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To whom it may concern

Review report of PhD thesis by Mgr. Klára Jiráková

The Ph.D. thesis presented by Klára Jiráková summarizes her efforts and successes during her Ph.D. study under the supervision of Dr. Eva Nohýnková at the First Faculty of Medicine, Charles University in Prague.

The thesis starts with an unpublished comprehensive review on the biology of *Giardia intestinalis* followed by the summary of the results presented in the appendix as three published papers and one submitted manuscript under review.

The review has the ambition to summarize current information available on the giardia cell biology. In general, Klára succeeded to provide the reader with the up to date understanding of giardia genetic system, cell cycle, cytoskeleton and secretion pathway. The referenced papers used for the review reflect the current stage of the field and are cited appropriately. While it is obvious that Klára understands well her field of expertise (e.g. the cell cycle) few parts of the review suffer from extensive simplification (e.g. the evolutionary aspects of the giardia biology).
However, this is a minor criticism of otherwise nicely built review. The main criticism comes on the quality of English writing. Klára rejected to use the definite articles all over the review and in some cases strange word ordering makes reading more challenging job. Some other minor mistakes appear throughout the review.
The references are listed according to Czech alphabet, *Ch* should be considered as *C*.

Summary of achieved goals is followed by three publications and one manuscript under review.

The first paper (Klára Hoštetrová as the first author) *Giardia intestinalis: aphidicolin influence on the trophozoite cell cycle* published in Experimental Parasitology describes the successful strategy of giardia trophozoites synchronization using DNA replication inhibitor aphidiolin. The paper is of considerable importance to the field as it provides a tool to synchronize the cell culture for various cell biology experiments. However, one should remember a cytotoxic side effect of the drug.

Q: At about the same time two papers from different labs were published on the same issue (Poxleitner et al, 2008, Reiner et all., 2008). Was this an unlucky coincidence or there are discrepancies in the obtained data?
Q: Can you think of a biological mean of cell synchronization? Or a technique without the toxic effect on the cells?

Q: Concerning the dissociation of the nuclear and cytoplasmic cycles, did you also observed increased number other organelles such as the ER, mitosomes or the peripheral vacuoles?

The second paper (Klára Hoštetrová as the second author) *Cytogenetic evidence for diversity of two nuclei within a single diplomonad cell of Giardia* published in Chromosoma describes heterogeneity of two cell nuclei. The enrichment of the cell culture for the mitotic cells was done by the inhibiting effect of albendazole. Apart from quite intriguing observation of different chromosome numbers in two giardia nuclei, the authors discuss a semi-open type of the mitosis as a constrain to genetic exchange between the nuclei.

The third paper (Klára Jiráková as the first author) *How nuclei of Giardia pass through cell differentiation: semi-open mitosis followed by nuclear interconnection* published in Protist extends the recent discovery of the genetic exchange in the cyst stage (Poxleitner et al, 2008). It shows, quite convincingly, the stages of nuclear division during encystation as well as nicely demonstrates the existence of the nuclear connection between each pairs of the nuclei.

Q: Can you discuss possible reasons for the presence or the absence of nuclear interconnections in the cyst or the trophozoite stage, respectively?
Q: Concerning the paper by Poxleitner et al, 2008 is there anything known on the propagation of the episomes in giardia nuclei?

The fourth paper (Klára Jiráková as the second author) is presented in the form of manuscript submitted for publication in Eukaryotic Cell. *Mitotic checkpoints in a binucleated protozoan parasite* Giardia intestinalis. Based on the genomic and functional data authors report on the lack of spindle assembly check point in giardia.

Q: Are there any other eukoryotes known to lack SAC?
Q: Technical question: What would be a feasible experimental set up to follow the whole cell cycle of individual cell in live imaging?

As a whole, PhD thesis submitted by Klára Jiráková represent a high quality scientific work, which fulfills the criteria for granting the PhD degree.

Pavel Doležal, Ph.D.