and GPI anchor. EPFv1 enters into the endosomal system and is overwhelmingly delivered to the lysosome for degradation. Selective inhibition of lysosomal thiol proteases rescues cell-associated EPFv1 (49%) with strong lysosomal localization. Treatment with the membrane-permeant bivalent F36V ligand, AP20187, also rescues cell-associated reporter (24%) with strong flagellar pocket and surface localization. Furthermore, EPFv1 is released from cells at elevated rates (43% versus <10%). Simultaneous treatment with FMK024 and AP20187 gives quantitative recovery of EPFv1 in cell (70%) and media (39%) fractions with concurrent localization in the lysosome and flagellar pocket. These results indicate that the prevalent fate of monomeric EPFv1, like native transferrin receptor, is lysosomal turnover, and that induced dimerization favors EPFv1 trafficking to the cell surface much like native VSG. These results are broadly consistent with the hypothesis that valence plays a prominent role in the trafficking of GPI-anchored proteins.

88A

Leishmania: trafficking and function of acylated proteins. D. F. SMITH, Immunology and Infection Unit, Department of Biology, University of York, York, United Kingdom.

The kinetoplastid genomes encode ~60 *N*-myristoylated proteins, downstream targets of the essential enzyme, *N*-myristoyltransferase.¹ *N*-myristoylation plays a role in targeting proteins to membrane locations and can also mediate protein-protein interactions and structural stability. The hydrophilic acylated surface proteins (HASPAs) are a subset of these kinetoplastid proteins that are expressed only in infective stages of *Leishmania* species, in which they are partially exposed on the external surface of the plasma membrane, a favourable site for host immune recognition.^{2,3} Dual acylation, by both *N*-myristoylation and palmitoylation, is required for transport to this location, which follows a non-classical secretory pathway that is conserved in mammalian cells.^{2,4} Genetic screening methods are being developed to identify the molecular components of this novel pathway. *Leishmania donovani* HASPB protein has been identified as a lead vaccine candidate for visceral leishmaniasis.⁵ Distinctive features within the central amino acid repeat domain of HASPB, characterized using biophysical methods, correlate with the induction of adjuvant-independent immune responses in the host. Another major group of kinetoplastid *N*-myristoylated proteins are the ADP ribosylation factors (ARFs) and their related proteins (ARLs), conserved molecules that function in a range of eukaryotic processes, including protein trafficking and membrane dynamics. Perturbation of ARF/ARL expression by RNAi in *Trypanosoma brucei* and gene targeting in *Leishmania* is revealing parasite-specific features that correlate with functional specialization in different stages of their life cycles. [1] Price, H. P. et al. (2003) *J. Biol. Chem.* 278, 7206; [2] Denny, P. W. et al. (2000) *J. Biol. Chem.* 275, 11017; [3] Prickett, S. et al. (2006) *J. Immunol.* 176, 4826; [4] Stegmayer, C. et al. (2005) *J. Cell Sci.* 118, 517; [5] Stager, S. et al. (2003) *Nat. Med.* 9, 1287; [6] Price, H. P. et al. (2005) *J. Cell Sci.* 118, 831.

89A

Host remodeling by malaria parasites. K. HALDAR, Northwestern University, Feinberg School of Medicine, Department Pathology, Chicago, Illinois.

Plasmodium falciparum is a protozoan parasite that infects human erythrocytes. It causes the most virulent of human malaria and the erythrocytic stages are responsible for all symptoms and pathologies of the disease. Critical to infection is the formation of a parasitophorous vacuolar membrane (PVM) at the time of entry and within which the intracellular parasite proliferates. Our studies reveal that entry and formation of the nascent vacuole requires activation of signaling via the erythrocyte raft-associated heterotrimeric G protein Gs (Harrison et al. 2003). Further Gs provides a novel target for malaria chemotherapy (Murphy et al. 2006). Parasite proteins delivered from the invading merozoite stage to the nascent vacuole are currently under investigation for their role in entry and vacuole formation. In addition we investigate parasite proteins secreted to the host erythrocyte during intracellular development. The best characterized of these virulence proteins belong to a variant antigen family of surface adhesins called *P. falciparum* erythrocyte membrane protein 1 (PfEMP1 encoded by VAR genes) that has been linked to both cerebral malaria and placental malaria as well as protein families like STEVOR, RIFIN also known for their antigenic variation. Our studies have identified a critical, conserved, host-targeting signal bearing a distinct, eleven amino acid motif shared by these and other virulence proteins that enables their export to the erythrocyte (Hiller et al. 2004). This led to identification of a (first) major host targeting pathway and "secretome" in eukaryotic pathogens that, we now show is shared by other microbes (Bhattacharjee et al. 2006).

90A

The *Toxoplasma gondii* parasitophorous vacuole membrane: transactions across the border. A. P. SINAI,^a A. M. MARTIN,^a T. LIU^b and B. C. LYNN^b, ^aDepartment of Microbiology Immunology and Molecular Genetics, University of Kentucky, Lexington, Kentucky, ^bDepartment of Chemistry, University of Kentucky, Lexington, Kentucky.

The obligate intracellular protozoan *Toxoplasma gondii* establishes its replication permissive niche within the infected host cell. This niche, the parasitophorous vacuole (PV), is delimited from the host cell cytoplasm by the PV membrane (PVM). In spite of this critical location very little is known about the biogenesis, composition or molecular basis of activities of the PVM. Among known functions are roles in the remodeling of host cell architecture (recruitment of organelles and reorganization of the cytoskeleton), nutrient acquisition and the manipulation of host signaling (including kinase activities directed at host substrates). These diverse functions suggest that the PVM may in fact be an "organelle" established outside of the parent organism. The paucity of knowledge regarding the biology of the PVM stems from difficulties inherent in its study. Among these are: (1) The PVM is formed only in infected cells. (2) Parasitophorous vacuole membrane-localized proteins derive from multiple parasite and potentially host sources. (3) The PVM cannot be purified for biochemical analysis due to its intimate association with host mitochondria and endoplasmic reticulum. In this presentation I will focus on the biology of the *T. gondii* PVM and present results regarding the development and utility of polyclonal antibodies directed against the PVM. These unique affinity reagents coupled with state of the art proteomic approaches is leading to the identification of the molecular bases underlying both identified and potentially novel PVM functions.

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Annual biomass and secondary production of soil ciliates in a subtropical moist forest. D. ACOSTA-MERCADO^a and D. H. LYNN^b, ^aUniversity of Puerto Rico, Mayagüez, Puerto Rico, ^bUniversity of Guelph, Guelph, California.

Protozoa, ciliates in particular, play a role in ecosystem functioning by recycling microbial biomass, and thus, nutrients and energy. However, data on their secondary production in aquatic ecosystems suggested that their overall contribution to annual production is minor, only becoming significant during bloom episodes. It is not known whether the ecological role of soil ciliates, based on estimates of their production is similarly minor. Temporal data on ciliate abundance among three subtropical plant species were used to estimate biomass and potential secondary production of soil ciliates in a subtropical terrestrial ecosystem. Ciliate abundance, biomass, and production were determined during months over the dry and wet seasons in the top 5 cm of soil. Mean ciliate abundances in these months were 5 ± 10.3 and 16 ± 16.1 ($n=93$) for nanociliates and microciliates, respectively. Mean ciliate biomass were $41,854 \pm 37,055$ pg C g⁻¹ dry wt. soil. Mean nano and microciliate biomass were 418 ± 704.4 pg C g⁻¹ dry wt. soil, and $45,315 \pm 39,466.8$ pg C g⁻¹ dry wt. soil, respectively. In contrast to plant species, time had a significant effect on ciliate abundance and biomass but this effect showed no predictable pattern suggesting that there was no seasonality. Mean daily ciliate production was $1.74 \times 10^4 \pm 1.95 \times 10^4$ pg C g⁻¹ dry wt. soil day⁻¹ (0.002 kJ m⁻² day⁻¹ or 0.047 kJ m⁻³ day⁻¹), ranging from 0 to $107,804$ pg C g⁻¹ dry wt. soil day⁻¹. Using the data on monthly precipitation, annual ciliate production was estimated to be 40.0×10^3 pg C g⁻¹ dry wt. soil yr⁻¹ (1.1 kJ m⁻² yr⁻¹ or 21.7 kJ m⁻³ yr⁻¹). Moderate production estimates in addition to low mean consumption of microbial biomass (0.01%) suggests that ciliates play a minor role in this subtropical soil ecosystem.

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Genetic characterization of *Cryptosporidium* spp. isolates from humans in north of Portugal. A. ALMEIDA,^a M. L. DELGADO,^a S. SOARES,^b A. CASTRO,^{a,b} E. SILVA,^b M. J. MOREIRA,^b C. MENDONÇA,^b N. CANADA,^b A. TOMÁS^c and J. M. CORREIA DA COSTA^{a,b}, ^aCentro de Imunologia e Biologia Parasitária, INSA, Portugal, ^bCentro de Estudos de Ciência Animal, ICETA, Portugal, ^cInstituto de Biologia Molecular e Celular, IBMC, Portugal.

Cryptosporidium spp. is an apicomplexan parasite that infects a wide range of vertebrates, and also humans. Cryptosporidiosis is a common cause of diarrhoeal disease in humans with a global distribution and cause significant morbidity and mortality in animals. Multiple transmission routes occur in *Cryptosporidium* epidemiology: animal-to-human, human-to-human, foodborne and waterborne. Thus, the epidemiology of cryptosporidiosis is difficult to study and is now best done by molecular techniques. These molecular approaches recognized *C. hominis* and *C. parvum* has the major causes of cryptosporidiosis in human cases, both in immunocompromised and immunocompetent persons. Although *C. hominis* is involved and maintained in the anthroponotic cycle of infection, other species infect both animals and humans, clearly showing zoonotic potential. *Cryptosporidium parvum* is the most reported zoonotic *Cryptosporidium* species, and is closely related to those infecting cattle. These animals are important sources of environmental contamination. The aim of this study was to identify the genotypes of *Cryptosporidium* present in the feces of adult immunocompromised humans by comparing two genetic loci. Fecal samples from immunocompromised and immunocompetent persons were collected by random

and delivered in our laboratory from a Portuguese hospital. These samples were processed by acid-fast staining and PCR. *Cryptosporidium* oocysts were detected in 14 people (2.8%). Positive fecal samples all came from immunocompromised persons, and were chosen for further evaluation. Cesium chloride-based gradients were made in order to isolate the parasite. For the genetic characterization, PCR-based procedures were used to amplify the two genetic loci: *hsp70* gene and the *18SrRNA* gene, and sequence analysis was performed. Five isolates revealed the presence of *C. parvum*, two isolates detected *C. meleagridis* and two detected *C. hominis*. The samples had 100% homology with *C. parvum* (AF221528 and AF112571), *C. hominis* (AF093492 and XM661662) and *C. meleagridis* (AY166839 and DQ201831), respectively. Sequencing of both the *hsp70* and *18SrRNA* genes showed the same *Cryptosporidium* species assignments. HIV-positive patients have higher percentages of *Cryptosporidium* infections, and *C. parvum* appears to be more frequent than *C. hominis* and *C. meleagridis*. This work was financially supported by Fundação Calouste Gulbenkian, project number 61018.

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Genetic characterization of *Giardia* isolates from humans in north of Portugal. A. ALMEIDA,^a M. L. DELGADO,^a S. SOARES,^b A. CASTRO,^{a,b} E. SILVA,^b M. J. MOREIRA,^b C. MENDONÇA,^b N. CANADA,^b A. TOMÁS^c and J.M. CORREIA DA COSTA^{a,b}, ^aCentro de Imunologia e Biologia Parasitária, INSA, Portugal, ^bCentro de Estudos de Ciência Animal, ICETA, Portugal, ^cInstituto de Biologia Molecular e Celular, IBMC, Portugal.

Giardia duodenalis is a protozoan flagellate commonly associated with intestinal disturbances and diarrhoea in a wide range of mammal hosts, including humans worldwide. Infection occurs through oral ingestion of vegetables or water contaminated with cysts, therefore, giardiasis is the most commonly diagnosed waterborne disease in developed countries. Furthermore, species of *Giardia* affecting humans also affect other animals. Several major transmission cycles of *G. duodenalis* are recognized, involving transmission between livestock cycles or wildlife cycles with the human cycle. Moreover, genetic and biological differences within the *G. duodenalis* complex exist and not all organisms have the same biological potential to pose a risk of human infection. Useful molecular characterization used in identifying genotypes or assemblages showed the genetically variety of the complex of *G. duodenalis*, but information about the molecular epidemiology are still insufficient. Assemblages A and B have the widest host ranges, encompassing the human infection and a variety of other animals. Assemblage A-1 appears to be the most common zoonotic agent, while assemblage A-2 appears to be human restrict, but has also been reported in cattle. The aim of this study was to identify the genotypes of *G. duodenalis* present in human feces by sequencing one genetic locus. Fecal samples were collected from 190 persons belonging to a Portuguese health department, ranging from children to adult persons, and delivered to our laboratory. Samples were concentrated by formalin-ethyl acetate sedimentation and processed by PCR. *Giardia* cysts were detected in seven people (3.7%), with a wide rate of cyst shedding, in children and adult persons. In the genetic assays, we used PCR-based procedures to amplify the beta-giardin gene of *Giardia*, and sequence analysis was performed. Five isolates revealed the presence of *G. duodenalis* assemblage B (four where assemblage B and one the assemblage B-1), and two isolates revealed the presence of *G. duodenalis* assemblage A (A-2 and A-3, each). The samples had 100% homology with *G. duodenalis* assemblage B (AY258616), B-1 (AY072725) and A-2 (AY072723), respectively. All isolates were from asymptomatic persons, and appears

that infections with assemblage B are more prevalent (71.4%) than assemblage A (28.6%). This work was financially supported by Fundação Calouste Gulbenkian, project number 61018.

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Cryptosporidium parvum: potential axenic development versus long-term survival of oocysts. M. J. ARROWOOD and L. T. XIE, Centers for Disease Control and Prevention, National Center for Zoonotic, Vector-borne and Enteric Diseases, Division of Parasitic Diseases, Atlanta, Georgia.

Parasites of the genus *Cryptosporidium* are more closely related to gregarines than to coccidian protozoa according to recently published phylogenetic studies. To support this position, other studies report the in vitro development and amplification of an isolate of *C. parvum* in the absence of host cells. This contradicts the conventional wisdom that *Cryptosporidium* species are obligate intracellular parasites. Our attempts to replicate the cell-free (axenic) culture of *Cryptosporidium* using the "IOWA" zoonotic isolate of *C. parvum* (routinely passaged in calves and mice in our laboratory) did not succeed. We duplicated the culture conditions previously described, but evaluated plasma supplementation as well as the serum supplementation of the basal culture media. To facilitate the differentiation of oocysts inoculated into the culture chambers from any potential de novo oocysts arising from parasite development, we pre-labeled the oocyst inoculum with fluorescein (fluorescein thiosemicarbazide). Parasite viability was verified in parallel cultures using host cells (MDCK and HCT-8). Culture preparations were monitored for 46 days post-inoculation (as previously described) with no evidence of parasite development. No morphological structures consistent with those previously described and interpreted as "unique" life cycle stages were observed and no evidence of new oocyst formation was detected. Further study included the evaluation of oocyst infectivity following long-term (46 days) incubation at 4, 18, and 37°C in a variety of buffers and media including water, Hank's balanced salts solution (HBSS), HBSS supplemented with glucose, culture media matching the above preparations. Oocysts were "re-isolated" based on surface labeling and light scatter profiles using flow cytometry to select for intact oocysts before inoculation into host cell-containing culture assays. Not surprisingly, the oocysts maintained at 4°C retained infectivity while oocysts held at higher temperatures declined significantly.

95A

Pneumocystis colonization and cigarette smoke exposure in the development of emphysema in a mouse model. J. M. BECK,^{a,b} A. M. PRESTON,^a J. E. PITZER,^a T. LING,^a R. PAINE III^{a,b} and P.J. CHRISTENSEN^{a,b}, ^aUniversity of Michigan Medical School, Ann Arbor, Michigan, ^bDepartment of Veterans Affairs Medical Center, Ann Arbor, Michigan.

Identification of *Pneumocystis* in respiratory secretions was previously thought to indicate pneumonia, but recent work using PCR to detect organisms confirms that colonization occurs. Colonization with *Pneumocystis* is common in patients with COPD, and is related to increasing severity of disease. Additionally, emphysema is an important complication of chronic HIV infection, particularly in individuals who smoke cigarettes. Our laboratory is interested in determining whether chronic *Pneumocystis* colonization and exposure to cigarette smoke interact to cause lung damage and emphysema. We exposed mice to daily cigarette smoke for periods up to 6 mo, and compared them with mice exposed to air alone. Cigarette smoke exposure caused airspace enlargement (measured by quantitative morphometry)

and increased functional residual capacity, both indicators of emphysema. We also established *Pneumocystis* colonization in mice by repetitive intranasal inoculation, and by co-housing with infected source mice. Using PCR, we compared the intensity of *Pneumocystis* colonization in mice exposed to cigarette smoke and in mice exposed to air. After 8 wk, the lungs of mice exposed to cigarette smoke contained significantly more *Pneumocystis* RNA than the lungs of mice exposed to air, indicating decreased clearance of the organism. Next, we determined whether exposure to cigarette smoke and colonization with *Pneumocystis* interact to accelerate the development of emphysema. Mice exposed to cigarette smoke and colonized with *Pneumocystis* developed significantly more airspace enlargement and significantly greater functional residual capacities than mice exposed to cigarette smoke or *Pneumocystis* alone. We conclude that chronic exposure to cigarette smoke slows the clearance of *Pneumocystis*, and that exposure to cigarette smoke and *Pneumocystis* colonization interact to accelerate the development of emphysema.

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Identification and characterization of a guanine nucleotide exchange factor in *Tetrahymena thermophila*. A. BELL, C. GUERRA and P. SATIR, Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, New York.

In 1999, a guanine nucleotide exchange factor (GEF) was identified in *Paramecium* from a screen for ciliary proteins and was named PSec7 (Nair et al. FASEB J.). Use of the *Paramecium* PSec7 antibody in *Tetrahymena thermophila* recognized a putative GEF protein, Gef1p, which localizes to the cilia in immunofluorescence, immuno-EM and Western blot experiments. Polymerase chain reaction methods and database analysis were used to identify *Tetrahymena* GEF1. A protein OF 2,053 amino acids in length, containing Sec7 motifs, truncated IQ motifs and PH domains, homologous to similar domains in PSec7, was cloned. Upregulation of the product of this gene was observed via RT-PCR following deciliation and subsequent regrowth of cilia in *Tetrahymena* cells. In order to confirm previous experimental results for Gef1p, a second antibody is being made against the cloned protein. In addition, the protein is being tagged with both hemagglutinin (HA) and green fluorescence protein (GFP). A proposed model for the role of GEFs in the cilium will be presented based on these data.

97A

Prevalence and preliminary genetic characterization of *Cryptosporidium* spp. from asymptomatic heifers in Galicia (NW, Spain). J. A. CASTRO-HERMIDA,^a A. ALMEIDA,^b M. GONZÁLEZ-WARLETA,^a J. M. CORREIA DA COSTA^b and M. MEZO^a, ^aCentro de Investigaciones Agrarias de Mabegondo, Xunta de Galicia, Spain, ^bCentro de Imunologia e Biologia Parasitária, INSA, Portugal.

The apicomplexan parasite *Cryptosporidium* spp. causes gastrointestinal infections in a wide range of vertebrate hosts, including humans. Three species of *Cryptosporidium* infect cattle: *C. parvum*, *C. andersoni*, and *C. bovis*. *Cryptosporidium parvum* infects the small intestine primarily of pre-weaned calves although also of humans and other animals, often causing diarrhoeal disease. *Cryptosporidium andersoni* infects the abomasums of juvenile and mature cattle; infection has been identified as a cause of reduced milk production but has not been associated with other signs of disease and nor is known to infect animals other than cattle. *Cryptosporidium bovis* was found to be the predominant species infecting calves between 2 and 11 mo old, but was not associated with overt disease. The aim of this study was to determine the

prevalence and intensity of infection of *Cryptosporidium* spp. in heifers (1–24 mo old) and identify which genotypes are present. Faecal samples were collected from 291 heifers selected at random from 58 dairy farms in Galicia and were processed by a direct immunofluorescence technique with monoclonal antibodies (IFAT) and PCR. *Cryptosporidium parvum* oocysts were detected in 41 heifers (14.0%) from 23 (39.6%) of herds and the rate of oocyst shedding ranged between 10 and 782 oocysts/g of faeces (mean 80.0 ± 126.7 oocysts/g of faeces). In these preliminary assays on genetic characterization, it was used PCR-based procedures to amplify and sequencing two genetic loci: the 18S ribosomal DNA and hsp-70 gene of *Cryptosporidium*, the isolates analysed revealed *C. parvum* presence. The samples had 100% homology with *C. parvum* isolate 11 (AF221528), and one of them had one polymorphism in the hsp-70 coding region (1,254 bp position in the isolate 11 hsp70 sequence), second codon position A to G, changing Lysine to Arginine. All animals sampled were asymptomatic indicating a real source of environmental contamination by *C. parvum*. The heifers may be an important zoonotic reservoir for this parasite. This work was financially supported by Xunta de Galicia through the Consellería de Innovación e Industria (PGIDIT05RAG50306PR).

98A

Prevalence and preliminary genetic analysis of *Giardia* from adult sheep in Galicia (NW, Spain). J. A. CASTRO-HERMIDA,^a A. ALMEIDA,^b M. GONZÁLEZ-WARLETA,^a J. M. CORREIA DA COSTA^b and M. MEZO^a, ^aCentro de Investigaciones Agrarias de Mabegondo, Xunta de Galicia, España, ^bCentro de Inmunología e Biología Parasitaria, INSA, Portugal.

Giardia is a protozoan flagellate infecting a wide range of vertebrate hosts, including humans, and related to intestinal disturbances and diarrhoea, particularly in young animals. The species affecting humans, *Giardia duodenalis*, is also present in livestock and pets. Adult animals are generally considered refractory to heavy infections and associated clinical disease owing to a strong immune response. Nevertheless, it has been shown that asymptomatic adult ruminants may act as healthy carriers and may be a source of contamination. Infection takes place through oral ingestion of vegetables or water contaminated with cysts. However, the information on the epidemiology, including the extension of this infection and genotypes involved, in general is scarce. Therefore, the first purpose of this study was to determine the prevalence and intensity of infection of *G. duodenalis* in adult sheep. The second objective was to identify which genotypes are present. Faecal samples were collected from 575 adult sheep selected at random from 68 herds in Galicia (NW Spain) and were concentrated and processed by a direct immunofluorescence technique using monoclonal antibodies (IFAT) and PCR. *Giardia duodenalis* cysts were detected in 188 sheep (32.7%) from 67 (98.5%) of herds and the rate of cyst shedding ranged between 10 and 4319 cysts/g of faeces (mean 177.1 ± 598.3 cysts/g of faeces). In these preliminary assays on genetic analysis, it was used PCR-based procedures in order to genotype with amplification and sequencing of the beta-giardin gene. Assemblage E was detected and the isolates had 100% homology to the CBHRG21 and CBHRG19 isolates (DQ116621 and DQ116620, respectively). All adult animals sampled were asymptomatic indicating a real source of environmental contamination by *G. duodenalis*. Nevertheless, the preliminary results of molecular biology showed that the sheep might not be an important zoonotic reservoir for this parasite. This work was financially supported by Xunta de Galicia through the Consellería de Innovación e Industria (PGIDIT05RAG50306PR).

99A

First molecular data on cryptosporidiosis in Venezuela. G. CERRAD,^{a,b} T. NGOUANESAVANH,^a A. HERNAN,^b E. ROJAS,^b R. CONTRERAS,^c L. POCATERRA,^b L. NUÑEZ,^b E. DEI-CAS^{a,d} and K. GUYOT^a, ^aEcologie du Parasitisme, Institut Pasteur de Lille, Lille, France, ^bCátedra de Parasitología, Escuela de Medicina ‘José María Vargas’, Universidad Central de Venezuela, Caracas, Venezuela, ^cSección de Geohelmintiasis, Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas, Venezuela, ^dParasitologie-Mycologie, Centre Hospitalier Régional et Universitaire de Lille, Lille, France.

There is not much information about the circulation and transmission of cryptosporidiosis in developing countries in which this disease remains as a clinically significant opportunistic infection, especially in those HIV-infected persons without access to HAART. In this study we included 37 HIV-infected patients from different regions of Venezuela. They underwent thorough clinical history evaluation and provided stool specimens for the diagnosis of *Cryptosporidium* sp. and other parasites. Genomic DNA was isolated from positive faecal samples for *Cryptosporidium*. A highly polymorphic section of the 18S rRNA gene was amplified by nested PCR. Different species of the parasite were identified by RFLP and confirmed by sequencing. Analysis of micro (ML1, ML2, MS9) and minisatellite (MS5) markers was performed when possible. *Cryptosporidium* sp. was found in 10 patients; all these patients came from urban communities. They were at an advanced stage of immunosuppression and only two persons were beginning HAART. Three genotypes were identified: eight isolates corresponded to *C. hominis*, one to *C. parvum* and another one to *C. canis*. In all *C. hominis* samples analyzed by micro- and minisatellite markers, we found the same combination of alleles (same multilocus genotype). Patients with *C. hominis* were all symptomatic adults with diarrhoea either acute or chronic. *Cryptosporidium parvum* was detected in a 2-yr-old child who was asymptomatic for cryptosporidiosis, and *C. canis* was found in a 73-yr-old woman with chronic diarrhoea as her first manifestation of AIDS. This patient confirmed during the epidemiological questionnaire that she currently owned dogs. This is the first molecular data on molecular cryptosporidiosis in this country. These preliminary results, suggest that *C. hominis* is the predominant species among HIV-infected patients in Venezuela and that species from animals can infect Venezuelan patients and may be linked to clinical disease.

100A

Barcoding protists: a trailblazing study on a ciliate genus of closely related species, *Tetrahymena*. C. CHANTANGSI, M. C. STRÜDER-KYPKE and D. H. LYNN, Department of Integrative Biology, University of Guelph, Guelph, ON, Canada.

A mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene has been proposed for use as a DNA barcode in identifying animal species. To test the applicability of this gene in identifying protists, the ciliate genus *Tetrahymena* was selected. *Tetrahymena* comprises about 42 species that are highly similar in morphology, and thus very difficult to discriminate. Some of these species belong to several sibling species complexes, while other species exhibit some morphological diversity in their polymorphic life cycles. In this study, the COI genes of all *Tetrahymena* species, whose cultures are available, and three non-*Tetrahymena* ciliates—*Colpidium campylum*, *Colpidium colpoda*, and *Glaucoma chattoni*—were amplified and sequenced. Nucleotide sequence analysis of 1,821 bp of the COI gene among these species showed an average 10.9% sequence divergence. Furthermore, sequence analysis of the

proposed barcoding region of about 650 bp, which is currently used as diagnostic site for identifying animal species, showed an average 10.4% sequence divergence. Amplification and sequencing of the COI gene of intraspecific isolates of the following species from geographically diverse regions—*T. borealis*, *T. lwof-fi*, *T. patula*, *T. pyriformis*, *T. thermophila*, and *T. tropicalis*—were also performed to determine the degree of intraspecific variation. Except for *T. tropicalis*, which showed a higher level of intraspecific variation and clustered with other species, the other five species showed low levels of intraspecific variation, and all intraspecific isolates grouped together. This study demonstrates the feasibility of the mitochondrial COI gene as a taxonomic marker for “barcoding” and identifying ciliated protists.

101A

18S rRNA gene sequences reveal diversity within the genus *Zoothamnium* (Ciliophora, Peritrichia). J. C. CLAMP, North Carolina Central University, Durham, North Carolina.

Zoothamnium is a genus of colonial, sessiline peritrichs with free-living and ectosymbiotic species occurring both in marine and freshwater habitats. It has a contractile stalk that, folds into a “zig zag” conformation rather than a helical one when contracted. The genus is the type of the family Zoothamniidae, which includes other genera with “zig zag” stalks. Miao et al. [2004. Phylogenetic relationships of the Subclass Peritrichia (Oligohymenophorea, Ciliophora) inferred from small subunit rRNA gene sequences. *J. Eukaryot. Microbiol.* **51**:180–186] demonstrated a close relationship between the freshwater species *Zoothamnium arbuscula* and *Carchesium polypinum*, a colonial member of the family Vorticellidae with a stalk that contracts into a helical conformation, using 18S ssu rRNA sequences. In the present study, the 18S rRNA genes of seven marine species of *Zoothamnium* (*Z. niveum*, *Z. alternans*, *Z. pelagicum*, and four unidentified species) were sequenced. Based on this evidence, the seven species constitute a poorly supported, possibly polyphyletic clade allied to the non-contractile, colonial genus *Epistylis* but separate from both *C. polypinum* and *Z. arbuscula*. However, within this clade, the species cluster into three, well supported, small clades of two to three species each. Future research is planned that will include gene sequences (including ones coding for proteins) from more species of *Zoothamnium* and more thorough description of morphological characteristics, especially the oral infraciliature, that are still incompletely known for most species in the genus.

102A

Combinations of sterol inhibitors show synergistic effects against *Pneumocystis carinii* viability in vitro. M. S. COLLINS and M. T. CUSHION, University of Cincinnati College of Medicine and the Cincinnati Veterans Administration Medical Center, Cincinnati, Ohio.

Members of the genus *Pneumocystis* are opportunistic fungal pathogens, which cause life-threatening pneumonia in immunocompromised mammalian hosts. Standard anti-fungal treatments have a very limited effect on these organisms. Growing extracellularly in the alveoli of the host lung, *Pneumocystis* may synthesize its own membrane sterols, scavenge and modify cholesterol, or a combination of these activities. Transcript analysis of *P. carinii* during fulminant infection identified several fungal gene homologs involved in sterol biosynthesis. Previous studies

have shown that some sterol biosynthesis inhibitors have moderate activity against *Pneumocystis* both in vivo and in vitro. In the present study, we explored these activities as potential drug targets by using combinations of inhibitors in a cell-free in vitro system. Viability of the organism pools was measured using an ATP-driven luciferin-luciferase assay. The results of the combination treatment were analyzed with normalized isobolograms (CalcuSynv2, Biosoft, U.K.). Six sterol biosynthesis and related enzymes were targeted with inhibitory compounds: HMG CoA synthase (*Erg13*) and reductase (*Hmg1*); DOXP synthase (*dxs*); squalene epoxidase (*Erg1*); sterol 14 α demethylase (*Erg11*); and sterol C24 methyltransferase (*Erg6*). The most efficacious combination was the *Erg6* inhibitor, berberine, with cerulenin, an *Erg13* inhibitor. Synergism was also attained with certain combinations of simvastatin and berberine, palmitine and terbinafine, cerulenin and terbinafine, simvastatin and tebuconazole, tolinaftate and tebuconazole. Equivocal or antagonistic results were achieved with combinations of simvastatin and tolinaftate, tebuconazole and berberine, berberine and terbinafine, palmitate and tebuconazole, cerulenin and tebuconazole. Surprisingly, fosmidomycin, though it had no effect as a monotherapy, showed synergism with simvastatin in this system. Combinations of sterol inhibitors show promise as potential anti-*Pneumocystis* agents and will be evaluated in a mouse model of infection.

103A

Analysis and functional classification of transcripts expressed by *Pneumocystis carinii* during fulminate infection. M. T. CUSHION,^a A. G. SMULIAN,^a B. E. SLAVEN,^a T. SESTERHENN,^a A. POROLLO^b and J. MELLER^b, ^aUniversity of Cincinnati College of Medicine and the Cincinnati Veterans Administration Medical Center, Cincinnati, Ohio, ^bChildrens Hospital Research Foundation, Cincinnati, Ohio.

In an effort to better understand the pathological process of *P. carinii* pneumonia, about 4200 Expressed Sequence Tags (ESTs) derived from *P. carinii* organisms during fulminate infection were analyzed by BLAST2GO then assembled using PHRAP. About 2,000 of the cDNAs were fully sequenced to build a unigene set of 905 members. BLASTx and BLASTn analysis of the ESTs revealed most (70%) had identity to existing polypeptides at <e-10. Most (68%) had identity with fungal or existing *P. carinii* sequences. Most other homologies were to genes of the filamentous fungi *Aspergillus* species (18%) and *Neurospora crassa* (13%) and to *Yarrowia lipolytica* (8%); *Schizosaccharomyces pombe* (7.5%); and *Cryptococcus neoformans* (6%). Highly expressed *P. carinii* genes were of the MSG and HSP70 families, (70%), subtilisin proteases, Ste11 and Erg6. Most transcripts were dedicated to cellular and physiological processes in the GO biological process classification, to binding and catalytic activity in molecular function, and organelle and cell compartments in distribution. KEGG analysis showed many pathways were functional including glycolysis, TCA cycle, fatty acid biosynthesis and metabolism, sterol biosynthesis, purine and pyrimidine metabolism, amino acid metabolism, N-glycan biosynthesis, aminosugars metabolism, pyruvate metabolism, and porphyrin biosynthesis. Oxidative phosphorylation represented the most active category. Several conserved anti-fungal drug targets were detected: *Erg1*, *Ram2*, *Nmt1*, and *Eft2*. Ornithine decarboxylase, a target of polyamine biosynthesis inhibitors, was also present. Interestingly, dihydrofolate reductase was expressed, but dihydropteroate synthase transcripts were not found. This study represents the first characterization of the expressed genes of a non-culturable fungal pathogen of mammals during the infective process.

104A

In vitro selection and in vivo efficacy of piperazine- and alkanediamide-linked bisbenzamidines against *Pneumocystis* pneumonia. M. T. CUSHION,^{a,b} P. D. WALZER,^{a,b} A. D. ASHBAUGH,^{a,b} S. L. REBHOLZ,^{a,b} R. BRUBAKER,^c J. J. VANDEN EYNDE,^d A. MAYENCE^d and T. L. HUANG^d, ^aVeterans Affairs Medical Center, Cincinnati, Ohio, ^bUniversity of Cincinnati College of Medicine, Cincinnati, Ohio, ^cThe Christ Hospital, Cincinnati, Ohio, ^dXavier University of Louisiana College of Pharmacy, New Orleans, Louisiana.

Bisbenzamidines such as pentamidine isethionate are aromatic dicationic compounds that are active against *Pneumocystis* and other microbes, but oftentimes toxic to the host. To identify potential anti-*Pneumocystis* agents, we synthesized bisbenzamidine derivatives in which the parent compound pentamidine was modified by a 1,4-piperazinediyl, alkanediamide, or 1,3-phenylenediamide moiety as the central linker. Several of the compounds were more active against *P. carinii* and less toxic than pentamidine in cytotoxicity assays. Here, we evaluated nine bisbenzamidine derivatives representing a range of in vitro activities from highly active to inactive for the treatment of pneumocytosis in an immunosuppressed mouse model. Six of these in vitro active compounds, 01, 02, 04, 06, 100 and 101, showed significant increases in survival versus the untreated, infected control mice. Compound 100 was highly efficacious against the infection at 20 and 40 mg/kg and had improved survival curves versus pentamidine-treated mice. All six bisbenzamidine compounds that exhibited high in vitro activity significantly decreased the infection in vivo; two compounds, 12 and 102, with marked to moderate in vitro activity had slight to no activity in vivo, while compound 31 was inactive in vitro and in vivo. Thus, selection of highly active compounds from the in vitro cytotoxicity assays was predictive for activity in the mouse model of *Pneumocystis* pneumonia. We conclude that a number of these bisbenzamidine compounds may show promise as new anti-*Pneumocystis* drugs.

105A

Golgi-associated genes in the oxymonad *Monocercomonoides* sp. suggest the presence of a cryptic organelle. B. DACKS,^a N. A. LIAPOUNOVA,^a V. HAMPL,^b R. P. HIRT,^c T.M. EMBLEY^c and L. GEDAMU^a, ^aUniversity of Calgary, Calgary, Canada, ^bDalhousie University, Halifax, Canada, ^cUniversity of Newcastle Upon Tyne, Newcastle Upon Tyne, United Kingdom.

Organelles such as mitochondria and Golgi are the hallmark of eukaryotic cells, and yet there are a few lineages, which appear to lack these organelles. In the past, this has been interpreted as implying the ancient nature of these taxa. More recently, however, this apparent absence has been proposed as secondary loss, suggesting that the modification of organelle morphology and function may well be an important aspect of eukaryotic cellular evolution. Previous work provided evidence of Golgi-associated genes in five of the seven major lineages lacking stacked organelles, suggesting the secondary loss of Golgi in these taxa. One lineage for which no such data currently exists is the oxymonads, a group of cell-biologically aberrant, anaerobic protists. This left open the possibility that they might be primitively lacking a Golgi body and raised questions of what Golgi homologue might be present in these organisms. From an exploratory EST survey of the oxymonad *Monocercomonoides* sp., we have identified several genes of Golgi function including components of the retromer and coatamer vesicle coats. This data suggests the presence of multiple Golgi-specific systems in oxymonads and thus further reduces the possibility of primitive organelle absence in this

taxon. Future work to characterize these genes and identify the physical Golgi homologue in oxymonads will allow investigation into how the Golgi has changed in this lineage and, more generally, into the diverse ways that this organelle may evolve over time.

106A

Geographic variation in epitopes of major surface glycoprotein as determined by reactivity to multiple MsgC clones. K. DALY,^a J. V. KOCH,^a E. CALDERON^b and P. D. WALZER^a, ^aVAMC, University of Cincinnati, Cincinnati, Ohio, USA, ^bVirgen del Rocío University Hospital, Seville, Spain.

We had previously shown that it was possible to differentiate HIV patients that had a previous bout of *Pneumocystis* pneumonia (PcP⁺) from those that did not (PcP⁻), based on Western blot analysis and ELISA performed with a recombinant major surface glycoprotein (Msg) fragment (MsgC1) from *Pneumocystis jirovecii*. The patient samples tested in our initial study were isolated in Cincinnati, OH, USA, and we were interested in determining the applicability of this finding to patient populations from other geographic locations. We have assayed patient sera derived in Seville, Spain for the ability to react to a panel of recombinant Msg fragments, and the results were remarkably different from those we obtained with our local samples. In Western Blot, we could find no significant differences in frequency of reactivity to MsgC1 in the healthy blood donors, PcP⁺ and PcP⁻ sera from the Spanish population. The results in ELISA were even more striking, in that the level of reactivity to MsgC1 in the European sera was extremely low compared with Cincinnati samples. We extended the study to include three other clones of MsgC (MsgC3, C8 and C9) as well as two other fragments of Msg (MsgA and MsgB). The reactivity of the healthy blood donors from Spain was significantly higher to MsgA and MsgC8 than was the response in the local US population. We have used the responses to MsgC1 and MsgC8 to profile HIV, cystic fibrosis and chronic bronchial disease patients that did or did not have PcP. While there was a consistently higher response to MsgC8 than MsgC1, there was no significant difference in the response of either clone across the different populations. The results suggest that the choice of recombinant Msg antigen most appropriate for a particular study will depend on the geographic site and on the MsgC clone.

107A

Cells infected with *Encephalitozoon* microsporidia show inhibition of p53-induced apoptosis. C. DEL AGUILA,^a F. IZQUIERDO,^a A. G. GRANJA,^b C. HURTADO,^b S. FENOY,^a M. FRESNO^b and Y. REVILLA^b, ^aFacultad de Farmacia, Universidad San Pablo-CEU, Madrid, Spain, ^bCentro de Biología Molecular "Severo Ochoa", Universidad Autónoma, Madrid, Spain.

Microsporidia are intracellular obligate parasites, which have recently been shown closely related to fungi. They have a unique extrusion apparatus, which is able to inject the sporoplasm directly into the target cell without using receptors. In recent years *Encephalitozoon* microsporidia have become a source of morbidity and mortality in humans, and it has been suggested that microsporidia may modulate the host cell cycle and apoptosis. Extracts of non-infected Vero cells and microsporidia-infected or African swine fever virus (ASFV)-infected cells, used as an apoptosis control, were prepared at various times after infection, separated by SDS-PAGE and blotted with specific antibodies related to apoptosis regulation. Inhibition of caspase-3 cleavage at different times of Vero cell infection by *Encephalitozoon*

microsporidia was observed. In addition, p53 phosphorylation during *Encephalitozoon* infection was also inhibited. Further, using confocal microscopy, we were able to observe that translocation of p53 to the nucleus did not occur after infection of Vero cells. Moreover, luciferase reporter assays showed that the transcriptional function of p53 was impaired during the infection cycle. Thus, to our knowledge, it was shown for the first time that an intracellular parasite may be able to multiply in the host cell without activating the p53 apoptotic pathway of that cell.

108A

Probable *Pneumocystis jirovecii* inter-human transmission in a French hospital pediatric unit and in the community. I. DURAND-JOLY,^{a,b,c} M. CHABE,^a N. GANTOIS,^a N. HEOUAINE,^b S. MOULRONT,^b D. CAMUS^{a,c} and E. DEI-CAS^{a,c}, ^aEA-3609-CH ET U-LILLE-2, France, ^bCentre Hospitalier de Dunkerque, France, ^cInstitut Pasteur de Lille, France.

We have shown experimentally that rodent *Pneumocystis* organisms are able to replicate in the lungs of immunocompetent hosts, which can transmit the infection to susceptible hosts. In the present work we investigated *P. jirovecii* carriage in a hospital in order to clarify the modalities of *Pneumocystis* inter-human transmission, and to determine if it can occur in a nosocomial manner. In a pediatric unit of a Dunkerque hospital, two brothers (7-mo-old and 2-yr-old) were hospitalized for acute bilateral pneumonia associated with chronic dry cough and repetitive bronchitis episodes. Additional diagnostic tests on both children revealed severe congenital agammaglobulinemia associated with *Pneumocystis* pneumonia (PcP). Before PcP or agammaglobulinemia diagnosis could be established, both children were co-housed in the hospital in the same conventional room two times for 6 days. In order to assess potential *P. jirovecii* nosocomial circulation from these untreated PcP patients, we explore *P. jirovecii* carriage in seven hospital staff members, who were or not in close contact with the two children. Also evaluated were their mother, father, and a third, older brother, who were apparently healthy. Nested-PCR at *Pneumocystis* mtLSU- and mtSSU-rRNA loci on oropharyngeal washing samples was used to detect *Pneumocystis* carriage, and direct sequencing was then performed in order to identify *Pneumocystis* species and genotypes. *Pneumocystis jirovecii* was detected in two nurses and in the oldest brother. Sequence analysis at the two loci revealed identical *P. jirovecii* genotypes in the two infected children and in the three carriers. These findings strongly suggested a direct inter-human transmission, probably by droplets, both in hospital and in the community, as the third brother has not been in the hospital. This contribution strengthened the idea of defining prevention measures aiming at limiting the circulation of *Pneumocystis* in hospitals.

109A

Protist parasites in bottle-nosed dolphins (*Tursiops truncatus*) in Florida and South Carolina. R. FAYER,^a M. SANTIN,^a J. P. DUBEY,^a P. FAIR^b and G.D. BOSSART^c, ^aAgricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, ^bNational Oceanic and Atmospheric Administration, Center for Environmental Health and Biomolecular Research, Charleston, South Carolina, ^cDivision of Marine Mammal Research and Conservation, Harbor Branch Oceanographic Institution, Fort Pierce, Florida.

Feces were examined from 99 Atlantic Bottlenose dolphins captured and released as part of the Dolphin Health and Risk

Assessment project in the Charleston, SC harbor estuarine environment and the Indian River Lagoon, FL during 2004 and 2005. Feces from rectal swabs or feces collected via enema were subjected to DNA extraction and PCR protocols for *Cryptosporidium* and generic Microsporidia. A two-step nested protocol was used to amplify a fragment of the SSU rRNA gene of *Cryptosporidium* and Microsporidia. Polymerase chain reaction products were purified, sequenced, and sequence data were examined to determine identity. *Cryptosporidium* was not detected in any specimens. Microsporidia were detected in specimens from 18 dolphins. Sequence data from 15 specimens were similar to but not identical to *Kabatana takedai*, *Tetramicra brevifilum* and *Microgemma tincae* reported from fish. Likewise, sequence data from three specimens were similar to *Enterocytozoon bienersi* (~87% homology), a species infecting humans and a variety of terrestrial mammals. The presence of fish-like Microsporidia might represent parasites of fish eaten by dolphins whereas the presence of *E. bienersi*-like organisms might represent a genotype of this species that infects dolphins. Sera from dolphins captured and released from the same locations in 2003 and 2004 were examined for exposure to *Toxoplasma gondii* utilizing four antibody-based tests; 147 sera were found positive. The present findings of *E. bienersi* and *T. gondii* are presumptive and should be confirmed by detection of these organisms in tissues.

110A

Wide occurrence of *Cryptosporidium bovis* and the deer-like genotype in bovines. Y. FENG,^a Y. ORTEGA,^b G. HE,^c P. DAS,^d X. ZHANG,^e R. FAYER,^f W. GATEI,^a V. CAMA^a and L. XIAO^a, ^aCenters for Disease Control and Prevention, Atlanta, Georgia, USA, ^bUniversity of Georgia, Griffin, Georgia, USA, ^cChinese Academy of Agricultural Sciences, Shanghai, China, ^dRajendra Memorial Research Institute Of Medical Sciences, Patna, India, ^eJilin University, Changchun, China, ^fU.S. Department of Agriculture, Beltsville, Maryland, USA.

Recent studies in the United States reported that of preweaned dairy calves infected with *Cryptosporidium* ~85% were infected with zoonotic *C. parvum* whereas of postweaned calves and those up to 1 yr of age that were infected with *Cryptosporidium*, only 1% were infected with *C. parvum*. *Cryptosporidium bovis* and the deer-like genotype were much more prevalent in the postweaned calves. It is not clear whether the disproportionately high prevalence of *C. parvum* in preweaned calves is influenced by intensive animal production methods in the United States or if it is primarily parasite-host age-related phenomenon. In this study, to determine whether the same *Cryptosporidium* infection pattern was present in other geographic areas, we genotyped *Cryptosporidium* specimens collected from pre- and post-weaned calves on two farms in China and India, using specimens from farms in Georgia, USA for comparison. *Cryptosporidium bovis* was the most common species found in pre- and post-weaned calves in all three areas. In Georgia, the deer-like genotype was found frequently in pre- and post-weaned calves, and *C. andersoni* was found in one weaned calf. There were no differences in the small subunit rRNA gene sequences obtained from *C. bovis* or deer-like genotype among the three areas. One adult yak in China, however, was infected with a species similar to *C. bovis*, with only three nucleotide mutations in the target gene. One of the nucleotide changes in the yak sequence produced a new *SspI* restriction site. All the five *Cryptosporidium* spp. could be differentiated from each other by restriction fragment length polymorphism analysis with enzymes *SspI* and *MboII*. Thus, both *C. bovis* and the deer-like genotype are found in cattle in diverse geographic areas and host adaptation of *C. bovis* might have occurred in yaks.

111A

A new generation archiving system for cilioprotists of southern New England. E. FREMEN and L. A. HUFNAGEL, University of Rhode Island, Kingston, Rhode Island.

Several years ago, the Rhode Island Coastal Micrograzers web site (<http://athena.cs.uri:8080/ricms>) was established to provide a portal for access to the Rhode Island Cilioprotist Micrograzer Survey (RICMS)©, a database resource for archiving information useful to anyone interested in the distribution, diversity, systematics and ecology of cilioprotists of Rhode Island and nearby regions of coastal southern New England. Initially, the plan was simply to archive digital videoclips collected during the past dozen years in a web-accessible database. However, additional goals emerged as we proceeded. These are: (1) to include other types of data, particularly for species identification, bar-coding and geographical localization; (2) to streamline the processing and entry of data already collected; and (3) to make the site more flexible, accessible and searchable for the end-user. To meet these goals, we have begun to develop an entirely new web-accessible database. The new Rhode Island Coastal Micrograzers site will enhance the ability of students, research assistants and others to process and upload video, photographic and other types of data, as well as provisional classifications, into a newly designed database. The data will then be reviewed by the principal administrator of the web site or an assigned curator, who will associate the approved data with one or more groups in the most up to date phylogenetic schema for the cilioprotists. The database will be searchable by a variety of morphological, molecular and geographical criteria. The system uses an extensible metadata system and integrated live search functions that allow all relevant data fields to be searched; search results can be filtered according to the administrator's permissions, so in-progress works will be available to end-users. A history of edits will be preserved, and no html coding or database experience will be required to use the system, for the administrator, those submitting data or the end-user.

112A

Detection and persistence of quahog parasite unknown in the coastal marine environment. R. J. GAST,^a D. M. MORAN,^a K. R. UHLINGER,^b D. R. LEAVITT^c and R. SMOLOWITZ^b, ^aWoods Hole Oceanographic Institution, Woods Hole, Massachusetts, ^bRoger Williams University, Bristol, Rhode Island, ^cMarine Biological Laboratory, Woods Hole, Massachusetts.

Quahog parasite unknown (QPX) is a significant cause of hard clam mortality along the northeast coast of the United States. The QPX organism is classified as a thraustochytrid protist. Although members of this protistan family are generally saprophytic and commonly found in the marine environment, no viable method was available to reliably survey the natural environment for QPX. It was unknown whether QPX could persist in the marine environment outside of its clam host, and whether there were other potential reservoirs of the parasite. Here we report on a PCR-based method that we have developed that permits sensitive detection of QPX in natural samples and seed clams. With our method, between 10 and 100 QPX cells can be detected in 1 L of water, 1 g of sediment and 100 mg of clam tissue. We have used the method to examine one hundred 15 mm seed clams, and found that 10–12% of the clams were positive for the presence of the QPX organism, although only 1% showed histological evidence of being infected. The method has also been successfully used to detect the presence of the pathogen in environmental samples (water, sediment, algae, invertebrates, detritus) from a site experiencing severe infection and a site with low to no infection. This survey has revealed the presence and year-round persistence of

QPX in local water, sediment, detritus and macrophytes, even in the absence of large-scale clam mortality events. The results of this study have led to suggestions for plot husbandry methods that may help to reduce the environmental level of the parasite and lessen the possibility of massive die-offs.

113A

Iron superoxide dismutase (FeSODA) of *Leishmania donovani* is targeted to the mitochondria via the N-terminal pre-sequence: role of positively charged amino acid residues. F. E. GETACHEW and L. GEDAMU, Department of Biological Sciences, University of Calgary, Calgary, AB, Canada.

Mitochondria are essential organelles that carry out many important functions such as ATP generation, lipid biosynthesis, amino acid metabolism and apoptosis. We have previously isolated a single copy FeSODA gene from *L. donovani*. Comparison of *L. donovani* FeSODA amino acid sequences with FeSODB and FeSODs and MnSODs from other organisms reveals a conserved putative 31 N-terminal amino acids extension in FeSODA. This N-terminal extension has similar properties to the mitochondria targeting sequence (MTS) and is predicted to form amphiphatic α -helical structure. Here, we show that FeSODA is localized to the mitochondria using enhanced green fluorescent protein (GFP) constructs, and that the 31 N-terminal amino acids are important and sufficient to target GFP to the mitochondria. Both the full length and N-terminal amino acids extension GFP fusion proteins show co-localization with the mitochondria specific probes Mito-tracker dye and TRYP2 antibody. Furthermore, we have assessed the attribution of hydrophobic region and the basic amino acid residues within the N-terminal extension using deletion and site directed mutagenesis respectively. The deletion of the hydrophobic amino acid residues (A9-L29) interestingly reveals that the first eight amino acids of the amino-terminal extension and the potential R-2 motif sequence of FeSODA might have the necessary information to target the GFP to the mitochondrion. The single substitution of each of the four basic residues within the putative N-terminal sequence does not affect mitochondrial import. However, the replacement of all the four basic amino acid residues (R3, R4, K8 and R30) with A (alanine) abolished mitochondrial targeting. These findings, for the first time in the Kinetoplastida demonstrate that the 31 N-terminal amino acids of FeSODA is important for targeting the protein to the mitochondria, and that the basic amino acids within this N-terminal extension are crucial for import into the mitochondria.

114A

Functional evidence for an aquaporin in the microsporidium *Encephalitozoon cuniculi*. K. GHOSH,^{a,b} C. CAPPIELLO,^a A. CALI,^b P. M. TAKVORIAN^b and L. M. WEISS^a, ^aAlbert Einstein College of Medicine, Bronx, New York, ^bRutgers University, Newark, New Jersey.

The microsporidia have attracted much attention recently as a result of their role as opportunistic pathogens in patients with immune suppression, such as those with HIV/AIDS. The infectious stage of these organisms is the spore. Spores possess a unique organelle, the polar filament, which lies coiled inside their cytoplasm. Upon appropriate stimuli, which lead to an increase in intrasporal osmotic pressure, the polar filament everts, forming an extruded extracellular tube. The infectious process is complete when the cytoplasmic contents are forced through this tube and into a nearby host cell. Because this extrusion process is almost certainly dependent on the rapid influx of water into spores, it has

been posited that aquaporins (AQPs), transmembrane channels that facilitate osmosis, are present in these organisms. To test this assumption, an AQP-like sequence of the microsporidium *Encephalitozoon cuciculi* (*EcAQP*) was cloned. *EcAQP* is within the expected size range and also possesses the canonical NPA motifs of an aquaporin. *EcAQP* exhibits sequence identity to AQP A of *Dictyostelium discoideum* (26%) and human AQP 2 (24%). In a standard functional assay, *Xenopus laevis* oocytes that had been microinjected with *EcAQP* mRNA were subjected to hypoosmotic stress and their swelling monitored by quantitative videomicroscopy. *EcAQP*-injected oocytes swelled significantly more than water-injected controls, indicating an increase in permeability of the oocyte plasma membrane. No permeability to the solutes glycerol or urea was observed. An *EcAQP*-GST fusion protein antiserum immunohistologically stains the oocyte and parasite plasma membrane. Further study of AQPs in microsporidia and their potential inhibitors may yield novel therapeutic agents for microsporidian infections.

115A

A web-based resource for studies on the microsporidia. C. GIRE,^a R. SCHEUERMANN^b and L. M. WEISS^c, ^aNorthrop Grumman IT Health Solutions, Rockville, Maryland, ^bUniversity of Texas, Southwestern Medical Center, Dallas, Texas, ^cAlbert Einstein College of Medicine, Bronx, New York.

The microsporidia have attracted much attention recently as a result of their role as opportunistic pathogens in patients with immune suppression, such as those with HIV/AIDS. The genome of the *Encephalitozoon cuciculi* was completed in 2001 (Katinka et al. *Nature*, 414:450–453, 2001). Additional partial genome data (genome sequence surveys) have been generated by different investigators for *Spragueia lophii*, *Antonosporea locustae* (*Nosema locustae*), *Encephalitozoon hellem*, *Nosema bombycis*, *Bracheola algerae* and *Enterocytozoon bienersi*. The National Institutes of Health has supported the development of Bioinformatics Resource Centers to create web-based resources for pathogenic organisms. A microsporidia web resource has been created at <http://www.biohealthbase.org>. The tools for the analysis of these organisms will be discussed and the functionality of the database will be discussed. The input of researchers involved with the Microsporidia will be critical in determining future directions for this web resource including issues such as taxonomy, community annotation and additional analytic tools.

116A

Placement of diverse amoebae on the eukaryotic tree of life. J. GRANT,^a E. BARBERO,^a J. COLE,^b E. JOHNSON,^c J. M. LOGSDON,^c D. J. PATTERSON^d and L. A. KATZ^a, ^aSmith College, Northampton, Massachusetts, ^bAmerican Type Culture Collection, Manassas, Virginia, ^cUniversity Iowa, Iowa City, Iowa, ^dMarine Biological Laboratories, Woods Hole, Massachusetts.

Amoebae are a polyphyletic group of eukaryotic microbes. The phylogenetic position of many amoebae is unknown, in part due to the few comparable morphological characters and limited molecular data for many groups. As part of an ongoing project "Assembling the Eukaryotic Tree of Life" we have sequenced the SSU-rDNA as well as three protein coding genes of several amoebae of *Incertae sedis*. For each taxon, we are assessing the number of paralogs for each gene and have uncovered unusually high diversity of actins within some lineages. We are constructing a multi-gene phylogeny of these taxa using a variety of evolutionary models. Our initial analyses show that some of these organisms do not fall consistently into any of the proposed

supergroups. Expanded taxon sampling is essential for constructing a robust eukaryotic tree of life.

117A

Is genetic variability of the alveolate parasite *Hematodinium* host-specific?. K. HAMILTON,^a D. MORRITT^a and P. SHAW^a, ^aSchool of Biological Sciences, Royal Holloway, University of London, London, United Kingdom.

Members of the genus *Hematodinium* (Alveolata: Syndiniales) are marine parasites that have been identified in a wide range of crustacean hosts. The species status and host-specificity of these pathogens have not been resolved, as the distinction between morphospecies is ambiguous and pathogenicity differs markedly between hosts. Furthermore, not all in vivo life-cycle stages have been identified and transmission routes need to be elucidated. We used molecular probes to detect ribosomal RNA genes of *Hematodinium*-like alveolates in the tissues of several crustacean species from the North-East Atlantic. The parasites' partial 18S, complete ITS1 and partial 5.8S DNA regions were sequenced, and ribotypes between and within host species were compared. We will discuss pathogenicity, seasonality and prevalence of *Hematodinium* infections in comparison to the parasites' rRNA types, and their phylogenetic position within protists.

118A

Detection and genotyping of *Enterocytozoon bienersi* in park pigeons. M. HARO,^a N. HENRIQUES-GIL,^a F. IZQUIERDO,^a F. ALONSO,^b S. FENOY^a and C. DEL ÁGUILA^a, ^aFacultad de Farmacia, Universidad San Pablo-CEU, 28668 Madrid, Spain, ^bFacultad de Veterinaria, Universidad de Murcia, Spain.

Microsporidia are obligate intracellular parasites that infect all animal phyla. The zoonotic origin of the human microsporidiosis is far from clear and, most certainly, the picture varies from one microsporidium species to another. In a previous study we identified several species of microsporidia including *Enterocytozoon bienersi* in public park pigeons (*Columba livia*) in Spain. We have now analyzed the ITS variability of *E. bienersi* isolates, and after sequencing, seven new genotypes have been detected from pigeon isolates. However, about 100 different ITS sequences have now been described by several authors, for *E. bienersi* isolates from wild animals, domestic animals (cattle, pigs and dogs) and humans. The sequences from the seven new genotypes revealed 21 polymorphic sites but compared with the whole ITS variability, they are quite similar. As *E. bienersi* is not a frequent bird parasite, these results suggest that *E. bienersi* spores could have been transmitted to pigeons from another reservoir host in a relatively recent past time. The observed variability could have been acquired while spreading within this new host. The strong polymorphism obtained for the ITS region suggests that other genome regions may also be variable, and with a high diversity of *E. bienersi* strains.

119A

Trichomonas vaginalis and *Giardia intestinalis* produce nitric oxide. K. M. HARRIS,^{a,b,c} B. GOLDBERG,^{a,b,c} G. BIAGINI^{a,b,c}, and D. LLOYD^{a,b,c}, ^aMicrobiology, Cardiff University, Park Place, Cardiff CF10 3TL, Wales, United Kingdom, ^bDepartment of Chemistry, New York University, Silver Building, New York, New York 10003, USA, ^cLiverpool School of Tropical Medicine, Liverpool, United Kingdom.

The relationship of nitric oxide (NO) to arginine metabolism for the production of ATP has been studied in the human patho-

gens *Trichomonas vaginalis* and *Giardia intestinalis*. A NO electrode indicated NO production by these parasites, which was stimulated by the addition of arginine and inhibited by NO-synthase (NOS) inhibitors in both organisms. Hydrogenosomal-enriched fractions from *T. vaginalis* were also found to produce NO, suggesting a discrete location for NO production in these organelles. NO-synthase activity was found in homogenates, and localized in subcellular fractions of both organisms. Confocal microscopy of *T. vaginalis* stained with DAF-FM, a NO-specific probe, showed a localization of NO production in the hydrogenosomes of *T. vaginalis*, providing further evidence for the presence of the NOS in the hydrogenosome. The production of NO increased markedly when *T. vaginalis* was exposed to starvation conditions, suggesting a correlation between the glycogen phosphorylase pathway and nitric oxide production by the parasite. Although heme proteins have not been found in these microaerophiles, and NOS is functionally linked with the heme protein guanylyl cyclase in higher eukaryotes, another mechanism may take on the role usually performed by guanylyl cyclases under aerobic conditions. The presence of NOS in hydrogenosomes of *T. vaginalis*, as well as the production of NO in both organisms, provides further evidence for the hypothesis that both may have been recent derivatives of aerobic lineages, and should thus be allocated to crown taxa.

120A

Effects of polyamine depletion by difluoromethylornithine on hydrogenosomes in *Trichomonas vaginalis*. K. M. HARRIS,^{a,b} B. GOLDBERG,^b A. HAYES,^a A. HANN,^a M. P. TURNER,^a R. E. LEWIS,^a K. M. LEMAR^a and D. LLOYD^a, ^aMicrobiology (Bios1), Main Building, Cardiff University, Wales, United Kingdom, ^bDepartment of Chemistry, New York University, New York, New York, USA.

The correlation between polyamine metabolism and the hydrogenosomes in *Trichomonas vaginalis* was examined using four distinct methods. When examined by confocal microscopy, *T. vaginalis* after exposure overnight to 5 mM difluoromethylornithine (DFMO) was stained with 5 nM Rhodamine123, and morphological clarity of the hydrogenosomes was lost due to a decrease in membrane potential. Difluoromethylornithine is an irreversible competitive inhibitor of ornithine decarboxylase (ODC). When examining hydrogenosome samples prepared for transmission electron microscopy, those from cells grown overnight in 5 mM DFMO had more congregations of electron-dense inclusions inside the hydrogenosomes. Utilizing flow cytometry, a decrease in fluorescence of polyamine-depleted cultures stained with 0.5 nM Dio-C₅(3) or 0.5 nM tetramethylrhodamine ethyl ester was observed in whole cells as well as in isolated hydrogenosomes. Thus, as fluorophore uptake correlates with the transmembrane electrochemical potentials of the organism, the hydrogenosomal membrane potentials decreased after arresting polyamine synthesis. Upon exposing these polyamine-depleted cells to 5 mM spermine or 5 mM spermidine for 7 h before using the same flow cytometry protocol, the fluorescence (and therefore hydrogenosomal membrane potential) recovered. The correlation of polyamine metabolism with the hydrogenosome was further confirmed by monitoring the amount O₂ consumed by both control and polyamine-depleted isolated hydrogenosomes. The rates of O₂ consumption by polyamine-depleted hydrogenosomes were approximately two times greater than the rates of consumption by those from control organisms. We conclude that the depletion of polyamines using DFMO disturbs hydrogenosomal structure and function. The presence of polyamines is essential in *T. vaginalis* in

order to maintain the normal, fully functional hydrogenosome, as well as a normal plasma membrane and hydrogenosomal membrane potential.

121A

Functional gene analysis of *Pneumocystis carinii* by complementation of yeast genes. P. M. HAUSER,^a L. CERUTTI^b and V. SIMANIS^c, ^aInstitute of Microbiology, University Hospital of Lausanne, Lausanne, Switzerland, ^bSwiss Institute of Bioinformatics, Lausanne, Switzerland, ^cSwiss Institute for Experimental Cancer Research, Epalinges, Switzerland.

In the absence of culture and genetic systems for *Pneumocystis carinii*, we thought to carry out functional gene analysis of *P. carinii* by complementation of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* deletion mutants. Out of the 1,790 *P. carinii* expressed genes identified by the “*Pneumocystis* Genome Project”, about 1,000 have good homologs in the databases from which the function of *P. carinii* genes may be inferred. Approximately 90 of these 1,000 have a *S. pombe* homolog with unknown function. As a first phase, we are focusing on a subset of these 90 genes, which have been selected in order to increase the probability for identification of new useful drug targets by the absence of homologous genes in vertebrates. Fifteen *S. pombe* genes will be deleted, the resultant phenotype determined, and the mutants complemented with the *P. carinii* homologous genes to assess function. In a complementary approach, the function of about 50 *P. carinii* expressed genes might be assessed by complementation of their *S. cerevisiae* homologs, which function is known and for which a deletion mutant is available. We are first investigating a subset of these 50 genes consisting of 14 genes, which *S. cerevisiae* homologs lead to death (essential genes) or to a severe growth defect upon disruption. Preliminary results will be presented.

122A

Investigation into the mechanism of IGTP-dependent IFN γ inhibition of *Toxoplasma gondii* in astrocytes as revealed by transcriptional analysis. S. HOGAN, T. WOODS, K. MCINERNEY and S. K. HALONEN, Department of Microbiology, Montana State University, Bozeman, Montana.

Toxoplasma gondii is a major pathogen in the central nervous system in AIDS patients that results from reactivation of a latent infection in the brain. IFN γ is the major cytokine controlling the chronic infection in the brain. Our previous studies established that IFN γ induces inhibition of *T. gondii* in astrocytes via a novel mechanism involving IGTP. The mechanism of this novel IFN γ -induced IGTP-dependent inhibition of *T. gondii* is not understood. The IFN γ results in a loss of parasitophorous vacuoles by 2 h after invasion in wildtype astrocytes but not Δ IGTP astrocytes. IGTP localizes to the vacuole upon invasion and associates with the parasitophorous vacuolar membrane during the first 4 h after invasion. The loss of parasitophorous vacuoles during the first 4 h after invasion was due in part to disrupted vacuoles in which cholesterol containing membranous whorls were found to emanate from the parasitophorous vacuole. Approximately 20–30% of the vacuoles were also found to fuse with the lysosomes during the first 4 h after invasion. In this study the mechanism of this IFN γ -induced IGTP-dependent inhibition of *T. gondii* in astrocytes was further examined via microarray analysis of IFN γ response genes in wildtype versus astrocytes deficient in IGTP (Δ IGTP astro-

cytes). Transcriptional analysis of IFN γ response genes in wild-type and Δ IGTP astrocytes identified over 2,000 genes that were differentially regulated. Genes involved in cholesterol and lipid metabolism and vesicular trafficking pathways were identified that were differentially regulated in IFN γ stimulated wildtype versus Δ IGTP astrocytes. This data is supportive of the hypotheses that effects on cholesterol metabolism and/or vesicular trafficking pathways to the parasitophorous vacuole may be the mechanism of IFN γ induced IGTP-dependent mechanism of inhibition in astrocytes.

123A

Intestinal expression of H⁺ V-ATPase in the mosquito *Aedes albopictus* is tightly associated with gregarine infection. C. G. HUANG,^a K. H. TSAI,^a W. J. WU^a and W. J. CHEN^b, ^aDepartment of Entomology, National Taiwan University, Taipei 106, Taiwan, ^bDepartment of Public Health and Parasitology, Chang Gung University, Kwei-San, Tao-Yuan 333, Taiwan.

Vacuolar ATPase (V-ATPase) is a family of ATP-dependent proton pumps expressed on the plasma membrane and endomembranes of eukaryotic cells. Acidification of intracellular compartments such as lysosomes, endosomes, and parasitophorous vacuoles mediated by V-ATPase is essential for the entry by many enveloped viruses and invasion into or escape from host cells of intracellular parasites. In mosquito larvae, V-ATPase plays a role in regulating alkalization of the anterior midgut. We cloned and expressed V-ATPase subunit-A protein with the full-length sequence of mRNA extracted from larval tissues of *Aedes albopictus*, which contains a poly-A tail and 2971 nucleotides. The fusion protein was then used to produce polyclonal antibodies in the rabbit; which were used as a tool to detect V-ATPase in the midgut and Malpighian tubules of mosquito larvae. A parasitophorous vacuole confining intracellular trophozoite(s) was formed in response to invasion by *Ascogregarina taiwanensis*; within the vacuole acidification was demonstrated using acridine orange staining. It is concluded that gregarine sporozoites are released by ingested oocysts in the V-ATPase-energized high-pH environment. The released sporozoites then invade and develop in epithelial cells of the posterior midgut. Acidification of the parasitophorous vacuoles may be mediated by V-ATPase and may facilitate exocytosis of the vacuole confining the trophozoites from the infected epithelial cells for further extracellular development.

124A

Pneumocystis dihydropteroate synthase (DHPS) mutations and HIV-associated *Pneumocystis* pneumonia (PcP). L. HUANG,^a D. WELSH,^b R. F. MILLER,^c C. B. BEARD,^d G. G. LAWRENCE,^d M. FOX,^d A. SWARTZMAN,^a M. BENSLEY,^a D. CARBONNET,^b J. L. DAVIS,^a A. CHI^a and J. L. JONES^d, ^aUniversity of California, San Francisco, California, USA, ^bLouisiana State University, Baton Rouge, Louisiana, USA, ^cUniversity College London, London, United Kingdom, ^dCenters for Disease Control and Prevention (CDC), Atlanta, Georgia, USA.

To examine factors predicting DHPS genotype and mortality among HIV-infected PcP patients. Prospective study at three medical centers: SFGH (May 1997–December 2004), Medical Center of Louisiana (June 2003–December 2004), and University College London Hospitals (May 1997–November 2001). DHPS genotyping was performed at CDC. Wildtype DHPS=Thr55/Pro57; mutant=amino acid substitution at one or both positions

(includes mixed). Two hundred and sixty-eight (72%) specimens contained mutant and 104 (28%) contained wild-type DHPS genotype. San Francisco patients were significantly more likely to have mutant DHPS genotype (79%) compared to patients from New Orleans (51%) or London (47%) ($p < 0.0001$). Patients receiving trimethoprim-sulfamethoxazole (TMP-SMX) or dapsone prophylaxis were also more likely to have a mutant genotype (90%) compared with patients on no prophylaxis (66%) ($p < 0.001$). San Francisco residence (OR=3.18, 95% CI=1.48–6.82, $p=0.003$) and TMP-SMX/dapsone prophylaxis use (OR=3.68, 95% CI=1.74–7.77, $p=0.001$) were both independent predictors of a mutant genotype. The majority of patients were prescribed TMP-SMX for PcP treatment. Thirty-six (10%) patients died within 6 wk of PcP diagnosis. In 29 patients, PcP was the primary cause and was the only cause in 20. There was a non-significant tendency for patients with mutant genotypes to have increased mortality (OR=1.74). San Francisco residence and use of TMP-SMX/dapsone prophylaxis were significant predictors of a mutant DHPS genotype. Although patients with mutant DHPS genotypes tended to have worse outcomes, the majority responded to TMP-SMX treatment and survived. The presence of a mutant DHPS genotype may be one of multiple factors impacting mortality in HIV-associated PcP. Larger studies are needed.

125A

The problems of developing molecular diagnostic tests for opportunistic pathogens: the example of *Pneumocystis jirovecii*. J. HUGGETT,^a R. F. MILLER,^b M. TAYLOR,^c A. COSTELLO^d and A. ZUMLA^a, ^aCentre for Infectious Diseases and International Health, University College London, London, United Kingdom, ^bCentre for Sexual Health and HIV Research, University College London, London, United Kingdom, ^cWellcome Trust Centre for Human Genetics, Oxford, United Kingdom, ^dInternational Perinatal Unit, Institute of Child Health, University College London, London, United Kingdom.

To develop a strategy for the design and optimisation of specific and robust molecular detection assays for diagnosis of pathogens on which there is limited genetic information. For our model we used the pathogen *Pneumocystis jirovecii*, the causative agent of *Pneumocystis* pneumonia (PcP), and report a flexible strategy that utilizes detailed phylogenetic and comparative genomic analysis to identify a suitable genetic target for diagnosis. We employed stringent assay design criteria and provide the optimum assay characteristics by real time PCR. Following sequence assessment of the related species *P. carinii* we identified a region of the heat-shock protein 70 (HSP70) gene likely to have species specificity suitable for our purpose. As the corresponding sequence was not available for *P. jirovecii* we amplified and sequenced this region. We then designed a 108 bp real-time PCR assay specific for *P. jirovecii*. This assay did not detect *P. carinii* or *P. murina* but was capable of detecting *P. jirovecii* template to a sensitivity of < 10 copies per reaction and had a reproducible reaction efficiency of $> 95\%$. Conventional and real-time PCR diagnostic procedures for diseases like PcP have generally not found favour in clinical diagnostic laboratories. There are many reasons for this but poor assay design and laboratory procedures are often key factors. This is confounded by the fact that PCR is seen as a single entity (i.e. poor efficacy of a particular assay is considered poor efficacy of PCR) and the stages required to get a result (sampling, extraction and detection) are rarely studied individually. We approach this problem by starting at the last stage of detection by PCR and use *in silico* methods to assist the assay design and suggest some basic factors that should be reported when optimising such an assay.

126A

Virulence shift in a sexual clade of wild *Toxoplasma gondii* infecting marine mammals. E. R. JAMES,^a M. A. MILLER,^b P. A. CONRAD,^c P. KEELING^d and M. E. GRIGG^a, ^aDepartment of Medicine, University of British Columbia, Vancouver, BC, Canada, ^bCalifornia Department of Fish and Game, Santa Cruz, California, USA, ^cSchool of Veterinary Medicine, University of California, Davis, California USA, ^dDepartment of Botany, University of British Columbia, Vancouver, BC, Canada.

Toxoplasma gondii-associated meningoencephalitis is a significant disease of California sea otters (*Enhydra lutris nereis*) and other marine mammals. *Toxoplasma* isolates have been obtained from a Harbor Seal, a California Sea Lion, and 52 California otters since 1998. Based on multi-locus PCR-RFLP and DNA sequencing at polymorphic genes (*B1*, *BSR4*, *SAG1*, and *GRA6*), two distinct lineages have so far been identified: Type II and a new type, called Type X, that possessed distinct alleles from archetypal strains at all four polymorphic loci sequenced. The majority (72%) of marine mammal *Toxoplasma* infections were of Type X, with the remainder being infected with Type II strains. No Type I or Type III genotypes were identified. Type X strains have also been identified infecting a variety of terrestrial animals in the US, including humans. Phylogenetic analyses separated the Type X *Toxoplasma* isolates from Types I, II and III. When assayed through mice, a number of Type X strains possessed differing degrees of virulence. The genetic basis for the altered virulence patterns among Type X strains is currently being assessed and will be presented.

127A

Characterization of the *Pneumocystis carinii* lanosterol synthase (*PcErg7*) gene and protein in yeast. T. JOFFRION, M. S. COLLINS, M. T. CUSHION, University of Cincinnati College of Medicine and the Cincinnati Veterans Administration Medical Center, Cincinnati, Ohio.

Pneumocystis carinii is a unique member of the fungal kingdom due to the apparent absence of ergosterol in its membranes. However, analysis of ESTs and gene sequences show that it encodes gene homologs in sterol biosynthesis and related pathways. Some of these appear to be functional as shown by inhibitor studies, transcriptional analysis and heterologous expression. One such enzyme, lanosterol synthase, (*Erg7*) was chosen as the focus of the present studies because it is responsible for the production of the first sterol intermediate in the sterol biosynthetic pathway and may offer promise as a drug target. The *PcErg7* was obtained as a cDNA clone and verified to be full length by sequence analysis. A *PcErg7* PCR probe hybridized to a chromosome of approximately 620 kb in eight karyotype forms of *P. carinii* on a CHEF Southern blot, corresponding to the third largest chromosome. A band of 550 kb was detected in *P. wakefieldiae*. To confirm function, *PcErg7* cDNA was ligated into the galactose inducible vector pYES2.1 and expressed in a haploid *Erg7* mutant of *Saccharomyces cerevisiae*. Western blotting showed the *PcErg7* to have a MW of 83 kDa, similar to the size predicted by the cDNA sequence. Yeast expressing the *PcErg7p* had a slightly decreased growth rate as compared with yeast expressing the *ScErg7p* from the same vector. Fluorescent antibodies to the V5 tag and fluorescent probes to lipid particles are being used to evaluate whether it localizes to lipid particles in yeast as does the wild type protein or whether it is mis-localized as reported by Milla et al. (2002). Antibodies will be generated to the *PcErg7p* to permit localization in *P. carinii*. Our studies show that the *P. carinii Erg7* gene produces a functional protein in *S. cerevisiae*, suggesting that it may serve a similar function in *P. carinii*.

128A

A new isolate of mammalian microsporidia and experimental infection of *Spodoptera littoralis* (Lepidoptera: Noctuidae). M. KAMLER,^a B. KOUDELA,^{a,b} D. MODRÝ^{a,b} and J. VÁVRA^{b,c}, ^aDepartment of Parasitology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, ^bInstitute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, ^cDepartment of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic.

Microsporidia are ubiquitous parasites almost all phyla of animal kingdom. They are important pathogens of man, particularly of immunocompromised patients. However, routes of transmission and modes of infection are not still clear. In some species of microsporidia, i. e. *Anncaliia (Brachiola) algerae* and *Trachipleistophora hominis*, possibility of vector-mediated transmission of microsporidiosis to man exists. We found a new isolate of *Trachipleistophora* in Madagascan endemic insectivore *Hemicentetes semispinosus*. Attempt to infect caterpillars of *Spodoptera littoralis* was successful. In infected caterpillars, infection was first visible in muscles of intestinal tissue and then developed into generalized diseases. The experiment suggests insect origin of this new mammal-derived tissue microsporidium. Moreover, our finding probably represents first record of *Trachipleistophora* in wild-originated mammal. Modes of transmission and consequences for epidemiology of similar infections are discussed.

129A

Recombinant *Pneumocystis carinii* S-adenosylmethionine:sterol Δ^{24} methyltransferase (SAM:SMT) expressed in heterologous systems. E. S. KANESHIRO, S. M. HUNT and S. W. NKININ, Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio.

The addition of methyl groups at the C-24 position of the sterol side chain is catalyzed by the enzyme S-adenosylmethionine:sterol Δ^{24} methyltransferase (SAM:SMT) which is coded by the *erg6* gene. Our group previously reported on the *Pneumocystis carinii* SAM:SMT expressed in *Escherichia coli*. The recombinant enzyme is capable of transferring one or two methyl groups to produce 24-methylene or 24-ethylidene alkylsterols. More recently, we found that the level of expression in the bacterial system was not consistent from culture to culture, probably due to variable levels of expressed protein stored in insoluble inclusion bodies. The *P. carinii erg6* gene was also inserted into the *erg6* null mutant of *Saccharomyces cerevisiae* and we confirmed that the mRNA was expressed by using RT-PCR. Transformed yeast whole cell homogenates, and the microsomal and soluble fractions were assayed for the enzyme activity using radiolabeled SAM and lanosterol as substrate. Again, enzyme activity levels varied from experiment to experiment and enzyme activity was generally far lower than expected. Protein analysis did not reveal a major band of expected size and intensity (43.2 kDa). Recently, *Tetrahymena thermophila* has been developed as a system to express recombinant proteins. The genome of this ciliate has been sequenced and, like *Pneumocystis*, its DNA is AT-rich. It may be that codon usage between *Pneumocystis* is closer to that of *Tetrahymena* than *Saccharomyces*. Furthermore, *Tetrahymena* lacks the *erg6* gene homolog. The ciliate synthesizes the pentacyclic triterpenoid alcohol tetrahymanol by cyclization of squalene and the ciliate does not contain sterols. These features are particularly attractive for analyzing the *P. carinii* recombinant SAM:SMT enzyme activity, therefore we are in the process of producing recombinant *T. thermophila* expressing the *P. carinii erg6* gene.

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Pneumocystis carinii PRT1/MSR/MSG gene clusters assembled from shotgun sequences. S. P. KEELY,^a B. SLAVEN,^b M. T. CUSHION,^{b,c} A. G. SMULIAN^{b,c}, and J. R. STRINGER^a, ^aDepartment of Molecular Genetics, Cincinnati, Ohio, ^bDepartment of Internal Medicine, University of Cincinnati, Cincinnati, Ohio, ^cDepartment of Cincinnati VA Medical Center, Cincinnati, Ohio.

Nearly 10% of the genome of *Pneumocystis carinii* is composed of members of three gene families, PRT1, MSR and MSG, which are clustered together at the ends of each of the 17 or so chromosomes. Complete sequences of six PRT1/MSR/MSG gene clusters were previously obtained from large segments (20–35 kb) of the genome cloned into cosmid vectors. However, the cosmid library did not contain most of the 34 expected telomeric gene clusters. By contrast, sequences from short segments (0.5–2 kb) of the *P. carinii* genome cloned into plasmids (shotgun library) were found to come from at least 80 MSG genes, 40 MSR genes and 30 PRT1 genes. Close to 10,000 sequence reads from the shotgun library contained a member of one or more of the three gene families. These reads were assembled using Arachne and PHRAP into 100 contigs, which is three times more than would be expected as gene arrays are restricted to the 34 or so chromosome ends. Analysis of these contigs will determine the accuracy of the assembly, which is potentially complicated by the repeated nature of the sequences assembled. We also amplified the first 350 basepairs from genomic MSG genes and sequenced 300 cloned copies of the amplified DNA. These studies produced more than 100 different sequences, but some were less than 1% different from others, suggesting that the number of different sequences might exceed the gene copy number due to allelic variation. This issue was addressed by using quantitative PCR and DNA hybridization, which indicated that there are between 80 and 90 MSG genes in the genome of *P. carinii*. Analysis of the expanded database of MSG sequences showed that the first 350 bp of MSG gene family members is organized into four variable regions separated by highly conserved regions.

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Multiple epitopes can be formed by limited amino acid variations in the major surface glycoprotein of *Pneumocystis jirovecii*. J. V. KOCH, K. DALY and P. D. WALZER, VAMC, University of Cincinnati, Cincinnati, Ohio.

We have identified two clones of the carboxyl terminal of the major surface glycoprotein (MsgC) of *Pneumocystis jirovecii* that differ from one another in amino acid sequence and in immune reactivity. These clones, MsgC3 and MsgC9, differ by only three amino acids, two of which reside in a 10 amino acid stretch at the amino terminal. Serum antibodies could differentiate between these amino terminal fragments, indicating that the amino acid polymorphisms are included in antigenic epitopes. In this work, we have demonstrated immune recognition of these fragments (MsgC3A and MsgC9A) by ELISA, and have used molecular genetic techniques to probe the contribution of each polymorphism to the epitopes recognized by serum antibodies. We used site-directed mutagenesis to change individual polymorphic amino acids creating MsgC3A/C9A chimeric molecules (93-1 and 93-2), and have analyzed the effect of the mutations on antibody recognition in sera that react with the parental MsgC3A and MsgC9A molecules. MsgC3A and MsgC9A are minor epitopes in terms of frequency and strength of recognition. Competition ELISA shows that they contain shared epitopes as well as unique epitopes, depending on the serum tested. The spectrum of epitopes expressed on MsgC3A was complex. Five different epitopes could

be identified: one epitope included the first polymorphic amino acid but not the second; another epitope had the opposite phenotype; one epitope was affected by both substitutions; a fourth epitope was not affected by either mutation but was not shared with MsgC9A; and a fifth epitope was shared with MsgC9A and is either outside the polymorphic region or not affected by either substitution. Only two epitopes were identified on MsgC9A. That such complexity could be generated in short fragments of Msg that differed by only two amino acids suggests that the potential for antigenic variation in *Pneumocystis* may be immense.

132A

Prevalence and incidence of toxoplasmosis in HIV-positive patients in the Czech Republic. P. KODYM,^a Š. HRDÁ,^a L. MACHALA,^b H. ROZSYPAL,^b M. STANKOVA^b and M. MALÝ^a, ^aNational Institute of Public Health, Prague, Czech Republic, ^bAIDS Centre, Bulovka Teaching Hospital, Prague, Czech Republic.

The anti-*Toxoplasma* antibody levels were followed up in more than 70% of all Czech HIV positive patients since 1988. The tests determined 42.4% prevalence of toxoplasmosis in men (N=467) and 39.5% in women (N=109). In contrast, in the general Czech population, even when stratified by age groups, toxoplasmosis is significantly less prevalent and the prevalence in men is lower than in women. If comparing the anti-*Toxoplasma* positivity in groups by year, when the patient was diagnosed as HIV-positive, no trend-indicating decline of prevalence in the course of the last 16 yr appeared. The following up of 335 persons, originally included as anti-*Toxoplasma* negative, (in total 530,312 person-days) revealed four cases of seroconversion. From this data, an incidence of 0.00275 per person per year was calculated, which is in accordance with the published risk of *Toxoplasma* infection in pregnant Czech women. In this study, the higher age (34–65 yr) of all HIV⁺ patients at seroconversion, as recorded after a 3–9 yr follow-up, was surprising, because in the general population it is typical to acquire toxoplasmosis in one's youth. As usual in HIV⁺ patients, anti-*Toxoplasma* antibody response was weak even in cases of recent infection. Clinical consequences and implications will be the subject of further studies.

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Subcellular centrin localization within distinct compartments of *Vorticella convallaria* contractile organelles. K. KONIOR, S. MCCUTCHEON and H. BUHSE, JR, Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois.

The stalked ciliate, *Vorticella convallaria*, is a good model system to study mechanochemical motility because its contractile organelles (spasmoneme and myonemes) use a mode of contraction that differs from most other eukaryotic motile systems. *Vorticella's* contractile cytoskeleton consists of a longitudinal lattice of filaments in the cell body (myonemes) that are bundled together to form the single contractile organelle (spasmoneme) of the stalk. Calcium triggers the cytoskeletal contraction. Upon binding calcium, the spasmoneme coils to 20% of its extended length in a few milliseconds. As calcium triggers this contraction, we have undertaken the molecular characterization of the calcium-binding proteins associated with these organelles. We have identified a multi-gene family of calcium-binding proteins using a degenerate PCR-based cloning method to the conserved calcium-binding protein, centrin. Many organisms have evolved centrin multi-gene families with the prediction of an increased range of functions within these organisms. Therefore, the *V. convallaria*

centrin multi-gene family is an attractive system to ascertain the various function of each centrin in the cell. We have isolated and identified seven unique centrin-like cDNAs from *V. convallaria*. We predict each centrin has a distinct function within the cell. To define these functions, we have initiated immunolocalization studies at the light and the ultra-structural level utilizing various anti-centrin antibodies. Western analysis indicates that each antibody recognizes a distinct subset of proteins in *Vorticella*. Using these antibodies, we have localized centrin to various contractile cytoskeletal structures within the cell. Preliminary data indicates that certain centrin proteins are further restricted in their localization within these organelles. This analysis allows us to begin to dissect the function of this multi-gene family.

134A

Survival pathway signal transduction is reduced in alveolar macrophages during *Pneumocystis pneumonia*. M. E. LASBURY, P. J. DURANT, D. TSCHANG and C.-H. LEE, Indiana University School of Medicine, Indianapolis, Indiana.

Our previous results indicate that alveolar macrophages undergo apoptosis during *Pneumocystis pneumonia* (PcP). Resistance to apoptotic stimuli is mediated in part by activation of the Akt survival pathway. Akt-1 is activated by phosphorylation, and phosphorylated Akt-1 promotes survival by inhibiting caspase-9 activation, forkhead transcription factor activity, and glycogen synthase kinase-3 β (GSK-3 β) activation; it also increases the activity of the mitochondria-protecting molecules Bcl-2 and Bcl-xl. PI-3K activation by phosphorylation is an upstream requirement for Akt-1 phosphorylation by the activated PI-3K. Our results indicate that PI-3K expression in alveolar macrophages was slightly increased during PcP, but activation of PI-3K was reduced by more than 30%. Calmodulin is required for recruitment of Akt-1 to PI-3K; immunoblot results indicate that alveolar macrophage calmodulin protein levels were significantly reduced during PcP. DNA microarray results indicated that the transcription of Akt-1 was reduced by more than twofold in alveolar macrophages during PcP. Enzyme linked immuno sorbent assay results revealed a reduction in total Akt-1 protein and a large decrease in activation of Akt-1 in alveolar macrophages of infected hosts. Levels of unphosphorylated (active) GSK-3 β , cleaved (active) caspase-9, and mitochondrial damage were increased in alveolar macrophages from PcP hosts. Together, these results indicate that activation of the Akt survival pathway is decreased in alveolar macrophages during PcP and one possible mechanism for *Pneumocystis*-induced apoptosis of these important immune cells is reduced cell resistance to apoptotic stimuli.

135A

Effect of gastrointestinal defenses on *Brachiola algerae* spore germination. G. J. LEITCH, Whitney Laboratory for Marine Bioscience, University of Florida, St. Augustine, Florida.

Brachiola algerae is a ubiquitous microsporidia species, known to parasitize insects. However, isolates of *B. algerae* infect mammalian cells at 37 °C and recently several clinical cases have been reported, but none involving the gastrointestinal tract as the major infection site. Cultures of human intestinal epithelial cells (Caco2) were readily infected with *B. algerae* or *Encephalitozoon intestinalis* and the resulting epithelial cell chemokine expression profiles were essentially the same with both parasite species. This led to a study to determine which innate gastrointestinal defense factor(s) could be responsible for preventing intestinal infection

by orally ingested *B. algerae* spores. Putative defense factors were tested for their ability to inhibit in vitro spore germination using an assay in which the germination stimulant was NaCl at a pH of 9.5. We tested pretreatment with HCl, with and without pepsin; pancreatic enzymes (pancreatin); lactoferrin, found in milk, saliva, pancreatic juice and neutrophils; lysozyme, found in saliva, neutrophils and Paneth cells; human α defensin 5, a product of Paneth cells; and human β defensin 2, an inducible product of epithelial cells throughout the gastrointestinal tract. Pancreatin and HCl, with or without pepsin, increased *B. algerae* spore germination. Lysozyme had no consistent effect. Lactoferrin and its peptide lactoferricin B were equally effective in inhibiting germination. These defensins inhibited germination in a salt sensitive manner, as reported for other defensin antimicrobial effects. In contrast to *B. algerae*, *E. intestinalis* spores germination, stimulated with H₂O₂, was not inhibited by lactoferrin or the defensins tested. These results suggest a role for a species-specific differential responsiveness to certain innate host defenses contributing to the apparent inability of *B. algerae* to infect the human gastrointestinal tract. Supported by R21 DK64573.

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Infectivity of microsporidian spores after exposure to temperature extremes and chemical disinfectants. X. LI^a and R. FAYER^b, ^aVeterinary Medical Teaching and Research Center, University of California-Davis, Tulare, California, ^bEnvironmental Microbial Safety Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.

Although *Encephalitozoon intestinalis*, *E. hellem*, and *E. cuniculi* infect humans and animals, reservoirs and routes of spread remain largely unknown. Detection of spores in surface, ditch, recreational, and drinking water and outbreaks (possibly water related) strongly suggest the importance of waterborne transmission. Rendering spores non-infective is extremely important for safety of water, food, and facility management but disinfection data are sparse. The present study investigated the effects of ethanol, chlorine, heating, and freezing on spore infectivity. After *E. intestinalis* and *E. cuniculi* spores were exposed to 70% ethanol for 5 min and *E. hellem* spores were exposed for 20 min spores were not infective for cultured MDBK cells. *Encephalitozoon hellem* spores exposed to laundry bleach diluted 1:50,000 for 10 min and *E. cuniculi*, spores similarly exposed for 30 min were not infective for cultured MDBK cells. To obtain 100% inhibition of *E. intestinalis*, spores were exposed to laundry bleach diluted 1:1,000 for 10 min. When *E. intestinalis* and *E. hellem* spores in water without cryoprotectants were held at -20 C for 24 h, and *E. cuniculi* spores were similarly held for 2 h, spores were not infective for cultured MDBK cells. Spores of *E. cuniculi* held at 100 C for 1 min failed to grow in cell culture but *E. intestinalis* and *E. hellem* spores had to be held for 5 and 10 min, respectively, to obtain 100% inhibition of growth in cultured cells. These findings provide basic data applicable to food, water, and environmental disinfection strategies.

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Inflammatory mononuclear cells are a source of polyamines in *Pneumocystis pneumonia*. C.-P. LIAO, M. E. LASBURY, C. ZHANG, S.-H. WANG, P. J. DURANT and C.-H. LEE, Indiana University School of Medicine, Indianapolis, Indiana.

Alveolar macrophages undergo apoptosis during *Pneumocystis pneumonia* (PcP). *Pneumocystis* organisms are known to release

polyamines, and polyamines may cause apoptosis in certain types of cells. To investigate the role of polyamines in apoptosis of alveolar macrophages during PcP, polyamine concentrations in bronchoalveolar lavage (BAL) fluids were determined. A 10-fold increase in the level of spermidine, acetylspermine, and acetyl-spermidine in BAL fluids from *Pneumocystis*-infected rats was observed. These BAL fluids also had the ability to induce apoptosis in alveolar macrophages from normal rats. Depletion of polyamines from these BAL fluids rendered them unable to induce apoptosis, and these polyamine-depleted BAL fluids regained their ability to induce apoptosis when polyamines were added back. Ornithine decarboxylase (ODC) is the rate-limiting enzyme in the polyamine biosynthesis pathway. To investigate whether various lung cells also produce polyamines during PcP, ODC expression in *Pneumocystis*-infected lung was determined by immunohistochemistry (IHC). Many ODC-positive cells were localized in perivascular and peribronchiolar areas in *Pneumocystis*-infected lung, but very few of these cells were seen in uninfected lung. Immunohistochemistry staining for spermidine/spermine confirmed that these ODC-positive cells produced polyamines. Staining of serial lung sections with cell-specific antibodies revealed that the majority of ODC-positive cells were monocytes or macrophages. Some ODC-positive cells were B-cells, and very few of them were T cells. Whether the polyamines produced by these cells contribute to apoptosis of alveolar macrophages during PcP is being investigated.

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Genotypes of *Enterocytozoon bieneusi* in mammals in Portugal. M. L. LOBO,^a L. XIAO,^b V. CAMA,^b F. ANTUNES^c and O. MATOS^a, ^aUnidade de Protozoários Oportunistas/VIH e outras Protozooses, UPM, Instituto de Higiene e Medicina Tropical, Lisboa, Portugal, ^bDivision of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA, ^cClinica Universitária de Doenças Infecciosas, Faculdade de Medicina (H.S.M.), Universidade de Lisboa, Portugal.

In the last few years, the use of molecular tools has provided convincing evidence suggesting that human infecting microsporidiosis due to *Enterocytozoon bieneusi* might have potential zoonotic origins. However, few studies have examined the genetic characteristics of *E. bieneusi* in companion animals, especially dogs and cats. The present work was undertaken to assess the zoonotic potential of *E. bieneusi* in mammals. *Enterocytozoon bieneusi* isolates in fecal specimens of several animals (dogs and cats from pet owners and shelters, calves from farms, and a marmoset from a Zoo) were genotyped using PCR-sequencing analysis of a fragment consisting of the partial SSU and LSU and the entire ITS region of the rRNA. The ITS-rRNA sequences obtained were aligned with each other and with ITS rRNA sequences from the GenBank database. Both host-adapted genotypes with narrow host ranges and zoonotic genotypes with broad host specificity were found. The former was represented by an *Enterocytozoon* sp. in a dog, one distinct *Enterocytozoon* genotype in the marmoset, two unique *Enterocytozoon* genotypes related to the WL4, WL5 and WL6 previously identified in muskrats, and two *E. bieneusi* bovine genotypes. The latter were represented by several genotypes in dogs, cats and cattle that are identical to or related to *E. bieneusi* that were previously found in humans. Most infected cats had *E. bieneusi* genotypes related to Peru2 and Peru3, which were previously found in HIV+ persons in Peru, indicating that cats may be a source of human infections.

139A

Cryptosporidium genotypes recovered from wastewater effluents entering a rural New York watershed. A. LUCIO-FORSTER and D. D. BOWMAN, Cornell University, Ithaca, New York.

Cryptosporidium oocysts pose a threat to our water resources through point and non-point sources of contamination. While livestock and wildlife contribute to the latter, wastewater treatment plants are frequently recognized as point sources of pollution. Prevalence of *Cryptosporidium* in non-point sources such as dairy farms and storm waters in upstate New York have been studied, however, there have been no studies to date determining the presence or genotypes of *Cryptosporidium* oocysts entering the Cayuga Lake watershed of upstate New York through wastewater effluents. In this study we report the detection and identification of five genotypes of *Cryptosporidium* recovered from wastewater effluents from six of the eight-wastewater treatment plants discharging into a rural watershed in NY. As all these municipal plants serve non-combined sewer systems (i.e. sewage and storm waters are not combined for treatment at the plants), we expected oocysts of the two species that are the cause of most human infections, namely *C. parvum* and *C. hominis*, to be the most prevalent. Oocysts recovered through continuous flow centrifugation (CFC) and immunomagnetic separation (IMS), were detected by polymerase chain reaction (PCR) amplification of the 18S SSU rRNA locus, and identified by restriction length polymorphism (RFLP) and sequencing, to determine phylogenetic relationships between the isolates and known *Cryptosporidium* species and water genotypes. The isolates identified grouped with three *Cryptosporidium* species, *C. andersoni*, *C. baileyi*, *C. parvum*, and two storm water genotypes, W4 (a cervid genotype) and W12 (an unknown wildlife genotype). Previous studies have determined that storm waters in New York State tend to be contaminated with animal, rather than human genotypes of this opportunistic protist, thus, our results seem to suggest that the oocysts present in our samples are likely reaching the treatment plants through storm water infiltration of the collection systems.

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The revised higher classification of eukaryotes: from the past to the present. D. H. LYNN, Department of Integrative Biology, University of Guelph, Guelph, ON, Canada.

The International Society of Protistologists recently published a higher classification of eukaryotes as the joint work of 28 scientists. This classification represents our current views on the relationships among eukaryotes, based fundamentally on significant discoveries made in the last quarter of the 20th century and the early years of this one. By this classification, our Society now formally recognizes the "protists" as pivotal organisms in the diversification of eukaryotes. Nevertheless, the path to this formal recognition has not been straight. In the late 19th century, Haeckel recognized the phylogenetic prominence of protists, but popular classifications, such as Bütschli's view of the protozoan protists, segregated the group along academic disciplinary lines. The development of a natural classification of eukaryotes involved the interplay of scientific concepts and technological advancements in the context of a developing sociological strengthening of protistology as a discipline. I will trace this development by commenting on these three aspects in four periods—1900–1950, 1951–1975, 1975–2000, and 2001–2006. I will conclude with a brief summary of the six major clades: (1) Amoebozoa; (2) Opisthokonta; (3) Excavata; (4) Rhizaria; (5) Archaeplastida; and (6) Chromalveolata.

141A

Marijuana exacerbates neuropathy and inhibits macrophage chemotaxis in mice infected with *Acanthamoeba culbertsoni*. F. MARCIANO-CABRAL, E. RABORN and G. A. CABRAL, Department of Microbiology and Immunology, Virginia Commonwealth University, School of Medicine, Richmond, Virginia.

The free-living amoeba, *Acanthamoeba culbertsoni* (*A. culbertsoni*), is the causative agent of Granulomatous Amebic Encephalitis (GAE), a disease occurring at increasing frequency in immune compromised individuals, including those with HIV infection and AIDS. Many of these also use marijuana illicitly for treatment of nausea, loss of appetite, or chronic pain. However, marijuana and its principal psychoactive component delta-9-tetrahydrocannabinol (THC) are immunosuppressive and modulate inflammatory responses. We have demonstrated, using a (B₆C₃)F₁ murine model of GAE, that treatment intraperitoneally with 50 mg/kg of THC followed by intranasal instillation of *A. culbertsoni* causes higher mortalities as compared to animals similarly infected and receiving vehicle. Histopathological analysis indicated that in THC-treated mice amoebae were present in the brain in the absence of granulomas whereas well-defined granulomas were identified in brains of similarly infected vehicle control mice. Thus, THC administration was associated with inhibition of accumulation of leukocytes and macrophage-like cells at focal sites of amoeba invasion. Using an in vitro migration assay, it was demonstrated that THC (10⁻⁶ to 10⁻¹² M) inhibited the chemotactic response of thioglycollate-elicited murine macrophages to *A. culbertsoni*. This inhibition was exerted comparably for whole cell amoeba extracts as well as for amoeba-conditioned medium. Comparable results were obtained when murine microglial-like cells and neonatal rat brain cortex microglia were used. Collectively, these data suggest that THC targets macrophage and macrophage-like cell chemotactic responsiveness to *A. culbertsoni*, articulating a mode by which this cannabinoid exacerbates invasiveness of the brain. Supported in part by: NIH/NIDA awards DA05832, DA15608, and DA05274.

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Discovery and "bar-coding" of new species of the trypanosomatidae by the analysis of spliced leader RNA gene sequences in environmental samples. D. A. MASLOV,^a X. XU,^a S. WESTENBERGER,^b V. A. YURCHENKO,^c J. LUKEŠ,^d N. R. STURM^b and D. A. CAMPBELL^b, ^aUniversity of California, Riverside, California, USA, ^bUniversity of California, Los Angeles, California, USA, ^cYeshiva University, Bronx, New York, USA, ^dInstitute of Parasitology, České Budějovice, Czech Republic.

The progress in investigations of diversity, biogeography and natural history of the Trypanosomatidae depends on the availability of tools and criteria for detection and recognition of species, new or previously described, in their environmental settings. Although hundreds of trypanosomatid species have been described earlier, identities of most of these organisms remain obscure, and many of them are expected to represent synonymous species. Sequence-based "bar-coding" of organisms directly from the environment represents a solution to this problem, especially because type cultures cannot be obtained in many cases. A PCR-based culture-independent approach was developed to investigate trypanosomatid diversity and ecology in insects of Costa Rica and Ecuador. Spliced leader (SL) RNA gene repeats were amplified from preserved extracts of the host's intestinal content. Conserved sequences in the exon-intron region served to relate the encountered trypanosomatids to previously known species, while highly variable intergenic region sequences were used to discriminate among closely related organisms. Cluster

analysis of the environmental SL sequences was used as an initial guide to new species discovery: separate sequence clusters often represented distinct species. This approach was validated by the analysis of morphology and phylogeny of several isolates for which axenic cultures could be obtained. Thus, in the Trypanosomatidae, SL RNA repeat sequences can be used for detection and identification of the organisms, as well as species vouchers.

143A

Molecular approaches to studying the genetic diversity and adaptation (ecology) of marine phytoplankton. L. K. MEDLIN, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven Germany.

The environment is experiencing rapid and accelerating changes, largely originating from human activity, whether coming from local impacts or from the more dispersed effects of global climate change. Only now are we developing the molecular tools required to adequately document microbial diversity on a routine basis and how it changes in space and time. Most cosmopolitan species when examined with molecular data reveal many cryptic species. A closer examination of morphological features within a molecular framework often identifies distinctive features that can be used to define the clades of the cryptic species and identify them in nature. When no feature can be found to define a clade, then a viable option to identify cryptic species is with molecular probes. Microarrays spotted with species-specific probes were developed to monitor biodiversity of organisms difficult to identify by traditional means (flagellated microalgae and picoplankton). The same probes when used in a biosensor format offer an early warning system for toxic algae. Enhanced genetic diversity below the species level defines the success or failure of a species to survive environmental change. Few studies of plankton have been attempted because of inherent logistic problems to sample and isolate enough cells for a true population study. Those studies that have been done show extremely high diversity within populations, often with reduced gene flow between close geographic areas. This contrasts with a belief that plankton populations have large population sizes. Studies of gene expression through the analysis of expressed sequence tags offer the easiest way to study how species are adapted to their environment. Expressed genes indicate how organisms respond to their environment. Examples of each aspect of molecular ecology are presented.

144A

Toxoplasma gondii and *Sarcocystis neurona* infections of pacific coastal sea otters in California, USA: evidence for land-sea transfer of biological pathogens. M. A. MILLER,^a M. E. GRIGG,^b W. A. MILLER,^c H. A. DABRITZ,^c E. R. JAMES,^b A. C. MELLI,^c A. E. PACKHAM,^c D. JESSUP^a and P. A. CONRAD^c, ^aDepartment of Fish and Game, Santa Cruz, California, USA, ^bUniversity of British Columbia, Vancouver, BC, Canada, ^cUniversity of California, Davis, California, USA.

Infection with the tissue cyst-forming coccidia *Toxoplasma gondii* and *Sarcocystis neurona* collectively are a major source of mortality for southern sea otters (*Enhydra lutris nereis*) in California. Sea otters do not prey on known intermediate hosts for *T. gondii* or *S. neurona*, so the most likely sources for infection are environmentally resistant oocysts and sporocysts shed in feces of cats and opossums, and transported via freshwater runoff into the marine ecosystem. Because sea otters occupy the nearshore environment and consume many of the same filter-feeding invertebrates as humans, they serve as ideal sentinels for land-sea transfer of biological pathogens. Long-term investigations of environmental patterns and pathophysiology of *T. gondii* and *S. neurona*

infection in otters have provided the following data supportive of terrestrial sources for these infections: (1) Otters sampled near large coastal freshwater outflows are nearly three times more likely to be infected with *T. gondii*, (2) Seasonal increases in *S. neurona*-associated mortality are associated with periods of peak runoff, (3) Based on necropsy, the prevalence of infection for both parasites is high and otters dually infected with *T. gondii* and *S. neurona* are common, and (4) There is little evidence to support transplacental infection as a primary means of parasite propagation within the sea otter population. Here we review and discuss current studies aimed at tracking potential land-sea connections by comparing *T. gondii* and *S. neurona* genotypes causing infection in marine and near-shore terrestrial animals.

145A

Seasonal variation in genotypes of *Pneumocystis jirovecii* isolated from HIV-infected patients undergoing diagnostic bronchoscopic alveolar lavage. R. F. MILLER, H. EVANS and J. CASSELL, Centre for Sexual Health and HIV Research, University College London, London, United Kingdom.

Seasonal variation in presentation of *Pneumocystis pneumonia* (PcP) has been described. To describe variation throughout the year of genotypes of *Pneumocystis jirovecii* in bronchoscopic alveolar lavage (BAL) fluid obtained from HIV-infected patients undergoing diagnostic bronchoscopy. One hundred and fifty-five isolates of *P. jirovecii* were obtained from 155 patients (154 adults) who underwent diagnostic bronchoscopy and BAL between 1989 and 2001. For each patient the month in which BAL was performed was recorded. Single round PCR, using primers pAZ102-H and pAZ102-E, was done in patients with PcP and nested PCR, (first round=as above) with second round primers pAZ102-X and pAZ102-Y, was done in those who had alternative diagnoses and who were colonized with *P. jirovecii*. After sequencing, polymorphisms at positions 85 and 248 in the mitochondrial large subunit rRNA (mt LSU rRNA) gene were used to distinguish genotypes of *P. jirovecii*. Sixty-one isolates of *P. jirovecii* were genotype 1, 40 were genotype 2, 30 were genotype 3, 8 were genotype 4 and 16 were 'mixed'—containing two genotypes (genotypes were as described by Beard et al. *Emerging Infect. Dis.* 2000; **6**: 265–72). There was monthly variation in frequency of detection of genotype 3, which was most frequently detected in January and September ($p=0.002$), but there was no significant association between month and genotypes 1 or 2 ($p=0.237$). After adjustment for month, the frequency of isolating genotype 2 was positively associated with maximum temperature ($p=0.013$) and with hours of sunlight ($p=0.04$), but not with rainfall. These data demonstrate that specific genotypes of *P. jirovecii* are more frequently detected at certain times of the year. Further, the data support the hypothesis that acquisition of *P. jirovecii* infection occurs by de novo airborne transmission.

146A

The quest for sensitive, specific, quantitative detection of fecal protozoa: *Cryptosporidium* in California mussels. W. A. MILLER,^a I. A. GARDNER,^a E. R. ATWILL,^a M. A. MILLER,^b M. HARRIS,^b J. AMES,^b D. JESSUP,^b A. MELLI,^a D. PARADIES,^c K. WORCESTER^d and P. A. CONRAD^a, ^aSchool of Veterinary Medicine, University of California, Davis, California, ^bMarine Wildlife Veterinary Care and Research Center, Santa Cruz, California, ^cBay Foundation of Morro Bay, Los Osos, California, ^dCentral Coast Regional Water Quality Control Board, San Luis Obispo, California.

In California, there is evidence of fecal pathogen pollution flowing from land to sea that affects marine mammal health.

While *Toxoplasma* and *Sarcocystis* spp. are important causes of encephalitis in sea otters and seals, methods for environmental detection of these parasites are not well developed. However, techniques are available for a related protozoan, *Cryptosporidium*, and so this was our starting point for molecular epidemiologic investigations of fecal protozoa along the California coast. *Cryptosporidium* parasites cause diarrheal disease and are shed by a variety of hosts, with some genotypes known to be zoonotic and others host-specific. We evaluated techniques to utilize mussels (*Mytilus* spp.) as natural concentrators of *Cryptosporidium* spp. in marine waters. Using spiked tissues and tank exposure experiments, we compared our TaqMan PCR assay with conventional PCR and fluorescent antibody techniques for sensitive and specific detection of *Cryptosporidium* spp. While all of these techniques were able to detect *Cryptosporidium* spp. in gill and digestive tissues, an extra concentration step using immunomagnetic separation (IMS) improved detection by one to two logs and may be important for environmental detection of fecal protozoa. Conventional PCR with DNA sequence analysis was the most useful technique for mussel hemolymph, and this technique was used in our 3 yr field study. We tested the hypothesis that if fecal pathogen pollution is flowing from land to sea, then *Cryptosporidium* may be detected more often in mussels collected near sources of human sewage or livestock runoff, compared to mussels collected distant to these sites. We deployed sentinel mussel batches and tested over 4,800 mussels for *Cryptosporidium* spp. Genotypes detected included *C. parvum* genotype 2, *C. andersoni* and *C. felis*. Risk factors associated with *Cryptosporidium* detection in mussels included exposure to freshwater outflow and precipitation in the week preceding mussel collection, but not exposure to human or livestock fecal sources.

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The diplomonad fish parasite *Spiroucleus vortens* utilizes glucose and produces hydrogen under anaerobic conditions. C. MILLET, J. CABLE and D. LLOYD, Microbiology (Bios 1), Cardiff University, Wales, United Kingdom.

The diplomonad fish parasite *Spiroucleus vortens* causes major problems in aquaculture of ornamental fish, while the related species *Spiroucleus barkhanus* affect wild and farmed salmonids, resulting in severe economical losses in the fish farming industry. The organism studied here was isolated from an angelfish (Sarah Poynton, 1995) and grown in bile supplemented (1%) TYIS33 medium with 250 µg/ml amikacin and 1000 U/ml penicillin G. A membrane-inlet mass spectrometer was employed to monitor, in a closed system, m/z peaks at 2, 32, and 44 for H₂, O₂ and CO₂, respectively. Under aerobic conditions, oxygen was consumed and CO₂ produced, but no H₂ was evolved. Addition of glucose accelerated the respiratory rate. Exhaustion of O₂ was accompanied by acceleration of CO₂ production (positive Pasteur effect). Hydrogen generation commenced under microaerobic conditions, slowed when full anaerobiosis was achieved, and further diminished as H₂ accumulated to a high concentration. Inhibition of H₂ production was accompanied by a decrease in H₂ levels in the system, thus suggesting that *Spiroucleus vortens* also utilises H₂. Addition of 0.5 mM metronidazole reduced H₂ production by 50% and 1 mM completely inhibited it. Ten millimolar Sodium azide did not affect anaerobic gas metabolism. Ten millimolar Iodoacetamide repressed H₂ production by 50% and 15 mM completely inhibited it, with no perceptible effect on CO₂ production. Thirty millimolar potassium cyanide did not affect oxygen consumption in the aerobic phase, but 15 mM suppressed CO₂ production and inhibited H₂ production by over 75%. The authors thank Prof. J. Kulda for providing the organism and his expertise on its culture.

148A

Evidence for unizoid cyst formation of *Cystoisospora canis* in vitro. S. M. MITCHELL, A. M. ZAJAC and D. S. LINDSAY, Virginia Tech, Blacksburg, Virginia.

Cystoisospora canis develops in the small intestine of their canine definitive hosts. Extraintestinal stages most commonly occur in the mesenteric lymph nodes (MLN) in both, definitive and paratenic hosts. The endogenous stages have been well studied but little is known about the biology of extraintestinal stages. Growing *Cystoisospora canis* in cell culture is difficult and few attempts have been made. Bovine turbinate (BT) cell or African green monkey kidney cell (CV-1) monolayers were grown on cover slips. Monolayers were infected with 1×10^5 excysted *C. canis* sporozoites. Cover slips were removed on various days, fixed and stained for light microscopy. A clear parasitophorous vacuole surrounded sporozoites that invaded both cell types on days 2 and 10 post-infection (PI). Cyst wall development around the sporozoite, appeared to fill in the parasitophorous vacuole in BT and CV-1 cells by day 15 and 16 PI, respectively. These stages structurally resemble the unizoid cysts seen in the MLN and other tissues in the paratenic hosts. Division of sporozoites was not observed in either cell type. Lengths and widths of sporozoites were the same from days 2–15 or 17 PI in BT and CV-1 cells, respectively. However, there was a slight increase in cyst wall dimensions from day 2–17 PI. Transmission electron microscopy is currently being done to further characterize these stages.

149A

Marrying function and genes in protists, an interdisciplinary approach. D. J. S. MONTAGNES and C. D. LOWE, School of Biological Sciences, University of Liverpool, Liverpool, United Kingdom.

We used an interdisciplinary approach to assess phylogenetic and functional variation in two free-living microeukaryotes. We assessed phylogenetic and functional diversity among 11 strains of the model flagellate *Oxyrrhis marina* using 5.8S ITS rDNA and growth responses to salinity; strains formed four phylogenetic clades and displayed two growth responses to salinity: one group achieved highest growth rates at high salinities, and the other grew best at low salinities. No clear correlation between molecular, ecophysiological, or geographical differences occurred. However, salinity tolerance was associated with habitat: intertidal strains grew best at high salinities; open-water strains grew best at low salinities. Extending this approach we examined salinity tolerance in the *Brachionus plicatilis* (Rotifera) species complex (admittedly, not protists but useful models, nonetheless). Ecophysiological and biochemical responses revealed an ion-regulatory mechanism, sensitive to salinity, which differed between sibling species. Molecular techniques indicated variation in, and changes in expression of, genes associated with this mechanism, again indicating sibling species differences. Such genetic variation might account for ecologically relevant differences in salinity tolerance between sibling species. We suggest that interdisciplinary studies like these offer a means to assess relationships between phylogenetic and functional variation and characterize the mechanisms underpinning important ecological traits.

150A

Calcium homeostasis and acidocalcisomes in *Toxoplasma gondii*. S. N. J. MORENO,^a K. MIRANDA,^{b,c} J. FANG,^a P. ROHLOFF^a and W. DE SOUZA^b, ^aDepartment of Cellular Biology and Center

for Tropical and Emerging Diseases, University of Georgia, Georgia, USA, ^bLaboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Ca²⁺ regulation in *Toxoplasma gondii* differs in several aspects from the processes that occur in other eukaryotic cells, providing great opportunities for targeting them for new therapies. Acidocalcisomes are distinct calcium-storage organelles present in *T. gondii*, in which calcium is mostly bound to polyphosphate (poly-P), although no information is available on second messengers involved in Ca²⁺ release from these organelles. Further studies are necessary to understand the biogenesis and function of acidocalcisomes in *T. gondii*. We do not know how acidocalcisomes multiply and distribute in daughter cells upon cell division or the reason for the morphological changes that occur in *T. gondii* acidocalcisomes during invasion of host cells. PP_i, poly-P, and cations are accumulated in large amounts in acidocalcisomes but their transport mechanism or their functions in *T. gondii* are largely unknown. Ca²⁺-ATPases are present in *T. gondii* but are apparently different from their mammalian counterparts. SERCA-type Ca²⁺-ATPases are sensitive to artemisinin, which does not inhibit the mammalian pump, while the PMCA-type Ca²⁺-ATPase, which also localizes in acidocalcisomes, does not possess a typical calmodulin-binding domain. New findings from our laboratory provide further evidence for the presence of these organelles in *T. gondii*, their interaction with other organelles and their composition.

151A

Distribution of *Acanthamoeba* in more and less polluted North Sea Coastal sediments. D. A. MUNSON,^a J.-P. DUCROTOY^b, and T. A. PAGET^b, ^aWashington College, Chestertown, Maryland, USA, ^bThe University of Hull, Hull, United Kingdom.

Acanthamoeba spp. are free-living amoebae that are ubiquitous in nature. Some species in the genus are opportunistic pathogens of humans. *Acanthamoeba* spp. have been isolated from a wide variety of sources including marine and fresh waters. These environments are often used for recreational purposes and may represent a potential source of infection. In this study we assess the impact of "pollutants" on the isolation rates and species/pathogen distribution of *Acanthamoeba* in six North Sea Coastal sites. Environmental data for each site was obtained from the Environmental Agency and the Scottish Environmental Protection Agency. Amoebae were isolated from sediments using bacterial overlay plates. From initial isolates, *Acanthamoeba* were sub-cultured and identified/speciated using cyst morphology and Hae III restriction of a ribosomal DNA PCR product. The pathogenicity of isolates was assayed by culture on high osmolarity plates and growth at 37 °C. *Acanthamoeba* were isolated from 34 of 71 sediment samples. The majority of isolates (65/77) were from Groups II and III based on morphology and 42 were speciated using restriction analysis. From the data collected it appears that environment has a significant effect on species diversity, with diversity being reduced in polluted environments. Interestingly the majority of pathogens were isolated from "clean" waters.

152A

Trypanosoma cruzi-adipocyte interactions: implications for HIV/AIDS. F. NAGAJYOTHI, M. S. DESRUISSEAU, L. M. WEISS, P. E. SCHERER and H. B. TANOWITZ, Albert Einstein College of Medicine, Bronx, New York.

Chagas disease, due to *Trypanosoma cruzi* infection, is a major etiology for heart disease in endemic areas. Chagas disease is now

appreciated to also be an opportunistic infection in immune-compromised individuals (i.e. those with HIV/AIDS). With 1.4 million infected people in endemic regions for Chagas disease and HIV/AIDS there is an increased risk of the reactivation of quiescent chronic *T. cruzi* infections. Through a network of endocrine, paracrine, and autocrine signals fat cells participate in the regulation of energy homeostasis, host defense, and reproduction, and can contribute to the development of pathological states. We determined the consequences of *T. cruzi* infection in adipocytes both in vitro and in vivo. Infected adipocytes exhibited changes in expression levels of a number of different adipocyte specific proteins. During infection in a murine model there was a unique metabolic profile associated with local inflammation in adipose tissue. Differentiated 3T3-L1 cells, a widely used adipocytes model, were used for in vitro studies. Forty-eight hours post-infection, adiponectin production was reduced while the levels of Toll-like receptor 2, TNF- α , interferon- γ , and IL1- β were up-regulated compared with β -actin. The mRNA levels of a number of chemokines and cytokines including ccl5, cxcl10, IL-10, TNF- α are upregulated 96 hr post-infection, while adiponectin expression of infected cells remained down-regulated. Electron micrographs of infected 3T3-L1 adipocytes revealed numerous intracellular amastigotes clustered around the lipid droplets, suggesting that parasites have a high affinity for host cell lipids. It is possible, that HIV-associated lipodystrophy could cause changes in adipocytes resulting in the release of quiescent intracellular parasites into systemic circulation, thereby triggering a new round of infection.

153A

Cryptosporidium population genetics: evidence of clonality in France and Haiti. T. NGOUANESAVANH,^a K. GUYOT,^a G. CERTAD,^{a,b} Y. LE FICHOUX,^c C. CHARTIER,^d R.-I. VERDIER,^e J.-C. CAILLIEZ,^f D. CAMUS,^{a,g} E. DEI-CAS^{a,g} and A.-L. BAÑULS^h, ^aEcologie du Parasitisme (EA 3609), Institut Pasteur de Lille, Lille, France, ^bCátedra de Parasitología, Escuela de Medicina "José María Vargas", Universidad Central de Venezuela, Caracas, Venezuela, ^cParasitologie-Mycologie, Centre Hospitalier Universitaire de Nice, France, ^dAFSSA Laboratoire d'études et de recherches caprines, Niort, France, ^eCentre GHESKIO, Port au Prince, Haïti, ^fLaboratoire Environnement et Santé, Université Catholique de Lille, France, ^gParasitologie-Mycologie, Centre Hospitalier Régional et Universitaire de Lille, France, ^hGénéétique et Evolution des Maladies Infectieuses, IRD, Montpellier, France.

Apicomplexan parasites of the genus *Cryptosporidium* can infect gastrointestinal or respiratory tracts of a wide range of vertebrates. *Cryptosporidium parvum* and *C. hominis* are the species most commonly found in humans. Understanding of population genetic structure of these widespread parasites is essential to clarify epidemiology and transmission dynamics but remains poorly documented. To study the *Cryptosporidium* populations in France and Haiti, a total of 117 parasite isolates from human and animal hosts from these two countries were genotyped using the 18S rDNA marker. Multilocus genotypes of 105 isolates were determined using a combination of four mini- and microsatellite markers. They were used for phenetic, genetic diversity, and population genetics analyses. *Cryptosporidium parvum*, *C. hominis*, *C. meleagridis*, and *C. felis* were identified in this study. Thirty-four multilocus genotypes were identified within the first two species. Analysis of linkage disequilibrium showed a predominant clonal structure of the parasite populations, of either basic or epidemic nature. Geographic factors seemed to play an important role in parasite diversity. The results emphasized the relevance of multilocus genotyping using mini- or microsatellite

markers and brought new data on *Cryptosporidium* population genetic structure in both developed and developing areas.

154A

The sequence of the *Pneumocystis carinii erg11* gene that codes for sterol 14 α -demethylase. S. W. NKININ,^a S. P. KEELY,^b J. R. STRINGER^b and E. S. KANESHIRO^a, ^aDepartment of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, ^bDepartment of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati, Cincinnati, Ohio.

The *erg11* gene codes for sterol 14 α -demethylase (14DM), a key enzyme in sterol biosynthesis and a target for azole antimycotic drugs. *Pneumocystis* is relatively insensitive to many commonly used 14DM inhibitors for clearing other fungal infections. We have cloned and sequenced the entire *erg11* gene from Form 1 and Form 6 of rat-derived *P. carinii*. The sequence analysis showed that the *erg11* genes are identical in the two-karyotype forms and that the gene has six introns. The sequence was also identical to that obtained from shotgun reads in the *Pneumocystis* Genome Project. The coding regions of the *erg11* gene sequence we obtained differed by a single base from that reported by Thomas et al. (Mayo group) who sequenced a *P. carinii erg11* cDNA that complemented the defect in a strain of *Saccharomyces cerevisiae* that lacked its own *erg11* gene. Our nucleotide sequence predicts a glutamic acid residue at a site where a lysine residue is located in the cDNA sequence from the Mayo group. It is not yet known if this difference affects the function of the enzyme. To address this issue, cDNA's encoding both forms of *P. carinii* 14DM will be transfected into a strain of *S. cerevisiae* that lacked its own *erg11* gene. Complementation for growth will be assessed and gas-liquid chromatography and mass spectrometric analysis will be performed to identify the sterols present.

155A

Evaluating support for eukaryotic supergroups and the search for sister groups of foraminifera. L. W. PARFREY,^a E. BARBERO,^b E. LASSER,^b J. GRANT,^b D. J. PATTERSON^c and L. A. KATZ^{a,b}, ^aProgram in Organismic and Evolutionary Biology, University of Massachusetts, Amherst, Massachusetts, ^bDepartment of Biological Sciences, Smith College, Northampton, Massachusetts, ^cBay Paul Center for Genomics, Marine Biological Laboratory, Woods Hole, Massachusetts.

Perspectives on eukaryotic diversity have recently shifted towards a supergroup system. We analyze two facets of supergroup robustness and find variable levels of support from molecular genealogies and considerable taxonomic instability (Parfrey LW, Barbero E, Lasser E, Dunthorn MS, Bhattacharya D, Patterson DJ, Katz LA, 2006, How robust are the eukaryotic supergroups?, submitted). Destabilized factors include issues of eukaryotic complexity, limited data, nomenclatural ambiguity, and sparse taxonomic sampling. We argue that low taxonomic sampling of diverse free-living microbial lineages is the most critical factor, as the resulting molecular genealogies substantially under-represent diversity. Hence, statements of monophyly may be premature. In conjunction with the EuTree consortium, we are testing organismal relationships within the putative supergroup "Rhizaria" by expanded sampling of understudied lineages and a multigene approach. "Rhizaria" encompasses Foraminifera, members of the polyphyletic Radiolaria, and a heterogeneous collection of amoebae and flagellates. Support for "Rhizaria" comes entirely from molecular studies, although the resulting tree topologies vary tremendously with taxon sampling and the method of tree construction. Our work aims to elucidate the sister groups of the

Foraminifera, as molecular studies have pointed to at least four groups as the sister taxon. This incongruity is largely due to highly divergent Foraminifera genes (Habura A et al. 2005, *Mol. Biol. Evol.*, **22**:2000–2009). Expanding sampling of taxa and genes may clarify these relationships.

156A

Using *Tetrahymena thermophila* to study the role of protozoa in inactivating viruses. M. D. O. PINHEIRO,^a M. E. POWER,^a B. J. BUTLER,^a V. R. DAYEH,^a R. SLAWSON,^b L. E. J. LEE,^b D. H. LYNN^c and N. C. BOLSA,^a ^aUniversity of Waterloo, Waterloo, ON, Canada, ^bWilfrid Laurier University, Waterloo, ON, Canada, ^cUniversity of Guelph, Guelph, ON, Canada.

The role of protozoa in inactivating viruses in water is largely unknown. Therefore we explored the interaction of *Tetrahymena thermophila* with bacteriophage T4 as a potential model system. T4 infectivity was assessed by the double agar overlay method using *Escherichia coli* B63 and monitored first in the absence of *Tetrahymena*, in three environments: (1) Osterhout's medium, which is a simple salt solution, (2) proteose peptone yeast extract (PPYE), which is a growth medium for *Tetrahymena*, and (3) conditioned PPYE, medium in which *T. thermophila* had been grown for various lengths of time and then removed. In these fluids, T4 showed no loss of viability for at least 4 days. T4 viability was then monitored in Osterhout's medium with either living or formalin-fixed *Tetrahymena*. For this type of experiment, a novel method of separating T4 from *Tetrahymena* was developed that involved placing 5 ml of the mixture (190,000 cells/ml *Tetrahymena*, and 1×10^5 PFU/ml T4) over 3 ml of Histopaque in a 15 ml centrifuge tube and centrifuging for 5 min at 300 g. When this was done immediately following mixing of T4 and *Tetrahymena*, all the *Tetrahymena*, whether living or fixed, were in the pellet and nearly all the T4 were in the Osterhout's medium on top of the Histopaque. As the time together with living *Tetrahymena* was increased, the T4 titre in Osterhout's steadily declined, reaching <0.1% of the starting titre by day 5. When formalin-fixed *Tetrahymena* were used, the T4 titre in Osterhout's medium did not decline with time. Likewise, when cytochalasin B was present together with living *Tetrahymena*, all the T4 continued to be viable. These results suggest that *Tetrahymena* actively removed T4 from fluid, likely by phagocytosis, followed by digestion in food vacuoles, although a role for adsorption is difficult to completely rule out.

157A

Knee arthritis associated with microsporidian-like spores in a patient with profound aplasia. M. RABODONIRINA,^a G. S. VISVESVARA,^b A. GENOT,^c J. Y. BLAY,^d L. X. XIAO,^b V. A. CAMA^b and T. FASSIER^e, ^aParasitology Unit, Croix-Rousse Hospital, Hospices Civils de Lyon, Lyon, France, ^bDivision of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, USA, ^cBiochemistry Laboratory, Croix-Rousse Hospital, Hospices Civils de Lyon, Lyon, France, ^dClaude-Bernard University and Medical Oncology Unit, Edouard Herriot Hospital, Hospices Civils de Lyon, Lyon, France, ^eMedical Intensive Care Unit, Croix-Rousse Hospital, Hospices Civils de Lyon, Lyon, France.

A 73-yr-old woman, without prior history of immunosuppression, was diagnosed as having an undifferentiated sarcoma of the right knee. Resection and replacement with prosthesis were performed, followed by chemotherapy. As she was in profound aplasia, she presented with febrile diarrhea, then septic shock with acute arthritis of the right knee. An infection of the prosthesis was

suspected. Bacterial and fungal cultures of the articular fluid were sterile. Amplification by PCR of the gene encoding the 16S rRNA using universal bacterial primers did not generate any PCR product. Even so, antibacterial treatment with ceftriaxon, vancomycin and ofloxacin was instituted. The patient became afebrile within a few days as she recovered from aplasia. The right knee remained inflamed and painful for 6 mo. Microbial etiology of this arthritis was not clear. However, after Giemsa staining, articular fluid was found to contain numerous polymorphonuclear neutrophil cells and monocytes, as well as many microorganisms with a morphological appearance strongly suggesting microsporidia, i.e. *Encephalitozoon*-like spores. Since there was no more fluid, DNA was extracted from a Giemsa-stained slide. The results of genetic analysis, which is currently in progress, will be discussed, in order to determine more precisely the taxonomy of the organism.

158A

Searching for alternative therapies against Cryptosporidiosis: in vitro and in vivo assays. C. RUEDA, S. FENOY and C. DEL AGUILA, Facultad de Farmacia, Universidad San Pablo-CEU, 28668 Madrid, Spain.

Cryptosporidiosis is an important cause of morbidity and mortality in animals and humans, mainly resulting in a diarrheal disease that can become chronic and life threatening in immunocompromised individuals. Owing to the lack of efficient treatments, new pharmacological studies are needed. We have studied the anti-*Cryptosporidium* activity of Bobel-24, a non-steroidal anti-inflammatory compound, which inhibits L-selectine. This action mechanism makes it a good candidate against cryptosporidiosis. To evaluate the efficacy of Bobel-24 we have used in vitro and in vivo models. For in vitro studies, HCT-8 confluent monolayers were inoculated with 8×10^5 oocysts/ml, and afterwards infected cells were incubated with different concentrations of Bobel-24. Paromomycin and mucine were used as treatment controls. For in vivo studies, two animal models were used. C57BL/6-Ifng^{tm1Ts} mice were infected with 1×10^3 oocysts as a model of acute cryptosporidiosis and SCID mouse were infected with 1×10^7 oocysts as a model of chronic cryptosporidiosis. In infected cells treated with 90 µM Bobel-24, *Cryptosporidium* growth was inhibited by up to 99%. Treatment with Bobel-24 of infected C57BL/6-Ifng^{tm1Ts} mice produced a 89% reduction in the oocyst shedding at day 6 p.i. However, all animals died on day 10 p.i. In the chronic infection model, significant differences in oocyst shedding were observed in comparison to the control group, when animals were treated with 125 mg/kg/day of Bobel-24.

159A

Genotyping of *Giardia duodenalis* isolates from dogs in the United States using a β -*Giardin* PCR-RFLP. A. V. SCORZA,^a S. M. CACCIO,^b R. BURNETT^a and M. R. LAPPIN^a, ^aColorado State University, Ft. Collins, Colorado, USA, ^bIstituto Superiore di Sanità, Rome, Italy.

Giardiasis is one of the most common parasites in domestic animals and can cause severe clinical symptoms in immunodeficient humans. Dogs and cats are infected with morphologically indistinguishable strains of *G. duodenalis*. Molecular genetic studies have demonstrated that *G. duodenalis* is a species complex comprising at least eight major assemblages. Assemblage A has been found in infected humans and in other mammals including dogs and cats. Assemblage B has been found in infected humans and dogs, but not cats. There are other genetic assemblages, within the *G. duodenalis* group that appeared to be confined to specific

animal hosts, like cats (assemblage F) and dogs (assemblages C and D). To date, only one genotype, genetic group I from Assemblage A, has been demonstrated to infect both humans and animals, thus proving zoonotic transmission. In the United States, the prevalence of giardiasis in dogs range from 7% to 26%, however in these studies genotyping of the isolates was not performed. The objective of this study was to genetically characterize *G. duodenalis* isolates of dogs from the United States at the β -giardin locus by PCR-RFLP and by sequencing analysis. Fecal samples from dogs that tested positive for *Giardia* by IFA, ELISA or, microscopic examination after Zn SO₄ flotation, were used in this study. *Giardia* cysts were concentrated following a standard sucrose centrifugal-flotation protocol before DNA extraction. The amplification of a fragment of the β -Giardin locus and the subsequent RFLP analysis was performed following a previously published protocol. DNA sequences were analyzed both forward and reverse direction. Thirty-three dog samples were analyzed, of these 31 (94%) correspond with the restriction pattern of assemblages D and two (6%) showed the assemblage C pattern. These results were confirmed by sequencing analysis. Results of this study showed that all the *G. duodenalis* isolates present in 33 dogs of the United States were dog-specific genotypes.

160A

Sequence of the mitochondrial genome of *Pneumocystis carinii*: implications for biologic function and identification of potential drug targets. T. M. SESTERHENN,^a M. T. CUSHION,^{a,b} B. E. SLAVEN^a and A. G. SMULIAN^{a,b}, ^aUniversity of Cincinnati, Cincinnati, Ohio, ^bVeterans Administration Medical Center, Cincinnati, Ohio.

With the exception of a few genes, most of the mitochondrial (mt) genome of *Pneumocystis carinii* has not been sequenced. Because of its potential for drug targeting as well as evolutionary importance, we assembled shotgun sequences generated as a result of the *Pneumocystis* Genome Project (PGP) for this purpose. Potential mt sequences were identified from the PGP database (<http://pgp.cchmc.org>) by BLAST analysis of known Pc mt sequences and those putatively identified as homologs to other fungal mt genomes. These sequences were assembled with the CAP3 assembler (X. Huang, MTU) (<http://bio.ifom-firc.it/ASSEMBLY/assemble.html>). The resultant contigs were then used to find more potential mitochondrial sequences in the PGP database in an iterative BLAST-type process resulting in a 27 kb contig. The size of the *P. carinii* mt genome was estimated to be about 40 kb by CHEF analysis, suggesting that there may be additional sequence not yet identified. Mapping and BLAST analysis of the contig identified 16 genes consistent with other fungal mt genomes and include: *cob*; *Atp6*, 8, and 9; *cox1*, 2, and 3; *Nad1*, 3, 4, 4L, 5, and 6; *var1* ribosomal protein; small subunit rRNA; and large subunit rRNA. Identification of *P. carinii* mt genes will facilitate the transcriptional analysis of effects of known and potential anti-*P. carinii* therapies.

161A

Large-scale analysis of introns and splice site elements in the *Pneumocystis carinii* genome. B. E. SLAVEN,^a A. G. SMULIAN,^a M. T. CUSHION,^a T. SESTERHENN,^a A. POROLLO^b and J. MELLER^b, ^aUniversity of Cincinnati College of Medicine and the Cincinnati Veterans Administration Medical Center, Cincinnati, Ohio, ^bChildrens Hospital Research Foundation, Cincinnati, Ohio.

The first large-scale characterization of *Pneumocystis carinii* (Pc) introns and associated splice site elements was conducted

using genomic and transcriptional sequence data obtained from the *Pneumocystis* genome project (PGP). Seven thousand five hundred and thirty-one cDNA transcripts were aligned against 4,272 non-telomeric genome contigs (~6.2 million bp) using GeneSeqer (Usaka et al. 2000). One thousand and seven hundred and eighty-one introns (86,694 bp) and 3,593 exons (705,555 bp) were identified after using strict cDNA to genomic sequence alignment criteria and removal of alternative and conflicting splice site alignments. A database was created for further characterization and analysis. Pc introns had an A+T content of 79.2%. Over 96% of the introns (1,710 of 1,781) had canonical (5'GU . . . AG3') splice sites and 1.12% (20 of 1,781) contained non-canonical (5'GC . . . AG3') sites. Exons had an average length of 196.4 bp with an A+T content of 66.7%, compared with an overall A+T content of 67.4% for the genome. The lengths of introns were tightly distributed with over 75% (1,344 of 1,781) between 40 and 50 bp; (av. Length=48.7 bp). The shortest intron was 36 bp, which we predict may be the minimum intron length that permits removal by the Pc spliceosome. The longest intron was 446 bp and contained a canonical (GU . . . AG) splice site. This large intron had greater than 100 perfectly aligned cDNA to genomic bases in its surrounding exons. The splice-branch site patterns showed a dominant polypyrimidine track between the 5'-splice site and the branch points of the introns with an overall branch site signal of 5 bp. The signal occurred between 8 and 17 bp from the 3' end of the splice site. These specific intron and exon characteristics will be applied to improve gene prediction accuracy levels for Pc. This strategy should lead to the identification of novel genes, which are not able to be identified by database homology methods.

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Draft assembly of the *Pneumocystis carinii* genome. B. E. SLAVEN,^a J. MELLER,^b A. POROLLO,^b T. SESTERHENN,^a A. G. SMULIAN^a and M. T. CUSHION^a, ^aUniversity of Cincinnati College of Medicine and the Cincinnati Veterans Administration Medical Center, Cincinnati, Ohio, ^bChildrens Hospital Research Foundation Cincinnati, Cincinnati, Ohio.

Pneumocystis carinii (Pc) presents many challenges to genomic sequencing and assembly technologies, highlighting current limitations. The genome A+T richness (67%), biased library construction, and subsequent genomic coverage necessitated a customized strategy. Lack of a viable culture system required purification of organisms from the lungs of immunologically suppressed rats during fulminate infection. Sequencing libraries were generated primarily from amplified DNA extracted from chromosome bands separated by CHEF. Cosmid end sequencing provided some scaffolding. Crossmatch (Green et al. 1996), an implementation of the Needleman-Wunsch algorithm, was used to compare sequences against host, bacterial and viral genomes to remove contaminants. The non-clonal nature of the Pc populations further complicated the assembly process since even slight genetic population drift inhibited genomic assembly merge operations. Approximately 10% of the Pc genome is composed of three telomerically located gene (PRT1/MSR/MSG) paralogs (Keely et al. 2006). Therefore, this draft assembly focused on the less repetitive portion of the genome. These Pc genome-specific challenges limited the effectiveness of heuristically based Arachne (Jaffe 2003), Phrap (Green and Ewing 2002) and Cap3 (Huang 1999) assembly systems and an alternative assembly strategy was implemented that took advantage of all three programs. Arachne was used to construct backbone contig-based scaffolds. Phrap was used to merge the contig-based scaffolds. Contigs were compared using Crossmatch to identify non-merged

contig overlapping areas. Overlapping contigs were binned and re-assembled in individual bins using Cap3. We tested various overall and localized assembly system results. Assembly system parameters were optimized, and merged contig constructions were computationally validated. The resulting iterative assembly process produced a draft of the *Pc* genome containing ~6.2 million sub-telomeric base pairs contained within 4,272 contigs. Nearly half of the contigs (2,010 of 4,272) were merged into 878 directionally oriented supercontigs using sister read names and cDNA alignments. Annotation of the genome is underway.

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Tetrahymena genome database (TGD): a resource for comparative studies with a model protist. N. A. STOVER, C. J. KRIEGER, G. BINKLEY, Q. DONG, A. SETHURAMAN, S. WENG and J. M. CHERRY, Department of Genetics, Stanford University, Stanford, California 94305-5120.

Tetrahymena Genome Database (TGD; www.ciliate.org) is a web-accessible resource containing information about the *Tetrahymena thermophila* genome sequence determined at The Institute for Genomic Research (TIGR). After completing the initial genome sequencing effort, TIGR performed a series of gene model predictions; these gene models have recently been submitted to Genbank. *Tetrahymena* Genome Database has performed sequence similarity-based comparisons for all of the predicted genes, and displays the results in a variety of ways to help researchers identify predicted genes of interest. The Quick Search tool at TGD allows researchers to search many types of gene annotations, including protein domains, homologs, and Gene Ontology (GO; www.geneontology.org) annotations detailing the gene product's function and localization, and gene descriptions written by the TGD curators. *Tetrahymena* Genome Database also maintains a BLAST/BLAT server, which can be used to search the *Tetrahymena* genome, gene model, and protein sequences directly. In addition to providing data and access to the genome sequence, genes, and proteins of *Tetrahymena*, TGD offers a powerful utility for searching the *Tetrahymena* literature. Textpresso, a full-text search program originally designed by Wormbase for searching *C. elegans* literature, has been adopted and modified by TGD to search full-text from the many *Tetrahymena* publications we have collected online. These features and others offered at TGD, including a graphical genome browser, community news section, and pages describing ciliate biology, make TGD a portal to an array of useful information about this powerful model organism and its research community. Here we will provide an overview of the many features and tools at TGD. *Tetrahymena* Genome Database is funded by a grant from the National Institutes of Health, as a component of the *Tetrahymena* Genome Sequencing Project at TIGR.

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Interaction of a synthetic azasteroid with several species of *Tetrahymena*. C. SUNDERMANN,^a S. LENAGHAN,^a N. DOORENBOS^b and J. BAKER^a, ^aDepartment of Biological Sciences, Auburn University, Auburn, Alabama, ^bPharmaceutical Sciences, Harrison School of Pharmacy, Auburn University, Auburn, Alabama.

Azasteroids are nitrogen-containing steroids that were first synthesized and reported in 1959. Derivatives of the original azasteroid have diverse uses such as antimicrobial, anti-androgen, and hair replacement therapies. One derivative, ND-497 (4-ethyl-4-aza-5 alpha-cholestane), was tested for its effect on cultures of *Tetrahymena*. ND-497 was solubilized in distilled water by drop-

wise addition of 6N HCl. Various concentrations of this stock solution were used as test solutions. Control solutions were unaltered growth medium and growth medium containing the same concentration of HCl as the experimental solutions. Ciliates from log phase cultures were counted, divided, and then placed in growth medium that contained an experimental concentration of ND-497, medium with solvent but no ND-497, or unaltered growth medium. The pH of all solutions was monitored every 24 hr when cells in each medium were enumerated. *Tetrahymena pyriformis*, *T. lwoffi*, and an SB mutant of *T. thermophila* were tested; all concentrations were tested in at least three separate trials. Originally, it was thought that ND-497 would be anti-protzoal, however, at low concentrations of ND-497, growth of ciliates accelerated (compared to control cultures) until 5 or 6 days after addition of the drug. Cultures that contained higher concentrations of ND-497 had little growth until 4–5 days after addition of the drug; at this time growth rapidly accelerated.

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High-voltage electron microscopy and computerized tomography: new approaches to study microsporidian spore internal organization. P. M. TAKVORIAN,^{a,c} K. F. BUTTLE,^b C. A. MANNELLA,^b L. M. WEISS^c and A. CALI^a, ^aRutgers University, Newark, New Jersey, ^bResource for Visualization of Biological Complexity (RVBC), Wadsworth Center, Albany, New York, ^cAlbert Einstein College of Medicine, Bronx, New York.

The Microsporidia are intracellular spore-forming parasites. Its spore is the environmental extra-cellular stage that contains the infective sporoplasm, a complex of membranous and tubular structures, and the extrusion apparatus (polar filament and anterior attachment complex), which facilitates transfer of the sporoplasm into a new host cell. The anterior portion of the spore contains the polaroplast, a bipartite system of lamellae (lamellar polaroplast) and tubular structures (vesicular polaroplast) that surrounds the straight or manubroid portion of the polar filament. The polaroplast is believed to play an important role in the extrusion process. Owing to the quantity of internal components, the extreme compaction of these structures inside the spore, and the density of the spore itself, it is very difficult to penetrate and observe spore structures with typical electron microscopes (EM). During the last 50 yr, thin sections have provided invaluable information for two-dimensional imaging; however, it has been difficult to use these images for three-dimensional interpretations of the relationships of the complex internal spore structures. High-voltage EM allows electron beam penetration of 300–500 nm thick sections. Projection images were recorded from thick sections of activated *Brachiola algerae* spores tilted around two orthogonal tilt axes, over an angular range of $\pm 60^\circ$ with a single and double tilt at one and two degree intervals, on an AEI EM7 HVEM operated at 1,000 kV. Fields containing polaroplasts were digitized, aligned using colloidal gold reference markers, reconstructed by modified back projection, and visualized by volume rendering. These tomograms are helping us define the 3D shape and organization of membranes and other internal structures at various stages of the extrusion process. This research was supported by NIH grant no. AI52035. Development of electron tomography at the RVBC is supported by NCRR/NIH grant RR01219.

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Spatial and temporal variation in populations of tide pool ciliates. F. TERRY,^a G. MCMANUS,^b B. COSTAS^b and L. A. KATZ^{a,c}, ^aDepartment of Biological Sciences, Smith College, Northampton,

Massachusetts, ^bDepartment of Marine Sciences, University of Connecticut, Groton, Connecticut, ^cProgram in Organismic and Evolutionary Biology, University of Massachusetts, Amherst, Massachusetts.

To assess levels of diversity and gene flow within the morphospecies *Strombidium oculatum* and *S. stylifer*, tide pool ciliates, we sequenced internally transcribed spacer (ITS) regions of the rDNA locus in samples from New England, the United Kingdom and Ireland. We tested the hypothesis that haplotype diversity is greater in the Irish Sea than on the Atlantic coast by characterizing polymorphisms from ~150 clones. Where previous studies showed 10 distinct contigs, the greatest diversity of which was present in the Irish Sea, our analysis finds considerable variation on the Atlantic side of Ireland, including the characterization of three previously unknown haplotypes. We are also testing the temporal stability of haplotype diversity at several sites in the Irish Sea. Preliminary data suggest the predominance of a single haplotype early in the spring and increasing diversity thereafter. We are investigating the potential ecological basis for this pattern. The discovery of both high diversity and high gene flow within *Strombidium* is inconsistent with both the "cosmopolitan" and the "endemism" hypotheses for microbial diversity, and implies the need for a restructuring of the current debate to include species diversity and gene flow as separate factors for consideration.

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Matrix-assisted laser desorption/ionization time of flight mass spectrometry-based analysis of *Giardia lamblia*. E. N. VILLEGAS,^a S. T. GLASSMEYER,^a M. W. WARE,^a S. L. HAYES^b and F. W. SCHAEFER III^c, ^aUS EPA, National, Exposure Research Laboratory, Cincinnati, Ohio, ^bUS EPA, National Risk Management Research Laboratory, Cincinnati, Ohio, ^cUS EPA, National Homeland Security Research Center, Cincinnati, Ohio.

Giardia lamblia is a zoonotic protozoan parasite that is a leading cause of drinking water related gastro-intestinal disease outbreaks worldwide. Owing to the genotypic complexity and high prevalence of this parasite in the environment, numerous research studies are being done to genotype the different *Giardia* species present in clinical and environmental samples. One approach used to genotype these pathogens is nested PCR in combination with restriction fragment length polymorphism (RFLP) analysis. However, this approach can be labor intensive, expensive, and prone to PCR contamination. In this study, we evaluated the utility of matrix-assisted desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) as an alternative method to identify different *Giardia* species. Matrix-assisted desorption ionization-time of flight mass spectrometry analyses of *G. lamblia* and *G. muris* reveal mass spectral fingerprints that contain genus and species specific peaks. Further analysis of flow cytometry sorted *G. lamblia* trophozoites identified trophozoite specific peaks that constituted a part of the total mass spectral fingerprint detected from live *G. lamblia* cysts. In addition, to determine the stability of these *G. lamblia* mass spectral profiles, live cysts and cysts killed by physical and/or chemical disinfection were also analyzed by MALDI-TOF MS. Results reveal the loss of specific peaks in killed cysts as compared to live cysts. These results suggest that a MALDI-TOF MS-based proteomics approach is effective at identifying unique mass spectral fingerprints from two different *Giardia* species and differentiating viable from non-viable cysts. This study presents an alternative approach for identifying various clinical and/or environmental *Giardia* isolates as well as a method to evaluate various drinking

water treatment and disinfection efficacies. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

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Toxoplasma gondii: RNA silencing pathway and applications. X. WANG, A. AL-RIYAH, F. AL-ANOUTI, G. SHAMIM and S. ANANVORANICH, University of Windsor, Department of Chemistry and Biochemistry, Windsor, ON, Canada N9B 3P4.

Recent advancement in genomic research on pathogenic apicomplexan parasites, namely *Plasmodium falciparum*, *Theileria parva* and *Toxoplasma gondii*, has revealed that these parasites harbor abundant antisense transcripts. Interestingly the frequency of some antisense transcripts is inversely proportional to the level of sense transcription detected, suggesting the presence of post-transcriptional control in these organisms. These findings, taken together with recent discoveries of functional RNA interference pathways in *P. falciparum* and *T. gondii*, suggest that these parasites have the ability to utilize post-transcriptional and RNA silencing pathway to modulate their gene expression. RNA silencing pathways are evolutionally conserved mechanisms among eukaryotes. These pathways include RNA interference (RNAi), RNAi-mediated chromatin silencing and DNA rearrangements and post-transcriptional regulation of mRNA by endogenously encoded microRNAs (miRNAs). These pathways use sequence-specific regulators that are small double-stranded RNA (dsRNA). Our group uses *T. gondii* as an experimental model for investigating RNA silencing pathways in apicomplexan parasites. Specifically we focus on the involvement of RNAi-related genes, namely Argonaute and Dicer in the silencing mechanism. Here we will present and discuss the RNA silencing pathway and its applications in *T. gondii*.

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In vitro culture of *Pseudoloma neurophilia*, a common microsporidian of zebrafish (*Danio rerio*). V. G. WATRALL,^a R. B. KAUFFMAN^b and M.L. KENT^a, ^aDepartment of Microbiology, Oregon State University, Corvallis, Oregon, ^bOregon Department Fish & Wildlife, Center for Fish Disease Research, Oregon State University, Corvallis, Oregon.

Pseudoloma neurophilia, a microsporidium from the central nervous system of zebrafish (*Danio rerio*), is the most common pathogen in zebrafish research facilities. Based on records from the NIH Zebrafish International Resource Center Diagnostic Service, the parasite has been detected in greater than 50% of these facilities. The infection is most common in the spinal cord and hind brain, and spreads to other tissues, particularly the somatic muscle. Infected fish are usually severely emaciated and may exhibit scoliotic changes. While it is possible to successfully transmit and maintain the parasite in vivo, obtaining adequate numbers of spores for experiments is problematic and time consuming. To address these issues we have developed a method for the successful in vitro culture of *Pseudoloma neurophilia* on various cyprinid cell lines. Preliminary results suggest that the parasite infects both EPC and FHM cell lines, and quickly develops aggregates of about eight spores per cell. The establishment of this culture system facilitates our ability to conduct in vitro experiments, such as testing of therapeutic and disinfectant agents for this microsporidium. In addition, this system allows for a more reliable source of spores for in vivo transmission experiments.

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An ecological perspective on protistan molecular genetics. P. C. Watts, C. D. Lowe and D. J. S. Montagnes, School of Biological Sciences, University of Liverpool, Liverpool, United Kingdom.

Molecular genetic techniques have revolutionized our understanding of ecological and evolutionary processes. The application of DNA sequence data provides a wealth of information on the diversity and function of free-living micro-eukaryotes and has revealed the presence of previously undocumented groups of organisms. To date, much of our applications of molecular methods to protists have been focused towards phylogenetic analyses. Now, the current rapid development of new genomic technologies is set to increase our ability to assess the roles of different protist groups in wider ecosystems by characterizing (i) functional biodiversity, (ii) ecological structure, and (iii) gene function. This brief review will present a synthesis of how new genetic techniques have been applied to uncover patterns of biodiversity and ecological function. We will outline the development of molecular approaches in the study of free-living micro-eukaryotes, compare this with the development of molecular methods applied to higher taxa, and highlight the need to combine more traditional ecological approaches with arising molecular methods. In so doing, we indicate that not only do protists present unique challenges, they also offer a series of unique opportunities. Thus, as more genomic information becomes available, free-living protists are likely to become increasingly useful as model organisms with which to assess a wide range of evolutionary and ecological processes. Protistologists consequently have an exciting opportunity to be at the vanguard of this rapidly developing field.

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Biochemical recognition of prey by planktonic protozoa. E. C. WOOTTON and E. C. ROBERTS, Department of Biological Sciences, University of Wales, Swansea, United Kingdom.

Through selective feeding, protists play a fundamental role in structuring bacterial and phytoplankton communities within the marine environment. Although recent evidence indicates that protozooplankton can select food based on cell surface properties of their prey, the underlying mechanisms are poorly understood. Previously, using haemagglutination experiments, we identified a Ca^{2+} -dependent, mannose-binding lectin (MBL) on the marine dinoflagellate *Oxyrrhis marina*. Feeding experiments, involving live and bead prey, demonstrated the employment of this lectin as a feeding receptor used for recognizing and selecting prey. Here, we present initial characterization of *O. marina* MBL, which will aid purification of this protein.

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Behavioral responses to serum in *Trichomonas*. R. YAMNIK, N. YARLETT and M. LEVANDOWSKY, Haskins Labs, Pace University, New York, New York.

Trichomonas vaginalis and *T. foetus* are important human and veterinary parasites, respectively. We have been interested in their behavioral responses to various chemical signals, some of which may have clinical relevance for infectivity or pathogenesis. In the presence of horse serum (10%), dynamic aggregates of cells appear in cell suspensions of both species. Swimming kinetics (speed and shape of swimming path) are essentially the same in single free-swimming cells in the presence and absence of serum. However in serum many cells form dynamic aggregates of swimming cells; cells in the aggregates are not motionless or quiescent,

but actively swim in contact with each other. Our tentative interpretation is that, in the presence of serum, some cells become attractive to others, forming groups of actively swimming, interacting cells.

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Polyamine biosynthesis by *Cryptosporidium parvum* and its potential as a chemotherapeutic target. N. YARLETT,^{a,b} M. MORADA^a and G. WU^a, ^aHaskins Laboratories, Pace University, New York, New York, ^bDepartment of Chemistry and Physical Sciences, Pace University, New York, New York.

Cryptosporidium parvum possess a high affinity polyamine transporter, which avidly takes up host derived polyamines, particularly spermine. Spermine is then retro-converted to the lower polyamine homolog by spermine/spermidine N¹-acetyltransferase (SSAT). The *C. parvum* SSAT was isolated and expressed and found to be a homotetramer with a subunit molecular mass of 18 kDa, and low sequence similarity with the host SSAT. CpSSAT had a K_M for acetyl-CoA of 24×10^{-6} M and for spermine of 50×10^{-6} M, with a K_{cat} of 9 s^{-1} . *Cis*-polyamine analogues exhibit mixed inhibition kinetics with low K_I values (15–30 μM) and long active site residence times ($\delta I_n E / \delta t$ 0.48–3.3 min^{-1}). These analogues were also effective in curing an immunocompromised mouse model infection without noticeable host toxicity confirming the potential therapeutic value of this target.

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Towards an understanding of the role of actin in *Tetrahymena* phagocytosis: comparative studies using inhibitors of PI 3-kinase and myosin light chain kinase. R. V. ZACKROFF,^a L. OCTAVIO,^b A. BIGDELI^b and L. A. HUFNAGEL^b, ^aMassachusetts College of Pharmacy and Health Sciences, Boston, Massachusetts, ^bUniversity of Rhode Island, Kingston, Rhode Island.

Wortmannin is a PI 3-kinase and myosin light chain kinase (MLCK) inhibitor. We previously reported that wortmannin mimics the inhibitory effects of latrunculins and cytochalasins on *Tetrahymena* phagocytosis. Like those actin inhibitors, wortmannin blocked nascent phagosome closure, as evidenced by the formation of oversized and/or elongated nascent phagosomes that failed to detach from the oral apparatus. However, while latrunculins or cytochalasins abolished actin antibody staining of the post-oral fiber, wortmannin had no detectable effect on post-oral fiber actin localization. In order to determine which of wortmannin's enzyme inhibitory activities might be responsible for the inhibition of nascent phagosome release, we compared its effects on phagocytosis with those of LY 294002 (a structurally unrelated drug which has been reported to specifically inhibit PI 3-kinase, but not MLCK) and ML-7 (an inhibitor specific for MLCK). Wortmannin inhibited the rate of phagosome formation by about 50% at a concentration of 0.1 μM , and induced oversized nascent phagosomes in most cells in the 0.2–2 μM concentration range. LY 294002 inhibited the rate of phagosome formation over the 4–40 μM concentration range, but did not induce formation of oversized nascent phagosomes. ML-7, up to 10 μM , did not inhibit the rate of phagocytosis or induce giant phagosomes. These results demonstrate that two different PI 3-kinase inhibitors block *Tetrahymena* phagocytosis, while a specific MLCK inhibitor does not. The results are consistent with the hypothesis that PI 3-kinase, but not MLCK, is required for *Tetrahymena* phagocytosis, and suggest that wortmannin's ability to mimic induction of giant phagosomes by actin inhibitors may be due to its recently reported

effects on still other enzymes, such as polo-like kinases, which influence actin dynamics in other cells.

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Genome diversity within the ciliate species *Chilodonella uncinata*. T. ROBINSON,^a R. A. ZUFALL^b and L. A. KATZ^a, ^aSmith College, Northampton, Massachusetts, ^bUniversity of Houston, Houston, Texas.

The relationship between germline and somatic genomes in eukaryotes is complex. It was initially believed the genome content of the germline was identical to the genome content of the soma. However recent data indicate that the relationship

between these genomes can vary due to epigenetic phenomena such as parental imprinting and X-chromosome inactivation. Our work on *Chilodonella uncinata* expands this relationship by demonstrating varying chromosomal content within macronuclei of a single species. To assess intraspecific variation in genome content, we characterized the ITS region of the rDNA locus, and the protein-coding genes β -tubulin and actin. While we found no variation in ITS sequence within this morphospecies, different paralogs of the protein coding genes were present in different isolates of *C. uncinata*. Furthermore, crosses between isolates indicate non-Mendelian inheritance of these paralogs. We present a model whereby epigenetics combined with chromosomal rearrangements during macronuclear development explain the presence/absence of paralogs in macronuclei.