

Interactive comment on “Testing the spatial and temporal framework of speciation in an ancient lake species flock: the leech genus *Dina* (Hirudinea: Erpobdellidae) in Lake Ohrid” by S. Trajanovski et al.

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We would like to thank the referee for his/her efforts in reviewing our manuscript. The comments are greatly appreciated. We carefully considered these comments and we have adjusted the manuscript accordingly. In the following, we list the individual comments of the referee as well as our replies.

1. Introduction: General comment: The Introduction is rather extensive, but does hardly help the non-leech specialist to get an impression of the organism's nature. It would be

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helpful to have – very briefly – information on hand describing general patterns in morphology, species diversity and ecology of the leech radiation. In a lake flock, one might expect differentiation along ecological axes (besides of the three depth categories considered) - are studies, data or preliminary observations available?

According to the suggestion of the referee, we added in the Introduction information on the biology and ecology of *Dina* spp. We also added short information on the habitat preference as another ecological axis.

2. P. 5015, l. 4-5: I wonder about the rather casual statement that species flocks from this lake have “provided first insights into patterns of allopatric and parapatric speciation”. To my knowledge, evidence for cases of parapatric speciation is still extremely scarce (probably because its so difficult to proof), and I think this issue should be introduced and discussed carefully. I also wonder why sympatric speciation is not incorporated as possible hypothesis or at least mentioned in this manuscript – I think the last years have shown that this mode of divergence should be incorporated as one hypothesis when testing for modes of (especially intralacustrine) speciation. If sympatric speciation within L. Ohrid is here excluded a priori, state why and show the apparent barriers for leech dispersal.

Our statement in the introduction is simply a summary of what is known in the literature about patterns of speciation in species flocks in Lake Ohrid. The fact that we don't mention sympatric speciation should, by no means, imply that this form of speciation does not occur in Lake Ohrid. Rather there is no information available about this mode of speciation. Also, we do not intend to test for modes of speciation in our papers (see the goals outline in the introduction). Instead our approach focuses on the role of gradients and barriers for speciation. Also, nowadays sympatric speciation is most often related to sexual selection. As our taxa are hermaphrodites, sexual selection appears to be unlikely.

3. P. 5015, l. 12-18: Consider revising this paragraph. Is it likely to expect congruent

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modes of divergence in all groups of organisms radiating in the lake? To me, it appears rather unlikely that gastropods, leeches, trouts etc. all show the same modes of species flock formation.

There must be a misunderstanding here. The paragraph referred to be the referee reads "A comprehensive picture of patterns and processes of speciation in Lake Ohrid, however, is still missing and the role of vertical and horizontal differentiation is still subject to controversial discussions." With "comprehensive" we mean "complete" rather than "congruent".

4. P. 5016, l. 15: Rephrase to "study the role of potential . . . barriers in Lake Ohrid . . .". At this point, existence of barriers for leech distribution first has to be demonstrated.

Done!

5. P. 5018, l. 2-3 (see also P. 5016, l. 12 ff.): It would be interesting to include a improved justification why this marker was used here. What are the expected advantages and limits for the present analyses?

We deleted this statement from the ms.

6. P. 5022, l. 21-23: It appears that specimen carrying a mtDNA haplotype not fitting the morphological expectation are labelled here as "cf.". Does that make sense? Usually, this epithet is used to show that a specimen or population is apparently similar to a certain species, but does for some reason not fit all characters unequivocally. In evolving radiations, mismatch between mtDNA haplotype and morphospecies is not uncommon. I think it would be straightforward and more informative to follow the morphological characters for identification, and show how phylogenetic structure and haplotype distribution fit morphological characters (as expressed by species determination).

We rephrased the relevant paragraph and now use the epithet "cf." according to its meaning specified by the ICZN. It now reads: "For subsequent analyses, we labelled specimens that resemble the known morphospecies closely but which could genetically

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not be assigned unambiguously to the named species with the epithet confer (cf.)."

7. P. 5021, l. 15-24 and p. 5024, l. 9-12 (Test for genetic structure): I have some problems following the logic here. Based on lacking intralake resolution in the mitochondrial tree, the authors argue that ". . . lineage sorting does not seem to be complete. . ." and test for habitat-specific signal without considering morphospecies. However, the haplotype networks appears to show quite clearly that haplotypes are by far not distributed randomly within L. Ohrid; there is obviously substantial signal related to the seven species distinguished in Fig. 2. If this is the case, I would recommend using a test incorporating these species (groups), calculating the test for the species one by one, or removing this approach completely from the analysis.

We do not dispute that there might be signal related to the species. But we feel that testing for genetic structure according to taxa would be circular because some of these taxa were identified based on genetic data. Moreover, our test shows that there is significant structure between spring and lake species. Therefore we feel that our test is appropriate. However, in order to address the concerns of the referee, we added to following statement on p. 13: "However, our approach tested only for differences among vertical zones but not for potential taxonomic structures (in order to avoid circularity since taxonomic identity here is partly based on the molecular findings of this study). Since both the zonation and a potential taxonomic structure may weaken each other in the test, a lack of differentiation may be due to type 2 error (false negative)."

8. Results: P.5022, l. 20: ". . . nominal taxa are, in part, not well resolved." According to the names used in Fig. 2, I do not see any species-specific resolution in the tree within Ohrid at all. Refer to the network here.

Done!

9. P.5022, l. 21-24: Move this section to "Material and Methods". P. 5023, l. 19: Change Fig. 2 to Fig. 3.

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Done!

10. P. 5023, l. 20-25, and elsewhere: The statement that “. . . lineage sorting. . . is still not fully complete” implies a “one-way road to speciation”, which must not necessarily be the case.

Throughout the ms, we removed the word “fully” relative to lineage sorting. We also raise the possibility of hybridization.

11. Discussion: General comments: What about potential axes of variation within the macrohabitats, e.g. gravel vs. mud in the sublittoral, or macrophytes vs. rocky areas in the littoral – are there any indications for ecological speciation which is not related to depth gradients ? In the revised ms, we now raise the question of a macrohabitat axis. But we also show that habitats in Lake Ohrid are strongly autocorrelated to depth zones (see Hauffe et al., 1010).

12. The Discussion is quite extensive; from a reader's point of view I would like to see it condensed substantially.

We shortened the Discussion by 23 lines.

13. P. 5025, l. 20-21: I think “incomplete lineage sorting” is not the only possible explanation for lacking congruence between morphology and mtDNA data as observed here. Maybe it would be worth considering also potential secondary hybridization, for example between lake- and spring-dwelling populations?

See our comments under 10.

14. P. 5026, l. 12-14: As mentioned above: physical barriers are not the only factor potentially driving speciation processes. . .

Thus might be true, but physical barriers and/or gradients are, according to the literature, the main drivers of speciation processes in lake Ohrid. Therefore, we specifically tested for these factors in the present paper, without suggesting that these are the only

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ones.

15. P. 5027, l. 16: Be specific: Explain why these populations are “highly interesting” or delete this.

We deleted this statement from the ms.

16. P. 5027, l. 23-27: Why did you hypothesize a reservoir function of populations from greater depths? Explain or delete.

We deleted this statement from the ms.

17. P. 5028, l. 18-20: I understood from the Results section that leeches from Lake Ohrid and the feeder springs share one mtDNA clade. Accordingly, I would say that speciation within this clade is not necessarily intralacustrine (inside a lake). Does amongspring separation (or the existence of other refugia), without necessarily assuming the permanent existence of the lake being filled with water, appear unlikely? Please explain.

Throughout the ms, we removed the term “intralacustrine”.

18. P. 5029, l. 26-27: Delete the last sentence (no relevant content).

Done!

19. Tables and Figures: Table 1: I would like to see reference also to voucher specimens, not only to DNA samples. The analyses and discussion of this work widely rest upon species determination, and judging from all the unidentified or questionable material there remains a lot to be done in terms of taxonomy in this group. Without vouchers on hand, later workers will hardly be able to link their data to the present study (which would be a pity).

We included information on voucher specimens in Table 1.

20. Figs 1-2: For readers not familiar with leeches some pictures or drawings inserted

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in the colour figures might help getting an impression of the organisms.

We provide a new Fig. 1 with a colour photograph of a live specimen as well as with a habitat photograph.

21. Fig. 3: Parts of the figure are minute and hence difficult to see (especially the codes for Littoral / Sublittoral / Profundal), its difficult to get an impression of the pattern presented. Please revise this.

Done!

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