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Review of the thesis: Population genetics of the fish tapeworm *Wenyonia virilis* (Caryophyllidea: Caryophllaeidae) and its fish host *Synodontis schall* (Siluriformes: Mochokidae)

PhD applicant: RNDr. Dagmar Jirsová

Reviewer: Doc. Mgr. et Mgr. Josef Bryja, Ph.D.

The PhD thesis of Dagmar Jirsová is focussed on important questions of current evolutionary biology, like host-parasite co-evolution, speciation and species delimitations, and the role of environment/evolutionary history on phenotype variability. The model system, the freshwater catfish and its tapeworm species in ecologically diversified environment of the lake Turkana, is well selected, as well as used genetic markers (combination of mitochondrial sequences with genomic screening by AFLP method). Sampling (despite relatively low number localities for some objectives) and genotyping are sufficient, however some analyses and interpretations of results are questionable (see more details below).

The thesis begins by 17 pages of the text, introducing the general context, model system, aims of the study and summary of main results. This part is followed by one published paper in PLoS ONE (PhD candidate as first author) and two unpublished manuscripts (one of them with D. Jirsová as first author), which is probably the necessary minimum for PhD theses at School of Doctoral Studies in Biological Sciences at USB in České Budějovice. The formal quality of the thesis, especially the introductory parts, is rather low. The text contains numerous redundancies, typos and the list of references includes numerous formatting errors (missing pages of papers, incorrect names of journals, etc.). Important information is missing in this part of thesis, e.g. the context of ecological speciation, species concepts, the map and details about the environmental variables of sampling localities (nowadays as well as in the past), or relevant information on the parasite population genetics (e.g. mating patterns, hypotheses about the dispersal of oncospheres and intermediate hosts, etc.). On the other hand, the detailed information about the use of various genes in studies of *Schistosoma* or about the Gibe dams are not directly relevant to this thesis.

Objectives of the study are clearly defined at page 4, i.e. before the description of the general context and summary of knowledge, which is not usual. In the summary of obtained results the PhD candidates clearly answers all asked questions. However, after reading the three papers, I would be much more cautious. While some results are relatively clear and their interpretations straightforward, many questions are still not resolved by current data and analyses (see below my opinions and suggestions for additional analyses). I still feel the space for alternative explanations, which are generally missing in Discussions.

The first paper was published in PLoS ONE, but I think that the reviewers probably overlooked some problems, especially in the presentation and interpretation of results. One of the main aims was to compare the differences in population structure between host and parasite taxa. The authors conclude that their study is the first example showing much higher genetic structure in endoparasite than in its host. However, I think it is not completely true.

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The main conclusion is based mainly on the analysis of cox I sequences (Fig. 4), but the sample sizes of parasite and host are not directly comparable (347 sequences of 990 bp in the parasite vs. 120 sequences of 604 bp in the host). This sampling bias itself could produce higher genetic diversity observed in the parasite taxon and some kind of correction (rarefaction?) should be applied. Even if the observed differences are real (and it is still possible), I do not believe that it is caused by faster rate of molecular evolution ("the mutation rates must differ dramatically"). Alternative explanations clearly exist, e.g. differing effective population sizes of hosts and parasites linked with faster lineage sorting in parasites, but they are not discussed at all. The analyses of AFLP data are even more confusing - they mix intraspecific level (e.g. what I would expect for population genetic analysis) with interspecific (I do not understand why other species of both hosts and parasites were included in this analysis), which violates assumptions of used approaches and may produce biased results. According the STRUCTURE analysis, there is no difference between *W. youdeoweii* and *W. virilis* from the Nile (and part of saline Turkana) - how you would explain it? Also, I do not see any clear difference of tapeworms between saline and freshwater habitats - in both of them the populations are clearly structured, but light blue and pink clusters are shared between two habitat types. It would be very helpful to see the model for K = 2 (including only *W. virilis* from Turkana), at least in Supplementary Materials. In *Synodontis*, there is clear substructure in both saline and freshwater habitats, but it is not analysed and commented in more details. How this substructure corresponds to two different phenotypes described in Paper 2? I have also numerous other questions that we can discuss during the defence. In total, I think that the main presented conclusions (i.e. opposing population histories of host and its parasite, lineage fusion in parasite and lineage fission in the host, and significantly faster evolutionary rate of the parasite) are not sufficiently supported by the data.

The second study used two mitochondrial and one nuclear gene to assess the species status of two main morphotypes of the host fish. The authors conclude that (1) *S. schall* and *S. frontosus* should be considered conspecific and (2) there is a possible new species in western Africa. I think again that neither of these conclusions is supported by the data. Ad (1): Fig. 8 shows much more variability in cytb compared with coxI (which is logical, taken into account longer cytb sequences), but there is no indication of morphotypes at this figure. How one can assess the distribution of morphological variability at this haplotype network? I suggest to perform the analysis of concatenated mitochondrial dataset (only for *S. schall* s.str./*frontosus*, ideally only from the lake Turkana), together with clear indication of phenotypes (*schall*/intermediate/*frontosus*) for each haplotype. Even if such analysis provides no differences at mtDNA among different phenotypes, it does not mean that there are not two species (mtDNA introgression is common in nature), and nuclear markers should be used. Nuclear marker (RAG) has very low variability, unresolved tree and cannot alone say anything about species delimitation. Why AFLP data were not used for this paper? I can easily imagine alternative explanations - for example *S. frontosus* is a separate species adapted to freshwater conditions of Omo River (no material from this river was analysed), while *S. schall* s.str. colonized the lake Turkana from the Nile and was forced to occupy the saline ecosystem (as the result of competition with *S. frontosus*). Because the two taxa were not completely reproductively isolated, the interbreeding led to mtDNA replacement and the hybridization can still occur in some extent, leading to intermediate phenotypes. Ad (2): I would be surprised by the lack of differences at mtDNA between western and eastern Africa - the observed pattern can be considered as typical within-species phylogeographic structure

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(especially if there are no morphological differences). The indication of two separate biological species is therefore very preliminary. Integrative taxonomic study (employing multiple nuclear markers, morphology, etc.) at the contact zone of two mitochondrial lineages (Chad basin) is required to test the hypothesis of two different species. What are the genetic distances between eastern/western mitochondrial phylogroups? Are there any similar phylogeographic patterns in other fish taxa?

The third manuscript (still in preparation, PhD candidate is the last author) is looking for the association between parasite morphology and genetic structure. It seems that some morphotypes are more prevalent in saline than in freshwater environments (based on Figs. 1 and 2). Anyway, I suggest following modifications of these analyses to make this conclusion stronger: (1) remove outgroup species (+ Nile population) from the analysis - it is confusing; (2) use haplotype networks instead of phylogenetic trees of coxI; (3) make the same orientation of both scatterplots at Fig. 2; (4) perform the model for K=2 in STRUCTURE for AFLP data of parasites and show the distribution of Q-values in different environments; (5) I would like to also see the association between host genetic structure (as visible at Fig. 6 of Paper 1) and distribution of morphotypes - may be it can also explain part of the variation in morphology. I also guess that the manuscript will be formatted properly, i.e. the references to "Paper 1, 2" will be replaced by "Jirsova et al. 2017", etc.

I have following general questions for the discussion:

- Turkana "almost" completely dried out in the Late Pleistocene, as stated at several places of the thesis - really nothing survived? Is it possible that one of the two Turkana haplogroups (either group 1 or group 4) for W. *virilis* is pre-desiccation origin?

- how do you define "allopatric differentiation in a sympatric system"

- at p. 8, you mention that one criterion for the selection of the parasite species was "complex life cycle". Isn´t it better to have direct life cycle if you would like to study the co-evolution of host and parasite?

- are you really able to distinguish isolation by distance from isolation by adaptation (e.g. p. 12 and other places) if the data were collected at two localities differing by ecological factors?

- I do not understand why two phylogenetic analyses were performed in the first paper - partitions were assessed by jModelTest and PartitionFinder. What was the purpose?

Conclusion:
During the preparation of the thesis the applicant collected relatively large amount of new genetic and morphological data on interesting host-parasite model, allowing to solve fundamental questions concerning its evolutionary history. Even if the author showed some ability to analyse the data, there are still drawbacks, especially in the interpretation of

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obtained results in wider context and their preparation into the form of high quality scientific publications. I look forward to the discussion during the thesis defence and if the applicant will be able to show her scientific maturity, I will suggest Dagmar Jirsová to get a doctor diploma (Ph.D.) of the University of South Bohemia in České Budějovice.

Studenec, the 12th of September 2017

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REVIEW OF THE DISSERTATION THESIS

AUTHOR: RNDr. Dagmar Jirsová

TITLE: Population genetics of the fish tapeworm *Wenyonia virilis* (Caryophyllidea: Caryophyllaeidae) and its fish host *Synodontis schall* (Siluriformes: Mochokidae)

The dissertation thesis is focused on morphological and molecular structure and population genetics of interesting host-parasite model, caryophyllidean tapeworm *Wenyonia virilis* and mochokid catfish *Synodontis schall* in Lake Turkana and River Nile using different molecular, morphological, phylogenetical and statistical approaches. The work is a result of extensive fieldwork, sampling, laboratory techniques and computational work. In this aspect, the author proved flexibility and capability to collect data from field, process them in laboratory, and evaluated by proper methods. What I consider to be a great benefit is a parallel analysis of both, fish host and its parasite, what enables better understanding of their ecology, distribution and environmental adaptation.

The dissertation thesis is based on three papers, one published in PlosOne (IF 3.5), one submitted to Molecular Phylogenetic and Evolution and one under preparation. I do not want to discuss the number of papers, since the number might be very relative depending on the complexity and spectrum of methods and analyses involved in the study. From this aspect, I guess that 3 papers are sufficient. However, regarding the start of PhD work (2009), it could be discussed, if the publication status of the two latter papers should not be more advanced.

The first part of the thesis involves Introduction (chapter 1. General introduction) and „current state of the knowledge" (3. Geographic and organismal models) which should represent comprehensive data related to the dissertation work, resulting to the definition of the aims. This part of dissertation work seemed to be written in last minute and without critical review of two supervisors, experts in their fields. General introduction provides rather chaotic summary mixing data on tapeworms, genes, mitochondrial DNA, whole-genome analysis methods, schistosomes, speciation processes, molecular taxonomy, biogeography, phylogeography, host-parasite relationships, co-phylogeny, evolutionary patterns and diversification. Introduction represents the first information to the reader and should be concise and informative enough to answer the crucial question: what was the purpose of this work? Introduction is aimed to attract the attention of reader (or reviewer) and inspire them to continue reading. Unfortunately, this was not the case of the thesis.

Technical note: there were some typing and spelling errors etc., introduction should refer to other relevant data published in the filed, not referring to the Papers 1, 2, 3 presented in the thesis.

Five partial objectives of the study apparently followed the chronology of articles involved in the thesis. More logical scheme would be to start with the aims nos. 4 and 5 (taxonomic identification,

morphological plasticity, molecular variation) of model organisms. Consequently, their population genetics can be assessed (aims 1 and 2). Aim no. 3 is purely methodological and it apparently resulted from problems related to the level of informational value of AFLP markers.

Model locality is explained in detail including geography, ecology, and palaeontology. What I initially missed in this part was informative map (found later on in Paper 1).

Model organisms were chosen according to good criteria ("wide distribution, high abundance, availability in Nile and Turkana"). *Wenyonia virilis*, the parasite model, is introduced by short description of order Caryophyllidea. However, members of this order are not only morphologically very specific (monopleuroid body, single set of reproductive organs), as indicated in the thesis, but they also represent evolutionary basal group of tapeworms and some members possess several molecular and genetic characteristics, such as NUMTs (numerical copies of mtDNA), divergent intragenomic ribosomal ITS copies (ITS paralogues), multiple NORs (nucleolar organizer regions), triploidy, parthenogenic mode of reproduction etc. Maybe the data on molecular and genetic characteristics of other members of the order Caryophyllidea would help to explain the difficulties encountered during the work (e.g. not sufficiently informative AFLP markers)?

Technical note: I missed some picture or drawing of model parasite, detailed taxonomic classification of model species and schematic map of its distribution.

Paper 1. The paper is dealing with population structure and a level of intraspecific genetic variation of parasite - caryophyllidean tapeworm *Wenyonia virilis* and host - mochokid catfish *Synodontis schall*, considering ecology of model locality (freshwater and saline) River Nile and Turkana Lake, and their migration routes. The paper was published in PlosOne, so it is irrelevant to review it again. However, I have some questions related to the paper. The study is based on two molecular markers, mitochondrial *cox*1 and AFLP data. Results based on *cox*1 data revealed significantly higher variability in parasite (out of 347 individuals, 209 mito haplotypes were determined) comparing to fish host (120 specimens shared 20 haplotypes). In haplotype networks, populations of *Wenyonia* from Nile contained pattern typical for older populations, while those from Turkana exhibited trait typical for young expanding populations. Tapeworms from Turkana possessed haplotypes specific either to saline or to freshwater environment. Contrary to this, haplotype network of *Synodontis* showed sporadic occurrence of freshwater haplotypes in saline locality and vice versa, what can be explained by unrestricted migration of host. In this aspect I have following comments and question:

The author claims that "Such absence of mixed infections probably indicates presence of reproductively isolated tapeworm subpopulation in the lake", "..... contrasting environmental conditions are more challenging for the parasite than for the host", "The salinity might affect the Turkana tapeworms indirectly by means of restricted distribution of alternative intermediate hosts....".

Q1: How would you explain a development of "reproductively isolated tapeworm population" considering that (i) fish – definitive host is able to migrate between ecologically different locations, and (ii) susceptible intermediate host (either one or more species?) has to be present in both, saline and freshwater part of the lake?

Q2: Is there any indication of specific intermediate host fixed to the saline or freshwater environment? If yes, what would be its selection mechanism for not ingesting *Wenyonia* eggs produced by adult tapeworm from "other part of the lake"?

Q3: Are data based on mtDNA sufficient to discuss reproductive isolation?

I like the results based on the Migrate analysis testing several scenarios of fish and parasite migratory routes. It was probably not surprising that populations of fish and parasite from White Nile represented ancestral populations and Blue Nile and Turkana were colonized separately, Turkana even multiple times.

Q4: How would you explain much higher haplotype diversity in Lake Turkana, comparing with that of Nile? Or was it a result of different sampling size?

Results of the AFLP analysis showed different picture of population structure than data based on mtDNA. Based on PCoA and Structure analyses, it is evident that while population pattern of *Wenyonia* from Turkana and Nile can somehow be distinguished, the population structure of *Synodontis* is unrecognizable based on both, PCoA and Structure outputs.

Q5: Is this a result of technical character (e.g. in design of AFLP markers) or do the data reflect low level of intraspecific variation in genomic DNA (contrary to high level of polymorphism in mtDNA)?

Q6: What was the purpose to include data on congeners (*W. youdeoweii* and *W. minuta* for parasite and *S. nigrita* for fish) in PCoA and Structure? Surprisingly enough, even congeneric species were not distinguished by different assembly in PCoA or by specific colours in the Structure.

Q7: Would you recommend to parasitologists to apply AFLP method? If yes, what would be your recommendation based on your own experience?

Paper 2 is focused on morphological and molecular characterisation of model fish host using AFLP and *cox*1 markers (I guess the data were adopted from Paper 1) and, in addition, mitochondrial cyt b and nuclear recombination-activating gene (RAG2). The results revealed that the single species present in Lake Turkana is *Synodontis schall*, while two species (*S. schall* and *S. nigrita*) are present in River Nile. The results were mainly supported by morphology and mito data, outputs coming from nuclear gene were less informative.

Q8: Why did you decide to use two mitochondrial genes and nuclear protein (enzyme) encoding gene, which low sequence variability could probably be expected in advance? Is there any reliable species-specific PCR-based method for *Synodontis* molecular identification (SSU, LSU rDNA)?

Paper 3 is focused on a correlation between five morphologically well-defined morphotypes of *Wenyonia virilis* and its population genetic structure in Lake Turkana. The mitochondrial *cox*1 and AFLP molecular tool were adopted from Paper 1. Two morphotypes (H and F) were excluded from the analysis due to small sample size. If I understand well, the results were aimed to show that (i) distinct morphotypes are correlated with mitochondrial haplogroups (Fig. 1), and that (ii) morphotypes are restricted to

freshwater or saline habitats in Turkana (Fig. S1). However, the second statement seems to be not so well supported. Besides, the distribution pattern of individual morphotypes displayed in PCoA analysis using AFLP data (Fig. S2) does not support the above statement. **Q9:** Could you explain it, please?

Comment: According to experience on molecular and morphological study on *Caryophyllaeus laticeps*, the well-defined borderline between morphotypes and phylogenetic branches based on analyses of different genes might change significantly according to the markers used (rDNA, mtDNA, microsatellites). Since AFLP data are in case of Paper 3 not so convincing and results are mainly based on *cox*1 data, the final conclusion should be claimed carefully.

Final conclusion. I am convinced that Dagmar Jirsová proved her ability to manage notably different parts of scientific work, starting with field expeditions, data collection, standard and routine but also more advanced molecular methods and statistical evaluation of data obtained. The two experienced scientists supervised the thesis and I truly believe that their expertise and knowledge was delivered to the author in every possible way. It is pity that not more attention was given to the final step of PhD work – endless, tiring and sometimes maybe eventful part of work – writing and summarisation of data. I suggest that the author keeps this in mind when progressing in her scientific carrier, especially during preparation of projects and guidance of students.

After successful defence and creative discussion **I recommend**

Dagmar Jirsová

to get an academic title **Philosophiae Doctor** (Ph.D.)

of the University of South Bohemia in České Budějovice.

In Košice, 2017 September 18th

RNDr. Ivica Hromadová, CSc.

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Report on the Doctoral Thesis

of Dagmar Jirsová
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Population genetics of the fish tapeworm *Wenyonia virilis* (Caryophyllidea: Caryophyllaeidae) and its fish host *Synodontis schall* (Siluriformes: Mochokidae)

The Ph.D. thesis of Ms. Dagmar Jirsova is a concise and dense piece of work. It includes three scientific manuscripts: one already published in a renowned international journal, one submitted manuscript and a third in advanced preparation.

The goals of this study clearly relate to the substantial gap in our knowledge on the population genetic patterns and genetic variability of fish parasites and their hosts from Lake Turkana and the River Nile – two allied/sibling basins, which have been separated by thousands of years but sharing a strikingly similar ichthyo- and parasitofauna. The specific objectives of the thesis are well formulated and fully justified in relation to previous research. The candidate discussed the results with authority and clarity and in an entirely convincing manner, as one out of three manuscripts is already published. All relevant references have thereby been quoted and correctly cited. Throughout the thesis, the tables and figures are clear and well presented; the illustrations and graphics are of superior quality and the candidate has demonstrated a detailed, clear and convincing style. All findings of the present study are original and their interpretation provides important insights into the population genetics structure of the caryophyllidean parasite, *Wenyonia virilis*, and its fish host, the mochokid catfish, *Synodontis schall*.

If I have any criticism about the present work, it would be its structure and concise nature. Although the candidate employed numerous approaches in modern population genetics with interesting findings, results and conclusions valuable for future studies in this field, the main body of the thesis seems very concise and certain parts could have been expanded, added and better structured. For instance, the general introduction focuses exclusively on the topics of molecular advances, phylogeographic/phylogenetic comparative studies and evolutionary patterns acting on host-parasite systems. However, in this chapter, a more detailed explanation of the study area (i.e. the two geographical sub-systems, Nile and Lake Turkana), the pre-historical (climatical and palaeohydrological) events and the host and parasite group in focus would have been beneficial. However, this has been described in chapter 3, thereby replacing a separate chapter on the materials used and methods employed. The only method described in detail, the amplified fragment length polymorphism (AFLP), was placed in the appendix. In addition, some parts of the manuscript seem very repetitive. For example, certain sentences have been repeated up to three times in different parts of the presented work with an identical wording (e.g., "geographic origin and environmental factors are not *a priori* decisive with respect to presence/absence in parts of the lake" – both in the discussion and conclusion of paper 3; chapter 5 conclusions). Also, various spelling mistakes were detected and most frequently definite and indefinite articles were absent. This could have been avoided by several more careful rounds of proofreading.

In what follows, I will provide a brief summary of the three included studies (i.e. papers), including questions, which arose while reading the thesis.

Study 1

The main aim of this study has been the assessment of the genetic diversity, population structure and shared evolutionary patterns of the host-parasite system in recently divided basins. In addition, evolutionary processes influencing this host-parasite system on population genetic levels were assessed. This study incorporated a combination of phylogenetic and whole genome scanning (AFLP) methods, including conventional DNA extraction/PCR (with newly designed primers), construction of statistical parsimony networks (haplotype networks), population genetic analysis (DNA polymorphism statistics), assessment of historical gene flow and migration pathways. Overall this study was well performed and the objectives sound. The sampling size and number of acquired cox-1 sequences for both parasite and host taxa are impressive (347 and 120 sequences, respectively) covering all sampling locations in the Nile and Lake Turkana for *S. schall* and including three species of *Wenyonia*. Novel insights of this study were astounding, revealing population genetic patterns that imply multiple colonization waves into Lake Turkana from the Nile. Parasites were shown to evolve more rapidly than their hosts, with higher molecular evolutionary rates (faster mutation rates) and higher morphological variability. Different parasite sub-populations were revealed in Lake Turkana; each restricted to saline or freshwater environments through physiochemical segregation.

Q1: In contrast to mtDNA data, AFLP failed to reflect interspecific genetic differentiation for all three members of *Wenyonia* included in the analysis. I wonder why the AFLP data, both in the Principle Coordinates Analysis (PCoA) and the analysis using the Structure software, grouped all species of *Wenyonia* together in one cluster. Why could the AFLP markers not differentiate between these parasite species? And what implications does it have on the population structure and differentiation of closely related parasite populations/taxa?

Study 2

This submitted study focused on the fish host, *Synodontis schall*, in the Nilo-Sudanian ichthyological province. Different morphotypes of *S. schall* have been detected in the Nile and Lake Turkana, but phylogenetic and haplotype analyses revealed these morphotypes to be conspecific. The mtDNA dataset, however, differentiated populations supposedly belonging to *S. schall* from the eastern and the western ichthyological province (*S. schall* sensu stricto vs. *S. schall* sensu lato), with an overlap in the Chad basin.

Q2: Specimens of *S. schall* sensu lato were reported to be morphologically "identical" to specimens of *S. schall* sensu stricto. However, in the molecular phylogeny based on the mitochondrial marker cox-1, specimens of *S. schall* sensu lato grouped with specimens of *S. oueensis*, *S.* aff. *bastiani* and *S.* aff. *haugi*. In this case, *S. schall* sensu lato might not even belong to *S. schall* and could be a species misidentification rather than a cryptic lineage hiding under the name of *S. schall*. Did the candidate observe specimens from Chad and the western ichthyological province to prove the 'conspecificity'? Or in other words, how trustworthy are these species allocations (supposedly from different

authors)? How can you exclude the possibility of having a different species of *Synodontis* in your analysis?

Q3: A potential conspecificity of *S. frontosus* with *S. schall* has been implied multiple times in the manuscript. This cautious approach is then revoked in the final sentence of the conclusions, where the authors stated the following: "Second, not only is *S. frontosus* conspecific with *S. schall* sensu stricto, but also *S. schall*, as it is currently defined, apparently includes two non-sister phylogenetic units, i.e. cryptic species, hidden under this name". This last sentence might need some revising in the proofreading stage. On the other hand, this study is based on a "dataset robust enough to allow reliable conclusions" (Discussion, 4.2). If the data presented allows for a reliable conclusion, I do not understand, why the authors chose such a conservative approach and why they have not synonymised *S. frontosus* with *S. schall*? Combined morphological and molecular data within the present study support this synonymization and it has even been adapted in the title (i.e. "overlooked morphological variability").

Study 3

In total, 298 specimens of *W. virilis* assigned to three out of five, previously recorded morphotypes have been included in a molecular phylogenetic study (based on cox-1) and AFLP analysis.

Q4: Specimens of *W. virilis* exhibit an extraordinary degree of intraspecific variability. Why did Schaeffner (2009) consider these different morphotypes of *W. virilis* to be conspecific rather than describing new species?

Q5: Schaeffner (2009) assigned specimens of *W. virilis* to five different morphotypes based on a set of morphological characters. In the present work, the candidate assigned specimens using only a limited set of morphological characters of Schaeffner's (2009) morphotype assignment. These four remaining characters were the 1) body shape; 2) scolex width/length ratio; 3) shape of the scolex margin; and 4) appearance of testes (spacing and number of layers). Most of these characteristics overlapped in certain morphotypes and specimens could therefore not be assigned properly. Other characters, such as the body part with maximum width, nature of the ovarian arms, characters of testicular and uterine regions have not been considered by the candidate and colleagues, due to the "tendency to reflect either conditions of fixation procedure or the degree of ontogenetic development". This statement, however, seems far-fetched and erroneous and a lack of these characters will not resemble the same morphotype assignment of Schaeffner (2009). The assigned morphotypes based on the earlier study were then provided as a figure in the supplementary data. Another morphological characteristic used previously – the distribution of postovarian vitelline follicles – could not be applied, because the posterior parts of specimens have been used for molecular analyses. Lacking the posterior parts, specimens of morphotypes E and H cannot be allocated with absolute certainty. Additional character sets (instead of relying on overlapping and matching characters, see morphotypes D and G) would have been necessary. How confident is the candidate with the allocation of specimens of *W. virilis* to individual morphotypes? What were the differentiating characters between the morphotypes D and G of

Schaeffner (2009), since they have been united in this study relying on a subset of characters?

Summary

As a whole, this work represents a valuable contribution to our knowledge of the genetic diversity and population structure of host-parasite systems. It successfully follows and further develops the high standards for rigorous population genetic approaches, analysis of metazoan evolutionary processes and host-parasite evolution. The study provides a firm foundation upon which future host-parasite comparative studies can build. I have no doubts that the results will be of considerable use for the scientific community. My confidence is based on the fact that the results of this work have already been published in a well-regarded international journal (PlosONE), where the candidate is the first author.
In my opinion, the candidate clearly has a detailed knowledge of the subject, has provided a thorough account of her research and achieved the initial objectives, thus demonstrating that she is capable of carrying out original and independent research to a high rating. Lastly, I would like to recommend the Ph.D. thesis for the Ph.D. defense and I wish the candidate every success in the next stages of her scientific career.

Bjoerg E. Schaeffner (Ph.D.)
České Budějovice, 18/09/2017