EDITORIAL INTRODUCTION

The First United Workshop on Microsporidia from Invertebrate and Vertebrate Hosts

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Abstract. The phylum Microsporidia is a large group of parasitic unicellular eukaryotes that infect a wide range of invertebrate and vertebrate taxa. These organisms are significant human and veterinary pathogens with impacts on medicine, agriculture and aquaculture. Scientists working on these pathogens represent diverse disciplines that have had limited opportunities for detailed interactions. A NATO Advanced Research Workshop ‘Emergent Pathogens in the 21st Century: First United Workshop on Microsporidia from Invertebrate and Vertebrate Hosts’ was held July 12–15, 2004 at the Institute of Parasitology of the Academy of Sciences of the Czech Republic to bring together experts in insect, fish, veterinary and human microsporidiosis for the exchange of information on these pathogens. At this meeting, discussions were held on issues related to taxonomy and phylogeny. It was recognized that microsporidia are related to fungi, but the strong opinion of the participants was that the International Code of Zoological Nomenclature should continue to be applied for taxonomic descriptions of the Microsporidia and that they be treated as an independent group emerging from a paraphyletic fungi. There continues to be exponential growth in the pace and volume of research on these ubiquitous intracellular protists. The small genomes of these organisms and the reduction in the size of many of their genes are of interest to many disciplines. Many microsporidia are dimorphic and the mechanisms underlying these morphologic changes remain to be elucidated. Epidemiologic studies to clarify the source of human microsporidiosis and ecologic studies to understand the multifaceted relationship of the Microsporidia and their hosts are important avenues of investigation. Studies on the Microsporidia should prove useful to many fields of biologic investigation.

Microsporidia are a group of eukaryotic obligate intracellular parasites first recognized almost 150 years ago with the description of *Nosema bombycis* as the cause of pébrine, a disease of economic importance in the silkworm industry (Sprague et al. 1992, Wittner and Weiss 1999). Members of the phylum Microsporidia (Sprague and Becnel 1998) are not limited, however, to infections in invertebrates, but instead infect virtually all animal phyla including other protists (Wittner and Weiss 1999). They are important agricultural pathogens in insects, fish, laboratory rodents, rabbits, fur-bearing animals and primates. Microsporidia have been investigated for use as biologic pesticides for the control of destructive species of grasshoppers and locusts (American Mosquito Control Association 1985). The phylum Microsporidia contains over 1,100 species distributed into at least 144 genera (Sprague et al. 1992, Wittner and Weiss 1999). Human infection with these pathogens was first reported in 1959 in a child with encephalitis (Matsubayashi et al. 1959), but subsequent case reports were rare until the recognition of diarrhoea due to microsporidiosis in the setting of HIV infection (Desportes et al. 1985). Since 1985 the number of reported cases of human disease has increased rapidly and they have been recognized in both immunocompetent and immunodeficient hosts (Wittner and Weiss 1999). In addition to

gastrointestinal tract involvement it is now appreciated that Microsporidia can infect virtually any organ system and cases of encephalitis, ocular infection, sinusitis, myositis and disseminated infection are well described in the literature (Cali et al. 1993, Desportes et al. 1985, Ledford et al. 1985, Terada et al. 1987, Rastrelli 1994, Field et al. 1996, Yachnis et al. 1996, Weber et al. 1997, Cali et al. 1998, Cali and Takvorian 2003, Coyle et al. 2004). Currently, the following species have been reported to cause human infections: Pleistophoridae: *Trachipleistophora hominis* Hollister, Canning, Weidner, Field, Kench et Marriott, 1996, *Trachipleistophora anthropophthera* Vavra, Yachnis, Shadduck et Orenstein, 1998, *Pleistophora ronneafiei* Cali et Takvorian, 2003; Encephalitozoonidae: *Encephalitozoon cuniculi* Levaditi, Nicolau et Schoen, 1923, *Encephalitozoon hellem* Didier, Didier, Friedberg, Stenson, Orenstein, Yee, Tio, Davis, Vossbrinck, Millichamp et Shadduck, 1991, *Encephalitozoon intestinalis* (Cali, Kotler et Orenstein, 1998); Enterocytozoonidae: *Enterocytozoon bieneusi* Desportes, Lechanpentier, Galian, Bernard, Cochandpriollet, Lavergne, Ravisce et Modigliani, 1985; and Nosematidae: *Brachiola vesicularum* Cali, Takvorian, Lewin, Rendel, Sian, Wittner, Tanowitz, Keohane et Weiss, 1998, *Brachiola connori* (Sprague, 1974), *Brachiola algerae* (Vavra et Undeen, 1970), and

Vittaforma corneae (Shadduck, Meccoli, Davis et Font, 1990). Moreover, single ocular infections by *Microsporidium africanum*, *Microsporidium ceylonensis* and *Nosema ocularum* have been described but the true systematic position of the causative agents is uncertain. It is believed that many of these pathogens are zoonotic and that water and foodborne transmission mechanisms are important for the transmission of these diseases (Deplazes et al. 2000). It has become evident that there is no clear barrier preventing several insect or fish microsporidian pathogens from causing infections in immunosuppressed human hosts.

As the Microsporidia infect a wide range of host species, scientists working on these pathogens represent diverse disciplines that have had limited opportunities for detailed interactions. Historically researchers dealing with the Microsporidia from various hosts have discussed these organisms within their segregated scientific communities. Reflecting these communities, modern microsporidology has been divided into three main branches focused on: (i) Microsporidia from Arthropods (mainly insects and crustaceans); (ii) Fish Microsporidia; and (iii) Mammalian/Human Microsporidia. Consequently, researchers working in each of the mentioned fields publish their papers in different journals and organize separate meetings. A NATO advanced research workshop (ARW) was, therefore, held July 12–15, 2004 at the Institute of Parasitology of the Academy of Sciences of the Czech Republic in České Budějovice, Czech Republic. This meeting entitled ‘Emergent Pathogens in the 21st Century: First United Workshop on Microsporidia from Invertebrate and Vertebrate Hosts’ brought together experts in insect, fish, veterinary and human microsporidiosis to discuss the state of the field and exchange data on the different microsporidian systems that they investigate. The meeting directors were Drs. Yuliya Sokolova (All Russian Institute for Plant Protection) and Oleg Ditrich (Institute of Parasitology, Academy of Sciences of the Czech Republic) and the international organizing committee was composed of Drs. Louis M. Weiss (Albert Einstein College of Medicine), Zuzana Kučerová-Pospíšilová (Center for Disease Control), Patrick Keeling (University of British Columbia), Judith Smith (Leeds University) and Jiří Vávra (Institute of Parasitology, Academy of Sciences of the Czech Republic). The meeting was supported by grants from the NATO ARW program, the National Institute of Allergy and Infectious Diseases (National Institutes of Health, United States of America) and The Ellison Medical Foundation. The current issue of *Folia Parasitologica* contains articles representing work presented and discussed at this conference. This article represents an overview of themes, discussions and consensus opinions of the participants of this meeting, but is not intended to represent an exhaustive review of all of the abstracts and data that were presented (citations for abstracts are indicated by [X], where X is

the abstract number and a complete list of abstracts 1 through 55 is provided at the end of this article).

Discussions were held on issues related to the taxonomy of the Microsporidia. There was general agreement that molecular phylogenetic data support that the Microsporidia are related to fungi and are not “primitive eukaryotes” (Edlind 1998, Keeling and McFadden 1998, Hirt et al. 1999, Weiss et al. 1999, Katinka et al. 2001). Microsporidia also display similarities to the fungi in mitosis, e.g., closed mitosis and spindle pole bodies (Desportes 1976), in meiosis (Flegel and Pasharawipas 1995), have chitin in their spore wall and store trehalose as do fungi. Keeling (2003) in a published analysis of β -tubulin data that included several species of Microsporidia and fungal phyla has suggested that the Microsporidia are a sister group to the Zygomycota. It was the opinion of the experts in the field gathered at this meeting that the International Code of Zoological Nomenclature should continue to be applied for taxonomic descriptions of the Microsporidia. There is no reason with our current state of taxonomic description of over 1,000 species and the ongoing evolution of molecular phylogenetic description of this phylum to apply the botanical code to this group of organisms despite their relationship to fungi. Microsporidia should be considered and treated as a separate monophyletic group, making the fungi paraphyletic. This is not unlike the situation seen with the phylogeny of reptiles, which must be considered as paraphyletic since birds are not included among them.

Phylogenetic studies of the phylum Microsporidia continue to evolve. Data suggest that the relationship of the host and its microsporidia (e.g. co-speciation) may be an important influence on microsporidian taxonomic relationships. It is clear that more environmental sampling will be needed to develop and extend the molecular phylogeny of these organisms as there has been only a limited sampling of the Microsporidia in many animal groups [35]. Nonetheless, molecular phylogeny is revealing new relationships among the Microsporidia for which 16S ribosomal sequences (or other gene sequences) exist [48, 50]. This information has illustrated that the traditional genera established by ultrastructural characteristics are often not monophyletic. It has been suggested that ultrastructural characteristics may have significant plasticity that could be altered by host range and environmental conditions [6]. Overall, the opinion of the group was that both traditional and molecular phylogenetic approaches are applicable for the description and classification of these organisms. In a description of a new species in the Microsporidia the majority of participants believed that it is important to provide ultrastructural and epidemiologic descriptions in addition to molecular data [6, 37, 50, 54, 51]. The ability of type specimens to be used to obtain molecular information as well as new ultrastructural data points out the utility of depositing reference slides [44, 49, 55]. In ad-

dition to the deposition of type slides it is critical for future taxonomic studies that any cultivated organism should be deposited in a reference culture collection such as the American Type Culture Collection (ATCC; <http://www.atcc.org/>). The participants believed it would be useful to establish a DNA depository for the Microsporidia and to encourage anyone describing a new organism to deposit or save DNA samples. Preservation of spores or tissue in ethanol represents a cost effective and easy method to store samples from which DNA can be recovered for future taxonomic studies.

The publication of the *Encephalitozoon cuniculi* genome in 2001 provided several insights into the organisation of the highly reduced (2.9 Mbp) genome of this microsporidian (Katinka et al. 2001). There are about 2,000 genes in the genome with evidence of a significant loss of biosynthetic pathways, resulting in dependence on the host cell pathways, (e.g. reductive evolution). Consistent with the high coding density there are almost no introns present in the genome, few repeats, and short intergenic regions. On average most genes are 15% smaller than their homologues in other eukaryotes and often display reduced complexity (e.g. a loss of protein interaction domains). The study of these genes should prove extremely useful for biologists interested in protein interactions and functions as they may provide insight into the minimal interactions and domains necessary to carry out a particular function. For example, the small subunit rRNA of the Microsporidia is missing domains seen in both eukaryotes and prokaryotes, yet is able to function in protein synthesis. These compact genomes appear to provide constraints for the evolution of genomes in the microsporidia. Data from genome sequence survey (GSS) of *Antonospora* (syn. *Paranosema/Nosema*) *locustae* that surveyed about 12% of this 5.4 Mbp genome demonstrated a significant preservation of gene order and or gene composition [1]. Studies involving cDNA (an EST project) are also yielding insights, having demonstrated the presence of multigene and antisense transcripts as well as demonstrating that transcripts can initiate in the coding region of upstream genes and terminate within downstream genes [1]. Studies on microsporidian chromosomes have suggested that these organisms are diploid and that significant chromosomal variability can be present [13, 15]. As more genetic information becomes available, techniques for genetic manipulation of these protists will be needed. Such techniques would greatly facilitate studies on important cell biology questions, such as differentiation.

Discussions indicated that several GSS projects were in various states of progress, including those on *Antonospora* (syn. *Paranosema/Nosema*) *locustae*, *Spraguea lophii*, *Nosema bombycis*, *Vittaforma corneae*, *Enterocytozoon bieneusi* and *Encephalitozoon hellem*. A microsporidium that undergoes several developmental cycles *Edhazardia aedis* has also been selected for a

genome project. The participants believed that this was an excellent choice as none of the current organisms displays the complex spore alternations between hosts that is a characteristic of many of the microsporidia found in insects [4, 5]. It is likely that, as in the *A. locustae* project, many developmental genes not present in the *E. cuniculi* genome will be present in this microsporidium [1]. Further discussions indicated that it would be useful to include *Brachiola algerae* in the group of microsporidia for which genomic data is obtained, given its broad host range and that it has now been demonstrated to infect humans [16, 18]. The research community strongly recommended that all organisms being used for genome projects (GSS, EST, or genomes) should be deposited in reference collections such as the ATCC so that these "genome type strains" would be easily available for correlative studies. The development of a web-based centralised microsporidian database similar to that available for *Toxoplasma gondii* (www.Toxodb.org) was felt to be an important community resource that would greatly facilitate research on these pathogens.

Many of the research presentations demonstrated the remarkable plasticity of the microsporidia in their adaptation to various environmental niches [4, 5, 31, 32, 33, 39, 41] as well as the profound influence of these organisms on the biology of their host cells. Many of the microsporidia cause feminisation of their hosts and this is often associated with a low-virulence phenotype [2]. Infection can significantly alter the physiology of the host [40, 45]. The development of hypertrophic host cells as well as xenomas is an important characteristic of many of the Microsporidia [14, 29, 30]. Study of the mechanism by which the Microsporidia cause such profound changes in their hosts and host cells should yield important insights into not just host-parasite relationships but also the fundamental biologic processes underlying the regulation of host cell size and proliferation. Microsporidia are also interesting model systems in which one can explore ecological issues. Studies have demonstrated the use of Microsporidia in the evaluation of the radiation of introduced species and their pathogens and in the effect of pathogens on emergent ecological relationships between native and introduced species [2, 3, 34, 36].

A defining characteristic of the Microsporidia is the presence of the polar tube, a unique mechanism of host cell invasion. Characterisation of the early events in the germination of microsporidian spores and infection of host cells is ongoing [8, 9]. Data suggest that the polar tube may also function as a mechanism by which these organisms escape phagosomes [7], providing an alternative mechanism for infection of host cells and dissemination of infection. Microsporidia have functional Golgi (Takvorian and Cali 1994) that are involved in post-translational modifications of proteins such as glycosylation [11, 12] (Xu et al. 2004). It is probable that dif-

ferent Golgi compartments are expressed in a time-dependent development sequence correlated with microsporidian replication and the formation of specialised structures such as the polar tube [11]. Previous data had indicated that the Microsporidia have relic mitochondrial organelles termed mitosomes (Williams et al. 2002) and additional characterisation of the genome suggests that some metabolic activities may continue to occur in these structures [10]. Discussions suggested that the polar vesicles that had been previously described in the Microsporidia are excellent candidates for mitosomes.

Microsporidia are excellent model systems for the study of immunology in both insects [43] as well as mammalian hosts [19, 27, 52, 53]. Murine models of microsporidiosis have indicated that intraperitoneal infection is highly dependent on CD8⁺ cells whereas oral infection is dependent on both CD4⁺ and CD8⁺ [52, 53]. Diagnostic assays for microsporidiosis continue to improve. Serologic markers of infection [22], immunofluorescence detection systems [22], as well as molecular diagnostic techniques for both insect [46, 47] and human infections [22, 25, 26] have been developed. Studies have illustrated that zoonotic transmission of human microsporidiosis is probable [25, 26] and that pigeon faeces can be a source of *Enterocytozoon bienersi* [26]. Microsporidiosis prevalence studies have been conducted and demonstrate the continued presence of these infections and the emergence of new microsporidian pathogens [17, 25, 26, 28]. Human infections have been described with microsporidia classically seen in fish and insects [16, 18]. A real-time PCR technique to assay drug efficacy against microsporidia has been developed [23]. Investigations into new therapeutic agents for microsporidia are ongoing and suggest that methionine aminopeptidase type 2 is a useful therapeutic target [20, 21]. A technique was described at this meeting for the purification of *E. bienersi* spores that allowed both the development of monoclonal antibodies and an initial characterisation of the genome size of this pathogen [17]. While many microsporidia can be cultivated *in vitro* and animal models exist, there remains a need for an *in vitro* cultivation system for *E. bienersi*.

It has been over 100 years since the description of the microsporidian polar tube by Thelohan (1894), a structure that continues to fascinate biologists to this day. Research on these enigmatic organisms has illuminated many other interesting areas of biology. As this recent meeting illustrated, the application of the tools of modern molecular and cell biology to these pathogens is highly likely to yield fundamental insights into eukaryotic functions and organisation. Taxonomic challenges exist in the integration of previous descriptions of these organisms with molecular phylogenetic studies. The sampling of species of the Microsporidia has tended to focus on a few host species groups. Expansion of available data sets on the Microsporidia of other hosts

should greatly facilitate the development of a comprehensive picture of the phylogeny for these pathogens. In addition, the generation of genome data (GSS, EST or full genomes) will yield fascinating insights into these organisms and will be a crucial resource for fundamental investigations. One should, however, not neglect the need for field investigations on these organisms. There is still a need for careful epidemiologic studies of transmission to clarify the source of infections and for ecologic studies to understand the multifaceted relationship of the Microsporidia and their hosts. The organizers and participants of this First United Workshop on Microsporidia from Invertebrate and Vertebrate Hosts agreed that future meetings and exchange of information between the various scientific disciplines would greatly facilitate progress on these pathogens and have formed a committee for planning future congresses.

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ABSTRACTS

Presented at the "First United Workshop on Microsporidia From Invertebrate And Vertebrate Hosts", Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, July 12–15, 2004.

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