

***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.***

**Anonymous Referee #2**

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In this paper, Trajanovski and co-workers use a comprehensive set of mitochondrial COI data to test the hypothesis that leeches of the genus *Dina* in ancient Lake Ohrid represent a lacustrine species flock, and to estimate the lake's maximum age. Both goals of this generally well-written paper are interesting to a broader audience of evolutionary biologists, limnologists and geologists. The main finding is that L. Ohrid's *Dina* species are monophyletic and show a pattern of morphospecies-specific differentiation which evolved most likely within the last approx. two MA, but no clear mitochondrial lineage sorting. This estimate is consistent with geological data and findings of biological

C2895

studies.

Below, I list some comments and suggestions which might be helpful for further improving this valuable manuscript.

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 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?

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.

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

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.

P. 5018, l. 2-3 (see also P. 5016, l. 12 ff.): It would be interesting to include a improved

C2896

justification why this marker was used here. What are the expected advantages and limits for the present analyses?

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).

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.

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.

P.5022, l. 21-24: Move this section to “Material and Methods”.

P. 5023, l. 19: Change Fig. 2 to Fig. 3.

P. 5023, l. 20-25, and elsewhere: The statement that “. . .lineage sorting. . .is still not

C2897

fully complete” implies a “one-way road to speciation”, which must not necessarily be the case.

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 ?

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

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?

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

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

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

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 among-spring separation (or the existence of other refugia), without necessarily assuming the permanent existence of the lake being filled with water, appear unlikely? Please explain.

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

C2898

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).

Figs 1-2: For readers not familiar with leeches some pictures or drawings inserted in the colour figures might help getting an impression of the organisms.

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.

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