

Review on the Diploma thesis of Bc. Nuria Vinuela Rodriguez

I read the Diploma thesis of Ms. Vinuela Rodriguez, entitled "Phylogeny and phylogeography of cyprinid fish genus *Pelasgus* (Teleostei: Cyprinidae)", with great interest.

This study is examining the phylogenetics relationships of cyprinid species of the genus *Pelasgus*, with appropriate methodological approaches for molecular phylogeny. The author has an extensive collection of samples (180 individuals), sufficiently covering the geographic distribution of the studied taxa in Southern Balkan Peninsula (mainly Greece, but also Southern Albania and FYROM), which is one of the strong points of the study. The author selected four commonly applied markers; one mitochondrial (cyt b) and 3 nuclear genes (S7, RAG1, rhodopsin), and subsequently analyzed their sequences for the calculation of genetic distances between taxa and the construction of phylogenetic trees and networks.

As expected from previous phylogenetic studies, the fast evolving mtDNA locus was more suitable on resolving the phylogenetic relations between closely related species, than the slowly evolving nuclear loci. Among the most important results, the authors expanded and updated our knowledge on the current geographical distribution of those cyprinids. Another interesting aspect of the study is the discovery of a group of haplotypes, named in the manuscript as *Pelasgus sp.*, which either correspond to *P. epiroticus* or a new undescribed species. As the author mentioned in the manuscript, the taxonomic status of this group of cyprinids should be resolved in the future with both sampling of *P. epiroticus* from type locality and appropriate morphological analyses (which was beyond the scope of this study). Furthermore, an interesting finding is the hybridization between allopatric cyprinid species, due to fishes transferred and released accidentally by fisheries.

Although the experimental part and the interpretation of the results seem to be conducted properly, the manuscript suffers from multiple typos. Among the most prominent typos: species names are not in italics in several tables (eg. pp. 58-59), numbers of studied individuals/haplotypes do not match in the tables/text (see p. 20, p. 24 and appendix 2). Important information is missing in some legends (eg. the species names in the figure with phylogeographic distribution in p. 67) and the bibliography section is not uniformly formatted. However, I am convinced that such mistakes are not due to lack of knowledge, but due to lack of proofreading before submission.

In addition, I believe that the discussion is focused mainly on the phylogenetic analyses, but the phylogeographic aspect is highly ignored. Based on the title of the

thesis, I would expect a section in discussion about the paleogeography and geology of Southern Balkans. The author should explore how geological changes and climate fluctuations of Pleistocene could lead to the geographical isolation of the ancestral populations, and subsequently to speciation.

Despite my criticism above, the results seem solid and convincing, the discussion is straightforward and the reader can easily follow the writing. The figures and tables, with the exception of some legends are informative and it is obvious that the author spent time and effort to prepare them. The whole research project is interesting for the scientific community and it is appropriate for publishing to international scientific journal even in the current form. However, I believe that the author should further expand the phylogenetic analyses prior to publication, by including a molecular dating approach (eg. by BEAST) and a standard population analysis (eg. AMOVA).

Sincerely yours,

Michail Rovatsos