Review of the MSc thesis

Thesis title: **Genetic variability of the genus *Alburnoides* in Azerbaijan**
Author: **Dmytro Omelchenko**

The aim of the thesis was to study the genetic variability within the genus *Alburnoides* in Azerbaijan using two mitochondrial and two nuclear markers, to study phylogenetic relationships between Azerbaijan *Alburnoides* and all species for which the comparable nucleotide sequences were so far published, to estimate the time of divergence between the lineages of *Alburnoides* from Azerbaijan and to reconstruct the biogeographical history of the species. The aims of the thesis were fulfilled. The results of the thesis are interesting and certainly worth publishing. A greater genetic variability of the representatives of *Alburnoides* from Azerbaijan than previously known was shown. Three well supported lineages of *Alburnoides* were revealed. Two of the lineages inhabit two geographically isolated regions of the country, while the third lineage comprises the individuals collected both in the south and in the north of Azerbaijan. The possible explanations of such distribution of the lineages were discussed. The time of divergence of the Azerbaijan lineages was estimated to be between 2.75 and 0.25 MYA.

Comments

Introduction. The results of the Stierandová et al. (2016) are not reflected in the Introduction where the author discusses the number of the European species of *Alburnoides*, although he further uses nucleotide sequences resulting from this work for his analyses.

Methods. Some information is missing in the Methods, but it is usually mentioned later in the Results. The author states that he constructed two haplotype networks, however, only one network appears in the Results.

Results. The author claims that the position of *A. kubanicus* is different in differently constructed phylogenetic trees for RAG1. In all cases, however, it is a polytomy, so the comment about the position of this taxon is not relevant.

Author should pay attention to the position of the *Rutilus rutilus* and *Alburnoides bipunctatus* in his rhodopsin trees.

Results in haplotype network are not described enough.

Discussion. Unfortunately, finalization of the thesis in hurry is probably the reason why some parts of the discussion are not as good as they could be. The discussion about the position of

Alburnoides sp. 1 and sp. 2 from two different published works concerning two different regions in the resulting tree is nonsense.
Some parts of discussion should be in the Results section (genetic distance; moreover the genetic distances should appear as a table in the Results or in Appendix).
The age of the genus *Alburnoides* can be much older according to some works (e.g. Perea et al. 2010). This could be reflected in the discussion.
The explanation of the distribution of the lineages of *Alburnoides* in Azerbaijan can be other than that the southern part was a glacial refugium.

The quality of the thesis is somewhat diminished by the obvious lack of time while finishing the thesis. This can be seen from the formal mistakes – typos, formatting of the references, sometimes not used italics for species and genera names, missing references to figures, worse quality of some figures, etc. The lack of time is reflected also in the quality of English at some places (e.g. not finished sentences), although the overall quality of the language is quite good. Some statements and use of some words are wrong or vague, but this can be due to English not being the native language of the student.

Overall evaluation

In spite of various flaws, the quality of the thesis is overall good and I recommend it for defence.

Questions:

1. The results of molecular dating depend on the calibration points you use. If you use the timing of Perea et al. (2010) of the emergence of the genus *Alburnoides*, the split between the species of *Alburnoides* in Azerbaijan will be much older. How would you explain the phylogeography of the Azerbaijan species then?
2. Do you know what the sea level drop of the Caspian Sea was during Pleistocene glaciations? Could the spread of *Alburnoides* in Pleistocene have been due to river confluences, which were enabled by the sea regression during the glacial periods?
3. How can you explain the distribution and phylogeography of the Iranian taxa used in your analyses?

Prague, September 7th, 2016

Jasna Vukić