



Poor taxonomic sampling undermines nomenclatural stability: A reply to Roxo *et al.* (2019)

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A recent study based on genomic data by Roxo *et al.* (2019) provided a phylogeny of the Loricariidae, the largest catfish family and second largest Neotropical fish family with approximately 1,000 species. The study represents a valuable and innovative contribution for understanding higher-level relationships within the family. The phylogenetic tree inferred by Roxo *et al.* (2019) thoroughly corroborates the monophyly and relationships of most currently accepted subfamilies of Loricariidae, based on a fair taxon sampling (nearly 14% of the species in the family) representing most genera of each but one of the subfamilies, the Lithogeninae, the sister-group of the remaining members of the family (Pereira & Reis, 2017; Reis *et al.*, 2017). In addition to a hypothesis of relationships, Roxo *et al.* (2019) also proposed a series of lower-level taxonomic changes, which are deemed premature considering that the taxonomic sampling of the study targeted higher-level clades, and go against one of the pillars of biological classification: nomenclatural stability (e.g., Heterick & Majer, 2018; Beninger & Backeljau, 2019). Here we (1) discuss implications of inadequate taxonomic sampling as a basis for changes in classification of species; (2) explain why the taxonomic sampling design of Roxo *et al.* (2019) is inadequate for the proposed nomenclatural changes; and (3) advocate that changes to classifications must be grounded on phylogenies with dense sampling of taxa at the relevant level.

Taxon sampling has been extensively discussed in the literature (Lacointre *et al.*, 1993; Swofford *et al.*, 1996; Hillis, 1998; Pollock *et al.*, 2002; Zwickl & Hillis, 2002; Smith & Craig, 2007; Heath *et al.*, 2008; Reddy *et al.*, 2017) with most of the debate focusing on the improvement of phylogenetic inference resulting from increasing density of taxonomic sampling. It must be emphasized, however, that we are not disputing the topology inferred by Roxo *et al.* (2019), but rather their taxonomic changes based on a rarified taxonomic sampling at species level. As in any scientific endeavor, sampling design is key in answering research questions, and thus phylogenetic scope must be carefully considered when defining taxon-sampling strategy (Zwickl & Hillis, 2002; Smith & Craig, 2007). If phylogenetic relationships among subfamilies of a given family are the goal of an investigation, sampling of a limited number of species representing most genera/clades is usually sufficient, without the need for inclusion of a dense sampling of species (Zwickl & Hillis, 2002). The resulting

hypotheses of relationships among species of particular clades, however, should be taken with a grain of salt as they may not be suitable for establishing lower-level classifications. Conversely, if relationships among species are being sought, data must necessarily include a dense sampling of species of the clade of interest, ideally all the species (Zwickl & Hillis, 2002). Phylogenetic analyses that fail to sample taxa according to the objective of the study are likely to produce taxonomic classifications at the relevant level that are not supported by the results. Nomenclatural changes based on such phylogenies are likely to be premature and prone to cause nomenclatural instability. As a simplistic example, a phylogenetic analysis including a minnow, a tetra, and a catfish will cluster the latter two in a clade. This is expected and reflects the high-level relationships among Ostariophysian fish, but it does not allow one to transfer the tetra into Siluriformes based on such phylogeny – the taxon sampling is just inappropriate for low-level taxonomic decisions.

Roxo *et al.* (2019) recovered polyphyletic genera among the Hypostominae, the Neoplecostomini, the Hisonotini, and the *Curumbataia* clade, but proposed taxonomic rearrangements only in the latter two. Roxo *et al.* (2019) reallocated single examined species of *Eurycheilichthys* (*E. luisae*, out of nine species), *Epactionotus* (*E. bilineatus*, out of three species), and *Otothyropsis* (*O. marapoama*, out of six species) into *Hisonotus* (seven species examined out of 34), and synonymized the four genera. Even the type species of *Eurycheilichthys* was not included in the study. As no other species of either *Eurycheilichthys*, *Epactionotus*, or *Otothyropsis* were included in the analysis, it is expected that those three species would cluster with each other or with species of *Hisonotus*, regardless of their relationships with the 42 species not included in the study. In addition, *Hisonotus* does not become monophyletic even with the inclusion of the genera above, as a few of its species are more closely related to some *Parotocinclus* or to a putative new genus. These four genera plus *Lampiella* (not included) are indeed closely related (Cramer *et al.*, 2011; Roxo *et al.*, 2014; Reis *et al.*, 2017), and the former two are demonstrably monophyletic and clearly diagnosable (Reis & Schaefer, 1998; Reis, 2017). If multiple representatives of all genera had been included in the analysis, the monophyly of each genus would have been properly tested and the species would be given the possibility to cluster with their congeners, most likely splitting *Hisonotus*, which is clearly non-monophyletic (Cramer *et al.*, 2011; Martins *et al.*, 2014; Roxo *et al.*, 2014; Calegari *et al.*, 2017), into separate genus-level clades. Roxo *et al.* (2019) ignored their own warning that “The tribe Hisonotini still lacks a morphological definition and further phylogenetic analyses are necessary to corroborate our *strong* molecular-based hypothesis” (Roxo *et al.*, 2019: 161; our italics). The authors also failed to produce a diagnosis for their definition of *Hisonotus*, and they did not discuss the extensive evidence previously presented by other researchers in support of those genera (*e.g.* Reis & Schaefer, 1998; Ribeiro *et al.*, 2005; Calegari *et al.*, 2017; Reis, 2017). The lumping of all four genera in one is likely to generate taxonomic confusion and create difficulties to much-needed studies of South American fish by obscuring existing information on relationships, diversity, evolution, biogeography, and ecology.

Other nomenclatural changes formally proposed by Roxo *et al.* (2019) include the reallocation of *Microlepidogaster longicolla* in *Rhinolekos* and the synonymization of *Gymnotocinclus* with *Corumbataia*. The latter change is plausible as there is previous compelling molecular and morphological evidence based on dense taxon-sampling that *Corumbataia* is paraphyletic without the inclusion of *Gymnotocinclus* (Cramer *et al.*, 2011; Martins *et al.*, 2014; Roxo *et al.*, 2017; Reis *et al.*, 2017). Transferring *Microlepidogaster longicolla* to *Rhinolekos* without additional taxonomic sampling, however, is premature. Roxo *et al.* (2019) again ignored their own warning: “Although relationships among species of *Microlepidogaster* and *Rhinolekos* remain unclear ([...]; Martins *et al.*, 2014), we transfer *Microlepidogaster longicolla* and *Microlepidogaster arachas* [not examined, our note] to *Rhinolekos*.” The authors justified this reallocation “based on the morphological evidence provided by Martins *et al.* (2014)”, paradoxically the same study they cite to support the notion that the relationships among these genera remain unclear, and neglected the reasons why Martins *et al.* (2014) did not implement such taxonomic changes themselves. They conclude that many species, including *Microlepidogaster arachas* “need to be analyzed in a phylogenetic context to better understand their placement in Hypoptopomatinae (...)”, blatantly underscoring our reservations here. Roxo *et al.* (2019) included a single species of *Rhinolekos* (*R. britskii*, out of four species) and two of *Microlepidogaster* (out of seven) in their taxonomic sampling. The two species of *Microlepidogaster* were not recovered as sister taxa in their phylogenetic hypothesis, but the three species are only two nodes apart, and separated by short branches.

The compositional limits of genera and other supra-specific taxa are based on hypotheses of entities that have reality in nature, comprising monophyletic groups of species. While there is flexibility in the absolute ranking implied by the hypothesis of relationships, taxa are customarily established considering the monophyly of the groups, the distinctness among clades, the species already included therein, and the principle of nomenclatural stability. The three alternative taxonomic actions that Roxo *et al.* (2019) could have taken given the topology of their *Hisonotus* subclade (which was represented by 10 species out of 52) were (1) to synonymize the four genera (*Eurycheilichthys*, *Epactionotus*, *Otothyropsis*,

sis, and *Hisonotus*) under the oldest genus-name available, as they did, (2) to maintain the established genera and create one additional genus for *H. laevior* and *H. leucophrenatus*, or (3) to refrain from proposing changes to an established taxonomy, acknowledging that their taxon sampling impedes sound nomenclatural amendment at that taxonomic scale. The latter option is illustrated by a recent molecular species-delineation study of the Neotropical fish *Astyanax*, whose authors opted to avoid making taxonomic changes, despite extensive sampling on a continental scale (Rossini *et al.*, 2016). Minimum number of taxonomic decisions should always be made to modify existing classifications (Farris, 1976; Wiley, 1979). Under this principle, the first option is the worst in terms of nomenclatural stability, as it synonymizes four genera, subverting an established classification, and disorganizes existing knowledge about morphological delimitation of individual clades produced in previous studies, which included wider sampling of species. The second option would require the description of only one new genus name and the transfer of three species to *Otothyropsis* in order to accommodate the implied phylogenetic information, but the taxonomic allocation of all species not included in the analysis would remain uncertain. These two options of taxonomic rearrangements have implications that reach beyond what the data allow and thus are premature and unnecessarily detrimental to nomenclatural stability.

Phylogenetic analyses are tools to convey information on genealogical relationships that can serve multiple purposes. One of the objectives of phylogenetic studies is to build up or modify existing classifications of organisms (Wiley, 1979), activities that belong to the realms of taxonomy and nomenclature and are regulated by internationally accepted rules and principles (ICZN, 1999). Nomenclatural stability is one of the most important of such principles and is the first general recommendation of the International Code of Zoological Nomenclature. Taxonomy is dynamic and taxonomic rearrangements reflect the progress of the discipline. Nomenclatural changes, however, must be performed as carefully as the supporting phylogenies are produced, or we fail the fundamental principles listed above.

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