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STATEMENT OF THE BACHELOR THESIS REVIEWER

Name of the student:
Kristýna Cimrhanzlová

Thesis title:
Characterization of new diplonemid species via electron microscopy, starvation experiments, and immunofluorescence studies

Supervisor:
Michael John Hammond, Ph.D.
Co-Supervisor:
Tashyreva Daria, Ph.D.

Reviewer: Eva Doleželová, Ph.D.
Reviewer`s affiliation: Biology Centre, CAS, Institute of Parasitology, Ceske Budejovice

Kristýna Cimrhanzlová performed an extensive analysis of four new species of marine diplonemids. The Bachelor thesis is very well written with excellent English and a large amount of experimental data. She showed exceptional abilities and skills, especially in the preparation of biological samples for different microscopical techniques. As a reviewer, I am impressed by the quality of figures and also by the way how they are presented. I really appreciate the graphical layout of individual images that meets the standards required in scientific publications.

The Introduction part is shorter but satisfactory. The references are well chosen. I would add description of features that diplonemids share with their related parasites or predators (the second paragraph).
The methods section is well organized. I have found just a few minor weak points. First, I miss the way how the sequences were obtained (e.g., which company was used). Second, I would recommend using a more precise description of the amount of material for DNA isolation – "5 ml of healthy culture" (page 6) is not suitable for a scientific text. Also, sometimes the concentrations are missing in the tables (primers – table 4, Ethidium bromide – table 6). For IFA analysis, an ATPase antibody was used; there I miss the reference about this antibody.

The Results and Discussion part is packed with results, and these are very well described. Despite the high quality, I have a few comments and questions. In the bachelor thesis, there should also be a picture of agarose gel with PCR products. More importantly, I would appreciate a better description of how the 18S sequences were proceeded, which parts were

chosen for phylogenetic analysis, and how long was the sequence used in the analysis. In the text - page 16, there is a statement that clone 9 cells are under stress more rounded (figure5), but there is no such description in the figure legend. I would appreciate the definition of “metabolic movement“, could you please describe it?
I would like to ask how it is possible that the dye SYTO24 stains bacteria and mitochondrial DNA, but it does not stain nuclei of diplonema.
Rhynchopus species have got two flagella. Do they possess the same length? Why did you measure the flagella length in only some cells (e.g., n=11 for flagella, n=50 for cells)?
The mean and the standard deviation should be used in a similar range, so when the mean is rounded to $1x10^6$, then the SD must be rounded accordingly. Instead, the SD is shown as exact number (e.g., mean=$1x10^6$ and SD=72157.96 cell/ml). Also, I would recommend replacing rather vague terms “a few“, “a minority“, “some cells“ with more accurate descriptions.
I would like to ask the author to explain the statement that the endosymbionts of cl. 10.3 and KQ12 are more related than their hosts to each other.
Could you comment on the presence of Holosporales as endosymbionts in the Diplonemida group?
I found the discovery of a new organelle in YPF1806 as exciting. Have you or your colleagues observed this structure in any other currently described species?

Conclusion:

In conclusion, I recommend the thesis for the defense, and I suggest the grade excellent.

České Budějovice Eva Doleželová