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## **Reply to Luzzatto *et al.* (2014): “Comment on ‘Genetic evidence and new morphometric data as essential tools to identify the Patagonian seahorse *Hippocampus patagonicus* (Pisces, Syngnathidae), González *et al.* (2014)’”**

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Luzzatto *et al.* (2014) criticize both methodological and other aspects of our work (González *et al.*, 2014). Our paper is a redescription of the Patagonian seahorse *Hippocampus patagonicus* Piacentino & Luzzatto 2004, based on the combination of morphometric and genetic analyses, which definitively contributes to clarifying the taxonomic status of *H. patagonicus* and validates the species. After criticizing several methodological aspects of our work, the authors concluded that ‘The work presented by González *et al.* (2014) is based on misinterpretations of the original study they claim to be improving and no new considerations are provided about the taxonomic status of *H. patagonicus*’. Below, we respond to these criticisms.

The original description of *H. patagonicus* by Piacentino & Luzzatto (2004) presents conceptual and methodological deficiencies that indicate a redescription was necessary. Our study clearly identifies these gaps, related with the improper selection of diagnostic characters. Our study demonstrates that the morphometric measures used as diagnostic characters in the original description of *H. patagonicus* overlap with those reported by other authors for *Hippocampus erectus* Perry 1810 (Vari, 1982; Lourie *et al.*, 1999 and see Table V in González *et al.*, 2014), specifically: (1) the number of rays in the pectoral-fin, (2) the number of rings on the tail and (3) the proportion of the snout length with respect to the length of the head. González *et al.* (2014) also present strong evidence (as well as evidence from other authors) that other characters given

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in the original description are not appropriate for diagnosis of seahorses (*i.e.* size and shape of the spines, colour patterns and pigmentation).

Another reason for the need of a redescription of *H. patagonicus* is the lack of genetic studies that demonstrate the validity of this species. The use of morphometric analysis alone for taxonomic studies in this group of fishes has led to much confusion. So, the combination of traditional methods with genetic studies has become a standard approach for taxonomic studies of seahorses. The original description of *H. patagonicus* (Piacentino & Luzzatto, 2004) was based only on morphology. Luzzatto *et al.* (2012) conducted a genetic analysis but, as outlined below, we consider that such a study does not provide conclusive evidence to the validation of *H. patagonicus*.

The criticisms of the morphometric analysis performed in our study are weak, unfounded and occasionally erroneous. For example, Luzzatto *et al.* (2014) state that ‘... referenced material was not examined...’ but data from the holotype and the type series were reviewed and measured (see Table III in González *et al.*, 2014). Luzzatto *et al.* (2014) also mention that the comparisons in our study were only made using information of *H. erectus* and that the statistical tests performed were not stated. Regarding this comment, it is important to highlight that the aim of our study was not to carry out a comprehensive taxonomic revision of the genus *Hippocampus*, but specifically to redescribe *H. patagonicus* and differentiate between the two sympatric species. Thus, in our opinion, there is no need to compare with other species from other geographic regions. Moreover, we consider that the information reported in Vari (1982) and Lourie *et al.* (1999) is reliable enough to make these comparisons. Regarding the need of a statistical test for comparisons, these were not made because morphometric ratios usually have larger sampling errors than the original measurements and have non-normal frequency distributions, violating the assumptions of standard statistical tests (Strauss & Bond, 1990).

Luzzatto *et al.* (2014) also criticize the origin of our samples. They suggest that we should have used specimens from different localities in order to validate the morphological data as diagnostic characters of the species. Because our study is a redescription of the species, however, we purposefully used only specimens from the type locality and not from other sites where the presence of this seahorse is very rare.

Luzzatto *et al.* (2014) also state that our genetic study lacks originality. We disagree because our analysis of *H. patagonicus* was based mainly on new sequences amplified by us from eight specimens. The other sequences used in our study were downloaded from GenBank ([www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank)), following the rules established by this public database.

Luzzatto *et al.* (2014) suggest that our paper does not contribute a novel phylogenetic analysis compared to that of Luzzatto *et al.* (2012). The genetic analysis presented in our paper, however, was developed from an updated and robust approach. Phylogenetic analyses of the molecular data were performed under maximum parsimony (MP) and Bayesian analysis, which are the most accepted methodologies today, and the analysis was complemented with *p*-distances. The study fulfils the basic requirement of replicability. It is our opinion that the phylogenetic analysis and tree presented by Luzzatto *et al.* (2012) are methodologically less robust than ours. Their phylogenetic analysis is unreproducible because they did not describe the model of molecular evolution used. There is a confusing lack of clarification of the meaning of the numbers on the branches of the tree and the lack of reference of the scale. Finally, the phylogenetic tree

presents one haplotype of *H. erectus* within the same clade as that of *H. patagonicus*, suggesting they might be conspecific.

In conclusion, we believe that the criticisms raised by Luzzatto *et al.* (2014) are in general unfounded, since they do not show significant methodological errors in our study nor throw doubt on the validity of our results.

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