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Dear Colleagues,

You will find below my review about the thesis of **Teresa Slámová** entitled **“Phylogenetic relationships within the *Gobius*-lineage (Gobiidae)”**.

The present report is dealing with the phylogeny of some goby (Teleostei) species from the *Gobius*-lineage including 26 genera. Phylogenetic relationships were investigated using two mitochondrial (the cytochrome *b* and cytochrome *c* oxidase I genes) and nuclear (recombination-activating gene 1 and rhodopsin) markers. Phylogenetic analyses were performed using two probabilistic methods: a maximum likelihood approach and a Bayesian inference. Networks were built for the *Gobius* and *Chromogobius* species from nuclear data. Inter- and intraspecific genetic distances were also estimated for each species of the *Gobius*-lineage. Phylogenetic trees did not confirm the monophyly of the genus *Gobius*, but they rather divided this genus into two clades. These two clades included also species from other genera (*i.e.* *Zosterisessor* in one clade and *Mauligobius* in the other one), while other genera appeared polyphyletic (*Vanneaugobius* and *Padogobius*). A new species of *Chromogobius* was also underlined.

The present report brings a new light on the phylogenetic relationships of the *Gobius*-lineage in taking into account a huge number of *Gobius*-like genera and species as well as several mitochondrial and nuclear markers. For these reasons, the work presented is interesting and important. The manuscript is written in a good English even if there are some grammatical problems. The introduction gives a good state of the art regarding the systematics of the family Gobiidae, and more precisely of the *Gobius*-lineage, with a relevant and up to date literature. The taxa, molecular markers (even some markers appeared less informative than others) and methods chosen are appropriate to investigate the phylogenetic relationships of the *Gobius*-lineage. Phylogenetic trees presented for each marker and each probabilistic method are clear, even some robustness values are not always easily readable. The phylogenetic results are discussed in relation to the most appropriate literature.

However, the manuscript, in a general manner, lacks clarity and precision that makes sometimes difficult to follow the ideas of the author. First, many sentences are too long and should be shortened by using punctuation marks (as . and ,). From my point of view, at least one figure/table summarizing the systematics and distribution of the group under study is missing in the introduction in order to help the reader to better apprehend the diversity of the group under study. A lot of previously published molecular studies are mentioned in the introduction but the molecular markers used in them are never mentioned. How can we estimate the relevance of the present work compared to these previous



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studies? For more clarity, the systematics should be also reported, at least, in the table 1 of the material and methods section. The modifications of the extraction protocol should be indicated, and the molecular markers should be presented in a more conventional way. In the results section, intraspecific genetic distances are presented in tables not numbered, while interspecific genetic distances are presented as supplementary information at the end of the report. This is not mentioned somewhere in the manuscript that the interspecific distances are in supplementary information. I discovered them after I checked the bibliography. It was however clear from the discussion that interspecific distances were also estimated because only these distances are discussed in this section. Regarding the discussion, it should be more structured with several sub-sections according to the group discussed. For me, this part was the most frustrating because, as many groups, the systematics of the group under study was first based on the morphology but nearly nothing is said about the morphological similarities or differences between species. Only the ecology is discussed but ecology results from convergence. For instance, p53, it is mentioned that *Zosterisessor ophiocephalus* and *Gobius bucchichi* were proposed to be placed in a separate subgenus of *Gobius* because of close morphological affinities. Which ones? This information could support and supplement the phylogenetic hypotheses proposed in the present work. In the manuscript, there is also a lot of abbreviation defined. Some are never used (for instance, Bl, I and G), others are never defined (BA) and others are not properly used (cytb, cyt b, Cytb).

However, my main concern with the present work regards one phylogenetic analysis that should be performed but was not. If the main aim of the present work was “to conduct a comprehensive phylogenetic study of species from the *Gobius*-lineage”, why the author did not combine in one dataset the mitochondrial and nuclear markers to get a more supported phylogeny for both deep (nuclear) and recent (mitochondrial) nodes? The phylogenetic hypotheses proposed would have had more impact. Could Teresa justify her choice?

Two questions:

1) In the material and methods section, p29, the author mentioned that 3,100,000 generations were performed for the mitochondrial markers, and 1,500,000 generations for the nuclear markers. Why this difference of generations between both types of markers? However, the burn-in is the same (25%). What can be the influence of this parameter on the results if the number of generations is different? Did you check what is the appropriate burn-in phase for each analysis?



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2) The author claimed that the nuclear markers are less informative than the mitochondrial markers, that the COI is less informative than the cytb or that the Rh is less informative than the RAG. Is there a way to quantify this? For the COI, p57, you said that the length of the marker could be an explanation (630 bp for COI *versus* 1118 bp for cytb)? Are you sure that the length of the sequence really matters?

I have also several specific remarks indicated directly in the pdf file that I sent back to the supervisor of Teresa Slámová. These remarks are related to the form (English, structure of the sentences, ...) not the scientific font.

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Despite my remarks mostly focused on the form and because a huge and interesting work was done, I recommend the thesis of Teresa Slámová for acceptance.

Sincerely yours,



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