

Perspectives of microsporidia as human pathogens: clues from invertebrate research (minireview)¹

Yuliya Y. Sokolova

*Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia,
and Louisiana State University, Baton Rouge LA, USA*

Summary

Microsporidia (Phylum Microsporidia Balbiani 1882) are ubiquitous parasites within the Animal Kingdom. The phylum includes 1400 described species belonging to 200 genera. The host range, as well as molecular data, strongly suggest that microsporidia evolved as parasites of invertebrates and, to a lesser extent, fish. Only about 1% of microsporidia species have been found in endothermic vertebrates, birds and mammals. Microsporidiosis in humans has been observed worldwide mainly in patients with HIV infection and now increasingly in other groups such as children, immunosuppressed individuals (e.g. organ transplant recipients), contact lens wearers, travelers, and the elderly. Among AIDS patients, microsporidiosis is listed as the third important opportunistic infection causing gastrointestinal disorders, after *Cytomegalovirus* and *Cryptosporidium*. In fact, only four species belonging to two genera can be considered true mammalian parasites: *Enterocytozoon bieneusi*, *Encephalitozoon cuniculi*, *E. intestinalis*, and *E. hellem*. These represent a serious threat to human populations as zoonotic infections. Findings of other microsporidia (as a rule, parasites of arthropods or close relatives of those) in humans, are accidental. At the same time these records demonstrate the consecutive stages of microsporidia adaptation to parasitism in humans: from transient arthropod-related microsporidia known by sequences in stools of AIDS patients, through accidental surface infections in immunocompromised patients (*Endoreticulatus*-like *Microsporidium* sp., *Tubulinosema*) and development in immune privileged tissues of eyes (*Vittaforma*), skin, and muscles due to accidental exposure to spores of a “generalist” microsporidium (*Trachipleistaphora*, *Anncaliia*), to specialized infections of gut epithelium (*Enterocytozoon*), and systemic microsporidiosis disseminated by macrophages (*Encephalitozoon*). The review is addressing the following questions. What is special about the microsporidia that are able to infect warm-blooded animals? How high are the risks of acquisition of new microsporidia

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parasites by humans, given abundance of microsporidia in invertebrates, many of which may traverse food chains leading to humans and other mammals?

Key words: microsporidia, microsporidiosis, molecular phylogeny, opportunistic infections, parasitism, invertebrates

Introduction

Microsporidia (Phylum Microsporidia Balbiani 1882) are ubiquitous parasites within the Animal Kingdom. They have been recorded from nearly every major animal phylum. However, distribution of microsporidia among host taxa is far from even. Of about 1400 described species (200 genera), nearly 70% parasitize invertebrates, predominantly arthropods of the classes Insecta and Crustacea, 10% - fish. Only about 1% of known species have been found in endothermic (“warm-blooded”) vertebrates - birds and mammals, including hominids (Becnel and Andreadis, 2014; Kent et al., 2014; Stentiford and Dunn, 2014; Vávra and Lukeš, 2013). Microsporidiosis in humans has been observed worldwide mainly in patients with HIV infection and now increasingly in other groups such as children, immunosuppressed individuals (e.g. organ transplant recipients), contact lens wearers, travelers, and the elderly. Among AIDS patients, microsporidiosis is listed as the third important opportunistic infection causing gastrointestinal disorders, after *Cytomegalovirus* and *Cryptosporidium* (Sokolova et al., 2011).

The host range strongly suggests that the Phylum Microsporidia evolved as parasites of invertebrates and, to a lesser extent, fish. However, a few species managed to establish themselves in the cells of birds and mammals in spite of the temperature barrier and advanced immune defenses. In *Homo sapiens*, the best studied representative of warm-blooded animals, as many as 14 species belonging to 8 genera have been reported (Table 1)². What is special about these species? How high are the risks of acquisition of new microsporidia parasites by humans, given abundance of microsporidia in invertebrates, many of which may traverse food chains leading to humans and other mammals?

² Though the Table lists in fact 16 species, the records on *Nosema ocularum* and *Microsporidium africanum* do not contain molecular data or electron microscopy, and thus do not allow proper identification.

ORIGIN OF MICROSPORIDIA

The evolutionary origin of microsporidia has been significantly elucidated during the last 2-3 years. The consensus tree, based on phylogenies inferred from several genes with high statistical support places Microsporidia within the Aphelidea-Rozellamycota-Microsporidia (ARM) clade, a basal Fungi or sister-to-Fungi lineage (Karpov et al., 2013, Letcher et al., 2013). Aphelids are parasites of algae, and the Rozellamycota lineage comprises species parasitizing fresh-water chitrids and amoebas, as well as numerous “cryptic” species known only by their sequences. Within the ARM clade, microsporidia cluster with rozellids. Association with rozellids has been proved recently by genomic and proteomic studies on *Paramicrosporidium* spp. and *Mitosporidium daphnia*, the “missing links” between rozellids and microsporidia (Corsaro et al., 2014; Haag et al., 2014). The intranuclear parasites of free-living amoebae, *Paramicrosporidium* spp., bear striking morphological similarity with hyperparasitic metchnikovellids (subphylum Rudimicrosporidia), presumably a basal lineage of Microsporidia³ (Corsaro et al., 2014; Sokolova et al., 2013; Sokolova et al., 2014). Hence, the common ancestor of *Paramicrosporidium* and Microsporidia may have been an intranuclear parasite of a protist. This inference is supported by (i) obligatory intranuclear development in three genera (*Nucleospora*, *Desmozoon* (*Paranucleospora*) and *Enterospora*)⁴, (ii) occasional development of some species within host nucleoplasm (YS, unpublished observations), and (iii) existence of

³ So far no rDNA sequences for metchnikovellids are available through public database, making open three possible positions of Metchnikovellids: as a basal taxon of Microsporidia, as a close sister group to Microsporidia, and as a sister to *Paramicrosporidium*.

⁴ All intranuclear microsporidia are parasites of enterocytes, and ability to develop in the nucleus could be considered a rudimental trait, a “pre-adaptation” employed by nucleus-dwelling microsporidia to avoid degradation by the lysosome system of enterocytes.

Table 1. Microsporidia discovered in humans, their relatives and host groups¹.

Genus and Species	Tissue tropism	Group of animal host	Closest relatives and their host groups
Encephalitozoon			
<i>E. cuniculi</i>	Brain, IGT, disseminated.	Mammals, birds, reptiles	<i>Mockfordia xanthoaeccilliae</i> (79-81%) ² , Insects , Psocoptera; <i>E. romaleae</i> (93-96%), Insects , Orthoptera; <i>E. lacertae</i> , <i>E. pogonae</i> (96-98%) ³ , Reptiles , Lacertilia
<i>E. hellem</i>	Disseminated		
<i>E. intestinalis</i>	IGT, gall-bladder, kidney, eye		
Enterocytozoon			
<i>E. bieneusi</i>	IGT, gall-bladder, kidney, eye	Mammals	<i>Paranucleospora theridion</i> (82%), Crustaceans , Copepoda, Fish ; <i>En. hepatopenaei</i> (84%), Crustaceans , Decapoda; <i>Nucleospora salmonis</i> (80%), Fish
Anncaliia			
<i>A. vesicularum</i>	Surface, eye, skin, muscle, disseminated (<i>A. connori</i>)	Insects (<i>A.a</i>); Primates (<i>A.v.</i> , <i>A.c.</i>)	<i>Anncaliia</i> spp. (97-99%), Insects , Diptera, Coleoptera; Crustaceans , Amphipoda ⁴
<i>A. algerae</i>			
<i>A. connori</i>			
Tubulinosema			
<i>T. acridophagus</i>	Muscle, disseminated	Insects, Primates	<i>Tubulinosema</i> spp (99%), Insects , Diptera, Lepidoptera, Coleoptera, Hymenoptera, Orthoptera
Trachipleistophora			
<i>T. hominis</i>	Eye, sinus, muscle	Unknown; Exp. infection in insects	<i>T. extenrec</i> (98%), Mammals , exp. infection in insects; <i>Vavraia culicis</i> (97%), <i>V. oncoperae</i> (96%), Insects , Diptera, Lepidoptera
<i>T. anthropopthera</i>	Eye, brain, disseminated		
Vittaforma			
<i>V. corneae</i>	Eye, bladder	Unknown; Exp.infection in mammals	<i>Endoreticulatus</i> spp. (89%), <i>Cystosporogenus</i> sp.(98%), Insects , Lepidoptera, Coleoptera, Orthoptera
Endoreticulatus group			
<i>Microsporidium</i> sp.	Muscle	Unknown	<i>Endoreticulatus</i> spp. (83-91%), Insects , Lepidoptera
Pleistophora			
<i>P. ronneafiei</i> *	Muscle	Unknown	<i>Pleistophora</i> spp., Fish
<i>Pleistophora</i> sp.*		Unknown	
Nosema			
<i>N. ocularum</i> *	Eye	Identification under question No EM or molecular data available	
Microsporidium			
<i>M. africanus</i> *	Eye		
<i>M. ceilonensis</i> *			

¹ Sources of information: Cali and Takvorian, 2003; Cali et al., 1998; Cali et al., 2005; Cheney et al., 2000; Docker et al., 1997; Franzen et al., 2006a; Franzen et al., 2006b; Koudela et al., 1998; Lange et al., 2009; Nylund et al., 2010; Pilarska et al., 2015; Plischuk et al., 2015; Richter et al., 2013; Sokolova et al., 2007, 2010; Sokolova et al., in press; Suankratay et al., 2012; Tourtip et al., 2009; Vavra et al., 2006; Vavra et al., 2011; Weiss, 2014.

² Percent of identity ("relatedness") inferred from SSUrDNA-based pairwise distance analysis (in brackets).

³ YS, unpublished data.

⁴ Tokarev et al., unpublished data.

* No molecular data available.

unusual metabolic relationships of cytoplasmic microsporidia with the host nucleus, i.e. targeting microsporidian hexokinase to the host nucleus during intracellular development (Senderskiy et al., 2014). *Paramirosporidium*-like hyperparasites of Archigregarina⁵ infecting gut lumens of the common ancestor of annelids and arthropods, probably gave

rise to some lineages, like metchnikovellids and *Chytridiopsis*-like microsporidia.

⁵ Archigregarines that parasitize annelids and occasionally harbor metchnikovellids, is the earliest diverging lineages within Apicomplexa, a "polyphyletic stem" from which all other gregarines evolved (Leander, 2008).

Insects and annelids are the major host groups for both Gregarina (Perkins et al., 2000) and Microsporidia (Becnel and Andreadis, 2014), and a hypothesis that cannot be excluded posits that gregarines might have functioned as a “Trojan horse,” enabling dispersal of microsporidia from marine and brackish water annelids to terrestrial arthropods and insects (Sokolova et al., 2013).

DISTRIBUTION AMONG INVERTEBRATES AND FISH

Estimates based on the analysis of distribution of microsporidia among hosts suggest that Microsporidia ancestors switched to parasitism in oligochaetes and polychaetes during their colonization of land, migrating from marine through brackish waters of river estuaries to fresh water basins during the Cambrian and Silurian. The radiation and flowering of Microsporidia likely took place during the Carboniferous and Triassic and was associated with diversification of Arthropods (Issi, 1986). Currently, 70% of microsporidia species parasitize aquatic hosts, mostly crustaceans and insects connected with aquatic habitats (Stentiford and Dunn, 2014). The distribution of microsporidia among groups of terrestrial and freshwater arthropods included numerous host switches via polyxenous life cycles, common parasites, and food chains. The result is the contemporary abundance of species, with evolutionary bonds that have been increasingly elucidated by SSUr-DNA-inferred phylogenies (Vossbrinck and Debrunner-Vossbrinck, 2005; Vossbrinck et al., 2014), though the whole puzzle is far from assembled.

Examples of adaptation of microsporidia to parasitism in invertebrates are numerous and exquisite, from bizarre ectospore appendages, multiple spore morphotypes, and polyxenous life cycles, to effects on host behavior, population dynamics and sex ratio (Becnel and Andreadis, 2014; Stentiford and Dunn, 2014; Vávra and Larsson, 2014). Microsporidia demonstrate an arsenal of adaptations to evade the innate immunity of invertebrate hosts including modification of the phenol-oxidase cascade, accumulation in specialized haemocytes and adipocytes, stimulating host cells to grow into gigantic cells with prolonged cell cycles (e.g., “cysts” in insects, and “xenomas” in fish), that form protected and nutrient-supplied niches for developing parasites. Biochemical and molecular studies have revealed that microsporidia are able to modulate host cell cycles by inhibition of

apoptosis, and influence host gene expression and metabolism by secreting diverse regulatory factors into the host cell (Senderskiy et al., 2014; Williams et al., 2014).

Tight ecological bonds within the aquatic habitats via numerous intersecting and overlapping food chains could have played a leading role in microsporidia host switches from invertebrates to fish (and in reciprocal host transfers), as suggested by phylogenetic analyses (Stentiford et al., 2013). Circumstantial evidence indicates that the most common parasite of the White Atlantic shrimp, *Agmasoma penaei*, cycles between shrimp and perciform fish feeding on juvenile penaeids (Johnson, 1995; Overstreet, 1973; Pasharawipas and Flegel, 1994, Sokolova et al., 2015). The microsporidia parasites could have been spread among aquatic inhabitants also by parasites similar to sea fleas, *Lepeophtherius* sp. (Copepoda), which are common fish ectoparasites related to free-living cyclopids. These crustaceans, like freshwater copepods, can be parasitized by several microsporidia species at least two of which display close evolutionary distances with a fish microsporidium, *Nucleospora salmoni* (Freeman and Sommerville, 2009; Jones et al., 2012). This suggests the presence of polyxenous fish-crustaceans life cycles now or in the past. Existence of such a cycle has been recently demonstrated for *Desmozoon (Paranucleospora) theredion*, a species that parasitizes simultaneously an Atlantic salmon and its copepod parasite (Nylund et al., 2010). The much broader distribution of microsporidia among fish versus birds and mammals can be explained by the fact that switching to parasitism in fish did not demand special adaptations to the elevated body temperatures, a major factor together with humoral immunity that has limited the spread of microsporidia among warm-blooded animals.

“HUMAN MICROSPORIDIA” AND RELATED SPECIES

The importance of the “clues from invertebrate research” for understanding the origin and accessing the threat of microsporidia to the human population are evident. Excluding two *Pleistophora* species, likely related to fish congeners, 12 species and 6 genera of microsporidia recorded as infectious to humans are either insect parasites themselves, like *Tubulinosema acridophagus* and *Anncaliia algerae*, or have close relatives among insect parasites (Table 1). The only exception, *Enterocytozoon bienersi*, is likely derived from a microsporidium infecting

a marine arthropod given the broad distribution of enterocytozoonids among marine crustaceans (Stentiford et al., 2013).

Mammals are the very recent hosts for microsporidia parasites from the evolutionary perspective. Microsporidia were adapted to intracellular parasitism in invertebrates well before switching to mammals, and a few lineages were apparently more successful in expanding their host range to vertebrates than others. Hence, addressing phylogenetic bonds between the species infecting humans and those parasitizing invertebrates might help to answer questions posed in the first paragraph of this short review.

CYSTOSPOROGENES/ENDORETICULATUS/VITTAFORMA CLADE

Vittaforma corneae was once isolated from corneal stroma of immunocompetent HIV-negative patient, and was the first human microsporidium placed in culture. However, some authors maintain that this species cannot be considered a true human pathogen (Van Frankenhuyzen et al., 2004). It shares 98.2% identity of its rDNA sequence with the lepidopteran microsporidium *Cystosporogenes legeri* and most likely is an unknown isolate of a closely related *Cystosporogenes* species accidentally developing in the immune-privileged site. Infection with *V. corneae* in immunocompetent patients is associated with self-limited short-term conjunctivitis caused presumably by traumatic inoculation of environmental spores of the insect pathogen (Weiss, 2014). Recently another species clustering within the same *Endoreticulatus-Cystosporogenes-Vittaforma* clade was found to cause myositis in the immunocompetent patient (Suankratay et al., 2012). Comparatively low identity (83–91%) of this *Microsporidium* sp. precluded the authors from assigning it to *Endoreticulatus*. The *Endoreticulatus-Cystosporogenes-Vittaforma* clade is composed predominantly of parasites of *Lepidoptera*. *Endoreticulatus* spp. though also has been isolated from two other orders of insects, *Coleoptera* and *Orthoptera* (Pilarska et al., 2015). *Cystosporogenes legeri*, a common parasite of insect rearing facilities, infects as many as 5 families of *Lepidoptera* demonstrating unusually broad host range (Van Frankenhuyzen et al., 2004). So, among representatives of this clade there are generalist parasites with broad host ranges among natural hosts. Another feature that might facilitate the transition to a new group of hosts is resistance of *Cystosporogenes*

spp. spores to high (up to 42°C) temperatures (Van Frankenhuyzen et al., 2004). Butterflies and moths, some of which are known as facultative blood and tear feeders (Plotkin and Goddard, 2013; Zaspel et al., 2014), could be vectors for transmission of microsporidia belonging to this clade.

ANNCALIIA/TUBULINOSEMA CLADE

Anncaliia/Tubulinosema clade is composed of two genera with extraordinarily broad host ranges for insect microsporidia. The host range of *Anncaliia* spp. includes representatives of at least two insect orders, *Coleoptera*, and *Diptera* (Franzen et al., 2006b), and also amphipod crustaceans (Tokarev et al., 2014). *Tubulinosema* spp. parasitize as many as 5 insect orders (*Lepidoptera*, *Orthoptera*, *Coleoptera*, *Diptera* and *Hymenoptera*) (Franzen et al., 2006a). *Tubulinosema acridophagus* from a grasshopper was found to cause myositis and disseminated infection in a patient with a bone marrow transplant (Weiss, 2014). This is a typical opportunistic infection, but as in the case involving the *Endoreticulatus*-related *Microsporidium* sp., it clearly demonstrates insignificance of temperature as a limiting factor for the parasite development. *Anncaliia* spp. probably diversified further as parasites of mammals. *Anncaliia (Brachiola) algerae*, a common mosquito parasite of several genera of mosquitoes (Andreadis, 2007), occasionally infects brain and eye tissues, and causes disseminated disease in immunocompromised individuals. It also may induce skin and muscle infections presumably transmitted by a mosquito vector in immunologically healthy humans (Weiss, 2014). *Anncaliia algerae* is known to develop infections in SCID mice (Koudela et al., 2001), and tolerate elevated temperatures; it can be cultivated in cell lines at >36°C (Trammer et al., 1999). Resistance of *A. algerae* to high temperatures could be an ecological adaptation, since this parasite in nature infects mosquito larvae inhabiting small pools heated during the summer period. The two other representatives of the genus, *A. connori* and *A. vesicularum*, have been recorded from humans with immunodeficiency, and their environmental source is unknown (Weiss, 2014).

TRACHIPLEISTOPHORA/VAVRAIA CLADE

Representatives of this clade, *Trachipleistophora hominis* and *T. anthropoptera*, the most widespread causative agents of human myositis due to micro-

sporidia, have been recorded from several immunodeficient individuals (Suankratay et al., 2012). Insect origin of these infections was suggested by successful experimental infection of insect larvae with human-isolated *T. hominis* (Weidner et al., 1999). One representative of this genus was described from a Madagascar insectivore, *Hemicentatis semispinosus* (Vávra et al., 2006). Interestingly, this mammal belongs to the peculiar family Tenrecidae (order Afrosoricida), which members are characterized by lower body temperatures. The spores isolated from the animal were also infectious to *Spodoptora littoralis* (Lepidoptera) larvae. It is unclear whether *T. extenrec* is a native parasite of tenrecs, or an insect pathogen. It may develop in both types of hosts, suggesting a potential transmission route from insects to insectivorous mammals. *Trachipleistaphora hominis* is a close relative of the mosquito microsporidium *Vavraia culicis*, sharing with the latter 98% of SSUrDNA sequence similarity. *Vavraia culicis* parasitizes mosquitoes belonging to 6 genera (Andreadis, 2007). Microsporidia from mosquitoes, as a rule, are species- or genus-specific “specialists.” Among >30 mosquito-infecting microsporidia a similarly broad range of hosts is known only for the above mentioned human-pathogenic species, *Anncaliia algerae* (Andreadis, 2007). One more *Vavraia* (*V. oncoeperae*) was described from a lepidopteran host (Malone and McIvor, 1995).

Interestingly, all three lineages of insect microsporidia that contain forms known to infect humans, include taxa of generalist pathogens as well as species tolerating high temperatures. Such consistency may suggest specific biochemical pre-adaptations required for transmission to a foreign warm-blooded host for these groups. Genes and regulatory factors responsible for these adaptations are yet to be identified by genomic and proteomic analyses. Factors analogous to LRR proteins or products of the *InterB* multigene family (Williams et al., 2014) might potentially play a role in regulating limits of host specificity within certain lineages.

ENTEROCYTOZON BIENEUSI AND *ENCEPHALITOOZON* SPP.

Enterocytozoon bienewsi, a specialized parasite of enterocytes, is the most common microsporidium known to cause diseases in humans, particularly in patients with AIDS. *E. bienewsi* is widely distributed among several orders of mammals, and also has been recorded in birds (Fayer and Santin-Duran, 2014).

The evolutionary history of *E. bienewsi* parasitism in vertebrates is probably relatively short, since the taxon has not diversified into separate species, but is represented by numerous genotypes with different levels of host specificity (Fayer and Santin-Duran, 2014). The closest relatives of this microsporidium infect fish and crustaceans (Stentiford et al., 2013), so *E. bienewsi* ancestors probably transferred to parasitism in vertebrates from these hosts via food chains.

Five of six species of the genus *Encephalitozoon* – *E. cuniculi*, *E. intestinalis*, *E. hellem*, *E. lacertae* and *E. pogonae*, parasitize vertebrates – mammals, birds and reptiles, and one species, *E. romaleae*, has been found in an insect, the lubber grasshopper *Romalea microptera* (Lange et al., 2009). *Encephalitozoon cuniculi* is the most famous and ubiquitous microsporidium of mammals. It has diverged into at least three mammalian host-specific genotypes (Didier et al., 1995). In reptiles *E. cuniculi* or morphologically identical species causes multi-systemic granulomatous disease (Koudela et al., 1998; Richter et al., 2013). Infection discovered recently in a bearded dragon *Pogona vitticeps* (Richter et al., 2013), were caused by a yet unknown genotype that occurred to be a new species *Encephalitozoon pogonae* (Sokolova et al., in press). *E. hellem* is a natural pathogen of birds, and *E. intestinalis* is more restricted to humans (Snowden, 2014). *Encephalitozoon* spp, unlike *E. bienewsi*, are not confined to infecting gastrointestinal tracts, but often cause disseminated microsporidiosis (Weiss, 2014). In phylogenetic reconstructions the *Encephalitozoon* lineage clusters within the Clade 4 of “Terresporidia” (Vossbrinck and Debrunner-Vossbrinck, 2005) composed of predominantly insect microsporidia. The *Encephalitozoon* branch forms a dichotomy with *Mockfordia xanthocaeciliae*, a parasite of *Xanthocaecilia sommermanae*, order Psocoptera. Psocoptera is considered to be the most basal order of hemipteroids, originating during the Permian Period 295–248 million years ago. Psocoptera are closely related to Phthiraptera, sucking lice, which parasitize warm-blooded animals including humans. These two orders are placed in the infraorder Psocodea and share a common ancestor, based on robust morphological and molecular evidence (Johnson and Mockford, 2003; for other references, see Sokolova et al., 2010). Though the majority of barklice are free living species, various species of Psocoptera inhabit plumage of birds and the pelage of mammals, as well as their nests. This short-

term commensal-type relationship presumably gave rise to obligate parasitism characteristic to Phthiraptera (Johnson et al., 2004). Evidence of a close relationship of *M. xanthocaecilliae* to *Encephalitozoon* spp. (Sokolova et al., 2010), ubiquitous parasites of birds and mammals, supports the idea that the association of ancestral Psocodea with mammals and birds could be one of the avenues of transfer of Microsporidia from arthropods to warm-blooded hosts. Within the *Encephalitozoon* clade the position of *E. romaleae* (Lange et al., 2009), which shares 96% of SSUrDNA similarity with *E. hellem*, certainly creates a problem. As an explanation of striking genetic relatedness of *E.romaleae* to *E. hellem*, perhaps this species evolved as a result of reciprocal transfer of the *E. hellem*-related bird-infecting microsporidium back to insects (Sokolova et al., 2010). The genomic survey revealed that the genomes of *E. hellem* and *E. romaleae* contained the gene for purine nucleotide phosphatase (PNP), a component of the purines salvage pathway, of insect origin (Pombert et al., 2012, Selman et al., 2011). This gene is absent in the genomes of *E. intestinalis*, *E. cuniculi* and other microsporidia with sequenced genomes, and likely was acquired from an insect host by a common ancestor of *E. romaleae* and *E. hellem*. The narrow distribution of this gene is most consistent with its recent gain (Selman et al., 2011) and conforms to the idea of reciprocal transfer that might have occurred relatively recently. Of note, the *Encephalitozoon* spp.-derived PNP genes cluster with the orthologue from *Pediculus humanus* (Phthiraptera) (Fig. 1 in Selman et al., 2011). This suggests that a lice-related ectoparasite of birds harboring an ancestral encephalitozoonid could have been a source for the PNP gene transfer from insects to the *E. hellem*-*E. romalea* lineage. Further molecular studies based on broader sampling and robust analyses could test this hypothesis.

Concluding remarks and further directions

In fact, only four species belonging to two genera can be considered true mammalian parasites: *Enterocytozoon bieneusi*, *Encephalitozoon cuniculi*, *E. intestinalis*, and *E. hellem*. They have evolved as parasites of warm-blooded vertebrates and might represent a serious threat to human populations as zoonotic infections (Fayer and Santin-Duran, 2014). Records of other microsporidia in mammals, including humans, are more or less accidental.

However, it is hard to argue the opinion expressed by Irma V. Issi, that “now microsporidia represent a numerous and aggressive group of parasites expanding the range of their hosts” (Issi, 1986). Recorded cases of microsporidiosis demonstrate the consecutive stages of microsporidia transforming into parasites of humans: from transient arthropod-related microsporidia known by sequences in stools of AIDS patients (Genbank accessions CQ408913, CQ408914; Sokolova et al., 2011), through accidental surface infections in immunocompromised patients (*Endoreticulatus*-like *Microsporidium* sp., *Tubulinosema*) and development in immune privileged tissues of eyes (*Vittaforma*), skin, and muscles due to accidental exposure to spores of a “generalist” microsporidium (*Trachipleistophora*, *Anncaliia*), to specialized infections of gut epithelium (*Enterocytozoon*), and systemic microsporidiosis disseminated by macrophages (*Encephalitozoon*). Potential sources of human infection with invertebrate microsporidia are likely associated with “generalist” parasites, and, particularly, with human (mammalian) hyperparasites. Further surveys of Microsporidia in Psocoptera, Phthiraptera and related orders, as well as in other ectoparasitic or bloodsucking insects (fleas, bed-bugs, dipterans and hematophagous lepidopterans) and acarines (ticks, mites and chiggers) will shed light on evolutionary routes of host transfers as well as on possible risks of infection.

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