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Review Report on Ph.D. thesis of Tomáš Skalický

entitled

Insight into insect trypanosomatid biology via whole genome sequencing

It's been a pleasure to read and evaluate the Ph.D. thesis submitted by Tomáš Skalický. The thesis includes two published research papers, one published review article and one recently submitted manuscripts of a research paper. The candidate is a shared first co-author on the three research papers and a co-author on the review. The number of papers the candidate co-authored justifies the format of the thesis, which is basically a compilation of the papers supplemented with a relatively short introduction and a summary of the results followed by a discussion. The introduction provides an overview of the current state of knowledge of the trypanosomatid diversity, phylogeny and some of the unique biological features of this group of obligatory parasites. Although this is not exhaustive, it is sufficient to provide the necessary background information relevant to the work presented. The introduction itself as well as the results' summary and the discussion reads well and is very clear and the discussion recapitulates well the major findings while putting them in context of the current knowledge.

Regarding the format and layout of the thesis, however, I have some objections. I would consider it appropriate to give a more detailed account of the candidate's share in the performance of experiments and analyses presented in the research papers, as these are results of a collective effort, with no less than three leading authors.

The inserted pdfs do not fill the entire page resulting in the font size being very small and barely legible. I must also object to the inclusion of a proofs pdf, complete with typeset marks and a 'submitted pdf' label written in large print across each page, thus obscuring the text of the paper and making it harder to read. Surely a regular text format would have served better in this case.

The grammar of the text is very good overall and largely devoid of typos and while I noticed several errors or awkward formulations these are rather rare and I do not intent to dwell on them at any length here. However, I do feel compelled to point out several instances where due to an unfortunate or wrong phrasing the resulting statement is confusing, incorrect or does not adhere to the best practice of scientific discourse:

For example on page 15 the candidate states that *Paratrypanosoma* "constitutes the earliest branch between free-living bodonids and parasitic trypanosomatids"; this is a nonsensical statement; one can either say that *Paratrypanosoma* constitutes the earliest branch of the parasitic trypanosomatids, or that it constitutes a branch between free-living bodonids and parasitic trypanosomatids.

On page 16: The candidate writes "Unfortunately, *T. cruzi*, *Leishmania major*, *Leishmania donovani*, *Leishmania infantum* or *Leptomonas seymouri* possess core components of the RNAi pathway" while he surely means quite the opposite.

Finally, I must object to the use of the word "prove" in the sentence "These modern techniques also proved that trypanosomatids really evolved from bodonids" on page 5 and even more emphatically to the phrase "... thus ultimately proving Léger's hypothesis that all dixenous flagellates are derived from their monoxenous predecessors from the insect hosts" on the page 11. Rarely in science are theories "proven" much less "ultimately proven" and this especially cannot be said about evolutionary history that took place hundreds of millions years ago and our attempts to reconstruct it.

Specific questions or comments:

Table on page 18 and the relating text in the discussion: The distribution of the genes for the components of the RNAi machinery is rather curious and the candidate argues this can be explained by the presence of the genes in the ancestor and their differential and independent loss in several trypanosomatid lineages. **(1)** I wonder if the candidate has performed or is aware of any phylogenetic analysis of the genes in question that could potentially uncover any indications of foreign origin and horizontal gene transfer.

The major point I would like to discuss is the allegedly "ultimately proven" fact that all extant trypanosomatids originated from a monoxenous parasite of invertebrates. I do not share the candidate's feeling of certainty regarding this issue due to the following reservations.

This rather grandiose conclusion is based on the discovery of a single trypanosomatid species that represents the most basal trypanosomatid lineage currently known and which supposedly is a monoxenous insect parasite. **(2)** Given that this insect host happens to be a mosquito how sure can the candidate be that this is a genuine monoxenous parasite and not an dixenous one for which we have simply not yet found the vertebrate host? Regardless of the previous point, it is not correct to assume that phylogenetically basal lineage equates, to all intents and purposes, the ancestor and that this ancestor must have necessarily shared the characteristics of the currently living members of this lineage. The next major lineage within the trypanosomatid clade is comprised of dixenous trypanosomes, which also contain many of the ancestral orthologous groups of proteins as revealed in one of the studies presented in this thesis. In the phylogenetic trees, the most basal trypanosomes are often those that circulate between a leach and a fish, evolutionary older taxa than insects or higher vertebrates (which host the phylogenetically more derived trypanosomes). This suggests that trypanosomes might have originated from

a dixenous ancestor in water environment and colonised the land later on together with the tetrapods, helped along by blood-sucking insects. I can easily imagine that an organism resembling the bodonid *Trypanoplasma* or an early branching *Trypanosoma* was an ancestor of all extant trypanosomatids. The monoxenous lifestyle in invertebrates would then have originated twice independently: Once in the ancestor of *Paratrypanosoma* and for the second time in the ancestor of the other monoxenous trypanosomatids. Present-day Trypanosomes would thus resemble the presumed ancestral trypanosomatid lineage quite closely. In contrast, it is hard for me to imagine that the ancestor of all trypanosomatids was associated with insect as this according to the molecular clock model presented in this very thesis (fig. 3) evolved after the paratrypanosoma lineage had separated from the rest of trypanosomatids. This brings me to another question: **(3)** Why exactly the discovery of a 150 million year old dixenous *Leishmania* fossil provides an additional support for the "insect first" hypothesis as stated on page 11?

The alternative "dixenous first" hypothesis outlined above would also help to explain why the vast majority of monoxenous trypanosomatids is found in the insect orders Heteroptera and Diptera (which contain most of the blood-sucking insect species) and others are found in phylogenetically distant blood-sucking fleas, while very few trypanosomatid species have been found in other groups of insects.

(4) I invite the candidate to ponder alternative hypotheses such as this one and explain more fully why he prefers the theory he presents in the thesis above other possible scenarios.

To summarise, the doctoral thesis submitted by RNDr. Tomáš Skalický represents an extensive body of solid research work which contributes greatly to the advancement of our knowledge and understanding of trypanosomatid evolution and biology. Despite my minor criticisms I have no reservations in recommending the thesis for a passing grade and as a basis for awarding RNDr. Tomáš Skalický the degree of Doctor of Philosophy.

I wish Tomáš every success in his future scientific career and look forward to reading many more of his inspiring papers.

Sincerely,

Luděk Kořený





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Warsaw, 14th of August 2017

Prof. Miroslav Oborník
Head of the Committee for PhD studies

Review Report on PhD Thesis of RNDr. Tomáš Skalický entitled *Insight into insect trypanosomatid biology via whole genome sequencing.*

The thesis of Tomáš Skalický addresses several interesting and important aspects of the diversity and evolution of insect trypanosomatids. The advancement of molecular techniques and next generation sequencing allows to explore a diversity of trypanosomatids and, as Tomáš's thesis shows, answer broad biological questions.

In comparison to vertebrate trypanosomatids, insect trypanosomatids are less studied, but Tomáš in his thesis has shown how important they are to our understanding of the evolution of parasitism in trypanosomatids and more broadly in eukaryotes.

The thesis contains an extensive introduction, summary of papers included in the thesis along with a statement of Tomáš's contributions, followed by three published manuscripts and one submitted manuscript.

In the first chapter, Tomáš introduces the reader to the dissertation themes. Structure and length of that part are appropriate, and the text is well written. Papers included in the thesis cover a broad spectrum of topics and methods, which makes it difficult to write an introduction covering all related topics. Tomáš highlighted the most important aspects of the evolution of kinetoplastids and relevant details of their nuclear genomes and RNA interference machinery. I think a broader discussion of the results beyond topics covered in the papers, mainly in the context of general trends observed in the evolution of parasitism in eukaryotes might be a valuable addition to this part of the thesis. Tomáš in this chapter demonstrated that he is familiar with the key and new literature in the field.

Short summaries of papers included in the thesis pointed out the primary results and impact of the papers on the field. In three out of four papers, Tomáš is the first co-Author, which indicates his substantial contribution. Tomáš participated in study design, performed



experimental procedures and bioinformatics analyses, prepared figures and tables, and contributed to writing manuscripts, all together that demonstrates that he has a comprehensive set of skills.

Three papers included in the thesis are already published in peer-reviewed journals with a high impact factor (including *Current Biology*), which reflects the quality of the research. Those papers have already been thoroughly reviewed. Therefore, I would mainly point out their impact on the field.

Paper entitled "*Paratrypanosoma* is a novel early-branching trypanosomatid" is a solid description (with TEM micrographs and phylogenomic analyses) of a new species of trypanosomatids, *Paratrypanosoma*. Strikingly, it was isolated in Prague not in some remote part of the world. New species is an insect trypanosomatid, most probably monoxenous one since it was never encountered in vertebrates. Its phylogenetic position suggests that it constitutes a missing link between free-living bodonids and derived parasitic trypanosomatids. Authors described the new species and resolved its phylogenetic position, as well as highlighted its importance for the understanding of the evolution of parasitism in kinetoplastids, which was further studied in the fourth paper included in the thesis.

Second paper "Evolution of parasitism in kinetoplastids flagellates" is a detailed, yet broad, review of current knowledge of the parasitic kinetoplastids and their evolution. Based on the description of contribution I assume that Tomáš performed phylogenetic analyses and prepared figures for that paper, which demonstrates his knowledge of phylogenetic and phylogenomic methods. The most interesting is a tree of kinetoplastid flagellates under the clock model. That tree enabled dating most important transitions in the evolution of trypanosomatids and correlate them with the evolution of their hosts.

Third paper "A tsetse and tabanid fly survey of African great apes habitats reveals the presence of a novel trypanosome lineage, but the absence of *Trypanosoma brucei*" is focused on the trypanosomatid species discovery in the Dzanga-Sanga Protected Areas in the Central African Republic, which hosts primates. I found fascinating the methodology for establishing putative vertebrate hosts, which were detected based on the provenance of blood meals of tsetse flies. The study designed to identify great apes trypanosomatids revealed that in fact, those apes are not a target for tsetse flies, perhaps due to some anti-tsetse behaviors or odor. In the presented study several new species of trypanosomatids have been described, with forest buffaloes and humans as a main source of blood.



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The manuscript entitled “Extensive flagellar remodeling during the complex life cycle of *Paratrypanosoma*, an early branching trypanosomatid” is a compelling study employing a variety of microscopy and molecular techniques, whole genome sequencing, and transcriptome profiling to understand the biology of *Paratrypanosoma*. Presented detailed studies revealed that *Paratrypanosoma* has three different morphological stages and has retained more ancestral genes than other trypanosomatids indicating that it might closely resemble the last common ancestor of trypanosomatids. Extensive loss of genes rather than gain throughout the trypanosomatid evolution together with the presence of extensive inter-stage morphological transformation suggests that most of the traits interpreted as characteristics for highly specialized parasites predated the two-host life style. However, some genes are unique for *Paratrypanosoma* or *Paratrypanosoma* and stercorarian trypanosomes and were lost in the other trypanosomatids. Among them, the most interesting example constitutes DFG1 proteins with a possible function in the host-parasite interaction. That paper represents a remarkable example of a very well conducted and broad study on the key organism, which helps to understand the transition from one-host to two-host life style and evolution of parasitism in trypanosomatids.

Overall, the thesis represents excellent scientific achievements, selected topics are interesting, and results are of broad interest beyond the field of protistology. In my opinion, this thesis fulfills all requirements for obtaining a PhD degree, and I enthusiastically recommend a defense of the thesis.

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Questions

1. The impact of the research on *Paratrypanosoma* on our understanding of the evolution of parasitism in trypanosomatids is indisputable. Could you comment and compare it with the results obtained from comparative genomics with the free-living relatives?
2. Could you give other examples of the newly discovered lineages of eukaryotes, which highlighted the evolution of parasitism? Can you draw any general conclusions about the evolution of parasitism, particularly related to the gene loss and gain?
3. I am intrigued by the discovery of the expansion of the gene family encoding DFG1 proteins. How would you try to reveal the function of DFG1 proteins and its possible role in the host-parasite interaction? Is it known what is the role of those proteins in stercorarian trypanosomes?
4. I am confused with the results indicating the high level of ancestral OGs in *Trypanosoma grayi* and losses of OGs shown on the tree in the ancestors of *T. grayi*. Could you elaborate more on the methodology and results of the analyses of shared OGs?