RESULTS:

The results of the Ph.D. thesis can be summarized into 4 thematic areas:

1) screening and experimental data concerning the prevalence, genus and species diversity, host and tissue specificity and of ecological, pathofyziological characters of infections and of their transmission.

2) classification and taxonomy studies

3) results of sequencing followed by phylogeny analysis

4) results of comparative study based on summarized data

Ad 1)
Twenty-four microsporidia from Trichoptera and Lepidoptera as well as 5 microsporidia from potential intermediate hosts (Crustacea, Amphipoda) were isolated and screened using light and electron microscopy and molecular biology methods. The screened organisms represented the following genera in the conventional classification of Microsporidia (Canning and Vávra, 2000) *Nosema* (7), *Endoreticulatus* (3), *Cystosporogenes* (2), *Thelohania* (4), *Gurleya* (1), *Cougourdella* (1), *Pyrotheca* (1), *Episeptum* (5), *Toxoglugea* (1), *Larssoniella* (2), unresolved isolates (2) (the number of species examined in respective genera is given in parentheses).
The publications (1-3) and the abstract (8) report the ecological and experimental data on the prevalence, host and tissue specificity and transmission of 10 examined microsporidia. The publication (4) and the abstract (7) describe ecological and experimental data on several [illegible] of microsporidia of the gypsy moth, *Lymantria dispar* (Lepidoptera) in the research of [illegible] author was engaged.
Summary: all studied microsporidia from Trichoptera beloning to genera *Episeptum*, *Paraepiseptum* n.g., *Zelenkaia* n.g. are host-species specific parasites infecting fat body and oenocytes of their hosts. Their spores are not infective for the original host, their life cycle involves probably an intermediate host and (or) transovarial transmission. Data obtained by rDNA sequencing (unpublished) showed that microsporidia (genera *Thelohania, Gurleya, Toxoglugea*) from several of *Gammarus* spp., occurring sympatrically with Trichoptera are not involved in the life cycle of trichopteran microsporidia.
All studied microsporidia from Lepidoptera and belonging to the genus *Nosema* a *Vairimorpha* are transmitted both horizontally and vertically (i.e. per os and transovarially). The species of the genus *Nosema* are highly host-species specific microsporidia infecting preferably salivary and silk glands or fat body. The studied isolates of *Vairimorpha disparis* n. comb. are not host specific, they are able to infect a wide spectrum of more or less closely related Lepidoptera, the part of their life cycle, producing uninucleate meiospores (octospores) is specifically located in the fat body.

Ad 2)
The publication (1) describes a new genus of a dispoblastic microsporidian from Trichoptera with the type species *Zelenkaia trichopterae* gen. et sp. nov.; publication (2) describes 4 new species of tetrasporoblastic microsporidia from Trichoptera (*Episeptum trichoinvadens* n. sp., *E. pseudoinversum* n. sp., *E. anaboliae* n. sp. a *Paraepiseptum plectrocnemiae* n. sp.), the genus *Episeptum* is redefined and tranfers the trichopteran microsporidia of the genera *Cougourdella* and *Pyrotheca* into a newly created genus *Paraepiseptum* gen. nov.; publication (3) describes a new microsporidian from Lepidoptera *Nosema chrysorrhoeae* n.sp.; publicaton (4) is a redescription of *Thelohania disparis* Timofejeva 1956 and its redefinition as *Vairimorpha disparis* n. comb.; publication (5) is the revision of the species *Marssoniella elegans* Lemmermann, 1900 infecting *Cyclops vicinus* (Crustacea, Copepoda),

Paraepiseptum gen. nov. from Trichoptera.

Ad 3)

Data in publications (1-3) and (5) resulted in the GenBank deposition of 23 sequences of partial rDNA genes from lepidopteran and trichopteran microsporidian species isolated either from fresh spores or from the syntype and archival slides (publication 6). The abstract (8) discusses phylogeny of trichopteran genera including *Issia* and *Thelohania* based on so far not released sequences.

Summary: microsporidia from Trichoptera including genera *Episeptum, Paraepiseptum, Zelenkaia, Issia* and *Amblyospora (Thelohania)* form several separate groups within a large clade uniting microsporidia from crustacea (first of all Copepoda and Cladocera) and insects with aquatic larval stages (Diptera - Culicidae, Simuliidae). This clade is a sister group of a well defined clade uniting microsporidia from mosquitoes (some having crustacea as intermediate hosts) clustered around the central genus *Amblyospora* ("*Amblyospora* group"). One of the trichopteran group is represented by phylogenetically close related tetrasporoblastic species of the genera *Episeptum* and *Paraepiseptum*, together with the copepode microsporidium *Marssoniella elegans*. This group is phylogenetically related to structurally strikingly different species of the disporoblastic genus *Zelenkaia*. The representatives of genera *Issia* and *Amblyospora (Thelohania)* from Trichoptera are closest to the group including microsporidia of the genus *Parathelohania*.

Microsporidia of the studied genera *Nosema* a *Vairimorpha* from Lepidoptera represent a very closely phylogenetically related group of parasites of the lepidopteran family Lymantriidae, situated within the microsporidia of Hymenoptera (species *N. ceranae, N. apis, N. bombi*). The second sister subgroup of the large clade ("*Nosema/Vairimorpha* group") is formed by microsporidia around *N. bombycis* with *Nosema* spp. and *Vairimorpha* spp. from amphipode and decapode crustacea. The phylogenetic distances among the species of the genus *Nosema* (also in relation to species of the genus *Vairimorpha*) are significantly smaller than distances among respective species of the genera of trichopteran microsporidia. However, the strict host specifity of *Nosema* spp. from family Lymantriidae indicates that the species involved are valid taxons.

Ad 4)

General conclusion to phylogeny and classification of microsporidia based on the data of the Ph.D. thesis:

(i) It occurs that the presently known microsporidia from Trichoptera and Lepidoptera have no common and direct ancestor in their evolution. It is hypothetized that microsporidia invaded both groups independently, probably from ancestors parasitizing crustacea. The key role in that probably played insects having aquatic life cycle stages. From these insects then microsporidia radiated into further different terrestric hosts.

(ii) The mosaic distribution of crustacean microsporidia in phylogeny trees suggests that the radiation model mentioned above probably was repeated in other groups of hosts who acquired microsporidia from crustacea living with them in the same environment.

(iii) The life cycles of microsporidia switching insects and crustacea as their hosts (e.g. mosquito *Amblyospora* spp.) are probably relics of the original radiation event. Phylogeny data suggests that microsporidia from Trichoptera could be of similar type.

phylogeny. The main structural distinguishing characters (especially for high level taxa) are plesiomorphic. The relative structural uniformity of microsporidia suggests that molecular characters providing signals appropriate for classification must be sought in future (for new classification).

REFERENCES:

Baker, M.D., Vossbrinck, C.R., Maddox, J.V. and Undeen, A.H. 1994. Phylogenetic relationships among *Vairimorpha* and *Nosema* species (Microspora) based on ribosomal RNA sequence data. J. Invertebr. Pathol. 64: 100-106.

Baker, M.D., Vossbrinck, C.R., Didier, E.S., Maddox, J.V. and Shadduck, J.A. 1995. Small subunit ribosomal DNA phylogeny of various microsporidia with emphasis on AIDS related forms. J. Euk. Microbiol. 42: 564-570.

Baker, M. D., Vossbrinck, C. R., Becnel, J. J. & Maddox, J. V. 1997. Phylogenetic position of *Amblyospora* Hazard & Oldacre (Microspora: Amblyosporidae) based on small subunit rRNA data and its implication for the evolution of the Microsporidia. *J. Eukaryot. Microbiol.*, 44:220–225.

Baker, M.D., Vossbrinck, C.R., Becnel, J.J., Andreadis, T.G., 1998. Phylogeny of *Amblyospora* (Microsporidia: Amblyosporidae) and related genera based on small subunit ribosomal DNA data: a possible example of host parasite cospeciation. J. Invertebr. Pathol. 71: 199-206.

Becnel, J. J., White, S. E. Shapiro A. M. 2005. Review of microsporidia-mosquito relationships: from the simple to the complex. *Folia Parasitologica* 52: 41-50.

Bell A. S., Aoki T, Yokoyama H. 2001. Phylogenetic relationships among microsporidia based on rDNA sequence data, with particular reference to fish-infecting Microsporidium balbiani 1884 species. *J Eukaryot Microbiol.* 48(3):258-65.

Brown, A. M., Adamson, M. L. 2006. Phylogenetic distance of *Thelohania butleri* Johnston, Vernick, and Sprague, 1978 (Microsporidia; Thelohaniidae), a parasite of the smooth pink shrimp *Pandalus jordani*, from its congeners suggests need for major revision of the genus *Thelohania* Henneguy, 1892. *J Eukaryot Microbiol.* 53(6): 445-55.

Brosson D., Kuhn L., Delbac F., Garin J., P. Vivares C., Texier C. 2006. Proteomic analysis of the eukaryotic parasite *Encephalitozoon cuniculi* (microsporidia): a reference map for proteins expressed in late sporogonial stages. *Proteomics.* 6(12): 3625-35.

Cali A, Takvorian PM, Lewin S, Rendel M, Sian CS, Wittner M, Tanowitz HB, Keohane E, Weiss LM. 1998. *Brachiola vesicularum*, n. g., n. sp., a new microsporidium associated with AIDS and myositis. *J Eukaryot Microbiol.* 45(3):240-51.

Canning, E. U. & Lom, J. 1986. The Microsporidia of Vertebrates. Academic Press, London, UK. 289 p.

Canning, E. U. 1990. Microspora. *In* Margulis, L., Corliss, J. O., Melkonian, M., Chapman, D., Jones, J., ed. Handbook of Protoctista. Bartlett, Boston. p. 53-72.

Canning, E. U., Curry, A., Cheney, S., Lafranchi-Tristem, N. J., Haque, M. A. 1999. *Vairimorpha imperfecta* n.sp., a microsporidian exhibiting an abortive octosporous sporogony in *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *Parasitology.* 119 (3): 273-86.

Canning EU, Curry A, Cheney SA, Lafranchi-Tristem NJ, Kawakami Y, Hatakeyama Y, Iwano H, Ishihara R. 1999b. *Nosema tyriae* n.sp. and *Nosema* sp., microsporidian parasites of Cinnabar moth *Tyria jacobaeae*. *J Invertebr Pathol.* 74 (1):29-38.

Canning, E. U., Vávra, J., 2000. Phylum Microsporidia Balbiani, 1882. In: J.J. Lee, G. F. Leedale, P. Bradbury (Eds). The Illustrated Guide to the Protozoa (2nd Edition), Vol. 1. Society of Protozoologists, Lawrence, Kansas, pp. 39-126.

Canning, E. U., Refardt, D., Vossbrinck, C. R., Okamura, B., Curry, A. 2002: New diplokaryotic microsporidia (Phylum Microsporidia) from freshwater bryozoans (Bryozoa, Phylactolaemata). Eur. J. Protistol. 38: 247–265.

Canning E.U., Curry A., Overstreet R.M. 2002b. Ultrastructure of *Tuzetia weidneri* sp. n. (Microsporidia : Tuzetiidae) in skeletal muscle of *Litopenaeus setiferus* and *Farfantepenaeus aztecus* (Crustacea : Decapoda) and new data on *Perezia nelsoni* (Microsporidia : Pereziidae) in *L.setiferus*. *Acta Protozoologica* 41 (1): 63-77.

Cavalier-Smith T. 1983. A six-kingdom classification and a unified phylogeny. In Endocytobiology II: Intracellular Space as Oligogenetic, ed. HEA Schenk, WS Schwemmler, pp. 1027–34. Berlin: Walter de Gruyter & Co.

Cheney, S. A., Lafranchi-Tristem, N. J. & Canning, E. U. 2000. Microsporidia based on small subunit ribosomal DNA sequences and implications for the source of *Trachipleistophora hominis* infections. *J. Eukaryot. Microbiol.*, 47:280–287.

Cheney, S. A., Lafranchi-Tristem, N. J., Bourges, D. & Canning, E. U. 2001. Relationships of microsporidian genera, with emphasis on the polysporous genera, revealed by sequences of the largest subunit of RNA polymerase II (RPB1). J. Eukaryot. Microbiol., 48:111–117.

Curgy, J.J., Vávra, J. and Vivares, C.. 1980. Presence of ribosomal RNAs with prokaryotic properties in Microsporidia, eukaryotic organisms. *Biologie Cellulaire* 38: 49-52.

Edlind TD, Li J, Visvesvara GS, Vodkin MH, McLaughlin GL, Katiyar SK. 1996. Phylogenetic analysis of tubulin sequences from amitochondrial protozoa. Mol. Phylogenet. Evol. 5:359–67

Fast, N. M., Logsdon, J. M. & Doolittle, W. F. 1999. Phylogenetic analysis of the TATA box binding protein (TBP) gene from *Nosema locustae*: evidence for a microsporidia–fungi relationship and spliceosomal intron loss. *Mol. Biol. Evol.*, 16:1415–1419.

Findley, A. M, Weidner, E. H, Carman, K. R., Xu, Z., Godbar, J. S. 2005. Role of the posterior vacuole in *Spraguea lophii* (Microsporidia) spore hatching. *Folia Parasitol* . 2005 52(1-2):111-7.

Fries, I., Paxton, R. J., Tengo˝, J., Slemenda, S. B., da Silva, A. J. & Pieniazek, N. J. 1999. Morphological and molecular characterization of *Antonospora scoticae* n. gen., n. sp. (Protozoa, Microsporidia) a parasite of the communal bee, *Andrena scotica* Perkins, 1916 (Hymenoptera, Andrenidae). *Europ. J. Protistol.*, 35:183–193.

Germot A, Philippe H, Le Guyader H. 1997. Evidence for loss of mitochondria in Microsporidia from a mitochondrial type HSP70 in Nosema locustae. Mol. *Biochem. Parasitol.* 87:159–68

Gill, E. E. & Fast, N. M. 2006. Assessing the microsporidia–fungi relationship: combined phylogenetic analysis of eight genes. *Gene* 375, 103–109.

Hall, B. D., Liu, Y. J., Hodson, M.C. 2006. Loss of the flagellum happened only once in the fungl lineage: phylogenetic structure of Kingdom Fungi inferred from RNA polymerace II subunit genes. BMC Evolutionary Biology 6: Art. No. 74.

Hinkle G, Morrison HG, Sogin ML. 1997. Genes coding for reverse transcriptase, DNA-directedRNApolymerase, and chitin synthase from the microsporidian *Spraguea lophii*. *Biol. Bull.* 193:250–51.

Hirt RP, Healy B, Vossbrinck CR, Canning EU, Embley TM. 1997. A mitochondrial Hsp70 orthologue in *Vairimorpha necatrix*: molecular evidence that microsporidia once contained mitochondria. *Curr. Biol.* 7:995–98.

Hirt, R. P., Logsdon, J. M., Healy, B., Dorey, M. W., Doolittle, W. F. & Embley, T. M. 1999. Microsporidia are related to Fungi: evidence from the largest subunit of RNA polymerase II and other proteins. *Proc. Natl. Acad. Sci.* USA, 96:580–585.

Huang, W. F., Tsai, S. J., Lo, C. F., Soichi, Y. & Wang, C. H. 2004. The novel organization and complete sequence of the ribosomal gene of *Nosema bombycis*. Fung. Genet. Biol., 41:473–481.