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INVITED COMMENT

Molecular versus morphological approach to microsporidian classification

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Classifications reflect the actual state of art and change with time. New tools and techniques generate new information and create platforms offering new points of view. This is excellent and the way science should operate, but sometimes the adoption of new techniques creates unnecessary problems. If the new techniques are used as substitutes, not as complements, to well-established and useful methods, comparison between old and new material is made impossible. The study of microsporidia has reached the breaking point where the classical cytology, which has been the base of microsporidian studies since the very beginning, has lost its importance and it has actually been replaced by the molecular biology. Drs. Vossbrinck and Debrunner-Vossbrinck have adopted the new view, and the result is a new phylogeny and a new classification for a selection of microsporidian species (Vossbrinck and Debrunner-Vossbrinck 2005).

The authors express the view shared by many molecular biologists "that structural and ultrastructural characters are unreliable for distinguishing among higher-level microsporidian taxa", and they give a number of examples of useless cytological characters. However, no serious cytologist would regard the examples given as characters with a potential to reveal relationships worth mentioning. For that purpose the cytological analysis must be considerably more detailed. It is not sufficient to count the number of polar filament coils and measure their width – the internal organisation must be evaluated. It is of no use to state that the exospore is electron-dense – it has normally a distinct structure. And to report that there is an interfacial envelope separating the parasite from the host is of no use at all. Nevertheless has the sporophorous vesicle, which is the parasite-derived interfacial envelope, a great potential for revealing relationships. It is easy to make a long list of fundamental varieties of construction and origin, but three examples will be sufficient to show that this is a cytological character of phylogenetic importance. Metchnikovellideans and chytridiopsids have a unique sporophorous vesicle where the plasma membrane of the sporont is incorporated into the wall of the vesicle, not into the spore wall as in all other microsporidia. Pleistophorids have a thick, merontogenetic sporophorous vesicle, i.e., it is formed prior to the sporogony. The most common type is the sporontogenetic sporophorous vesicle. It is formed as a secretory product from the plasma membrane of the sporont, but the initiation, structure and inclusions vary greatly between taxa. The real and obvious problem with the cytological characters is not that they are useless but that the evaluation is time-consuming – a time molecular biologists, used to rapid results, apparently are not willing to spend.

The authors conclude that the ecology has been the important factor in the evolution of microsporidia, and they distinguish clades of microsporidia with marine, freshwater and terrestrial origin. None of the clades is strictly confined to the particular habitat. A close look at the cladograms reveals a number of microsporidia with erroneous habitat classification. *Flabelliforma montana* uses a terrestrial host (not freshwater). The amphipods hosting *Dictyocoela cavimanum* (*Talitrus* sp., *Orchestia cavimana*), *D. deshayesum* (*Talorchestia deshayesii*) and *D. gammarellum* (*Orchestia gammarellus*) are all living in the sand on sea shores (not freshwater species), and the host of *Kabatana takedai* is a freshwater fish (not marine). Personally I have never considered *Glugea anomala* to be a freshwater parasite. I have never found it in sticklebacks from freshwater biotopes, but it can easily be collected from sticklebacks living in brackish water. Even if these mistakes are corrected, mixing of habitats remains.

Some of the genera are split, and their species distributed into different clades. One explanation could be polyphyletic genera or, expressed by other words, that some of the species have been assigned tentatively to these genera. Another explanation might be that the gene sequences published actually are from other microsporidia than the species reported. Practically all genera of microsporidia are defined by cytological characteristics, but the cytological evidences for the identification of the species are lacking in many molecular studies.

The authors have obvious problems with explaining why some of the taxa branch in the "wrong" place. In Clade III, containing microsporidia of marine origin, a subclade is formed by the genera *Pleistophora*, *Heterosporis*, *Ovipleistophora*, *Vavraia* and *Trachipleistophora.* This group causes embarrassment. As neither the *Vavraia* species nor *Trachipleistophora hominis* occur in marine environment, "the anomalous placements

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…cannot be explained so easily". However, for a cytologist this subclade of the cladograms is the only possible place for *Vavraia* and *Trachipleistophora*. All genera of this subclade share the unique synapomorphy of the family Pleistophoridae: the thick merontogenetic sporophorous vesicle.

It is apparent that also Clade I, with freshwater microsporidia, is well supported by cytological and developmental characteristics. This is the clade of the polymorphic species using intermediate hosts and with up to three spore morphs in the life cycle. One obvious synapomorphy of this clade is the lanceolate spore with the unique polaroplast, composed of rounded, bladder-like compartments. Another is probably the thick, layered exospore of the meiospore. It is a complication that the life cycle has not been worked out for all species included in this clade. *Amblyospora* sp. 2 is represented by the copepod morph while e.g. *Amblyospora connecticus* has been sequenced from the culicid morph. It is possible, or even probable, that the life cycles of *Larssonia obtusa*, *Berwaldia schaefernai* and *Trichotuzetia guttata* are more complex than known today. The suggested cytological synapomorphies of the clade are not present in the morphs described, but they might be present in stages unknown at present time. What is hiding behind *Vairimorpha* sp. and *Amblyospora* sp. 3 is unknown to me.

The authors suggest that the ancestral microsporidian was aquatic and had a complex life cycle. While nothing speaks against aquatic origin, it is difficult to believe that the complex life cycles, with up to three hosts, are ancestral. In gregarines and coccidia, parasitic protists where the phylogeny is more obvious, the aquatic (in those cases marine) origin is apparent, but it is also apparent that the complexity of the life cycles has increased successively.

The taxonomic discussion and conclusions are the controversial part of the paper. It is correct that previous attempts to classify microsporidia using cytological characteristics have failed. The cytology of microsporidia is far from well known. It is not correct that three *Nosema* species have been renamed. The species have been removed from *Nosema* and three new genera (*Brachiola*, *Vittaforma* and *Paranosema*) have been established with these species as type species. And I doubt the authors really believe that the temperature causes certain *Vairimorpha* species to loose their diplokarya – their genes for diplokarya remain even if they are expressed only at certain temperatures.

The authors have based their analysis on about 10% of known microsporidia. They are well aware that this is a small fraction. They admit that there are problems with the molecular characters ("there may not be total phylogenetic agreement among genes") and that their conclusions "may require adjustment of the taxonomy presented here". They summarize that "neither the taxonomic designations given here nor the phylogeny presented in Figs. 1 and 2 represent a final classification". Under these circumstances it is surprising to find that three new classes are established and named. It has not been stated how the new classes relate to existing classes. Actually this action is not as problematic as it might appear. None of the new classes has been described. There are no diagnoses telling what characters, genetic or cytological, that differentiate the new nominal taxa. The three names are *nomina nuda* and hence can be made available at a later time, for these or other groups, by any taxonomist.

In my opinion it would have been better if this phylogeny had been presented as an opinion, without unsupported taxonomic statements and creation of naked names. The cladograms do not contain much that is controversial, even if there are uncertain branches here and there, and many of the problems are easily explained with cytological knowledge. Even if the base of the phylogeny is a molecular analysis, cytological characters would give a similar result, and that could also be expected. The cytology is the visual expression of the genome, and if both the cytology and the genetics have been evaluated correctly, cladograms based on the two sets of characters should correspond.

References

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