

# Barcoding piranhas and pacus: Species diversity and morphological convergence of reofilic taxa

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Piranhas and pacus (Characiformes: Serrasalminidae) are a charismatic but understudied family of Neotropical fishes. Here, we analyse a DNA barcode dataset comprising 1,122 specimens, 69 species, 16 genera, 208 localities and 34 major river drainages, in order to make an inventory of diversity and to highlight taxa and biogeographic areas worthy of further sampling effort and conservation protection. Using four methods of species delimitation, we report between 76 and 99 species-like clusters, i.e. between 20% and 33% of *a priori* identified species were represented by more than one mtDNA lineage. There was a high degree of congruence between clusters, with 60% supported by three or four methods. Pacus of the genus *Myloplus* exhibited the most intraspecific diversity, with six of the 13 species sampled found to have multiple lineages. Conversely, piranhas of the *Serrasalmus rhombeus* group proved difficult to delimit with these methods. Overall, our results recognize substantially underestimated diversity in the serrasalmids, and emphasizes the Guiana and Brazilian Shield rivers as biogeographically important areas with multiple cases of across-shield and within-shield diversifications. We additionally highlight the high diversity and complex phylogeographic history of rheophilic taxa in particular, and suggest multiple colonizations of these habitats by different serrasalmid lineages.

Piranhas are iconic animals of the Amazon, and as vividly expressed by Theodore Roosevelt (1914), they are frequently demonized in popular culture as voracious man-eaters. Yet despite their infamy, piranhas are poorly known in terms of diversity, and in common with Amazonian ichthyofauna, are insufficiently investigated taxonomically (Reis et al. 2016).

Together with the mostly herbivorous pacus, piranhas comprise the family Serrasalminidae (Ostariophysi: Characiformes), currently with 94 valid species in 16 genera (Eschmeyer et al. 2017). Endemic to the Neotropics, extant serrasalmids are native to all major drainages east of the Andes (Jégu 2003).

Serrasalmids are a morphologically, ecologically, and behaviorally diverse group with a variety of feeding strategies and associated morphological adaptations (Winemiller 1989, Sazima 1990). For example, *Colossoma* and *Piaractus* are fruit and seed eaters inhabiting large rivers and important dispersers of seeds in flooded forests; *Tometes*, *Mylesinus*, *Utiaritichthys* and *Ossubtus* are rheophiles and specialist consumers of aquatic plants (Podostemaceae) growing in rapids; while *Acnodon normani* and *Catoprion mento* specialize in eating the scales of other fishes.

## Results

We collected a total of 975 serrasalmid individuals from 168 unique localities in 30 major river drainages. With the addition of data from GenBank, this increased to 1,122 specimens from 208 unique localities in 34 major drainages. Upon morphological assessment, a total of 68 species level taxa were identified, and we were able to assign names to 60 of these (i.e. valid nominal species), with a further eight being identified to genus level only, i.e. putative new species (six pacus, two piranhas). Data for 13 species were obtained from GenBank, but only one of these (*Serrasalmus marginatus*) was not already present in our dataset. Overall, the sampling covered 61 (19 piranha species and 42 pacu species) of the 94 (65%) currently valid serrasalmid species representing all 16 genera. Per species, 63 of the total 69 (91%) were represented by more than one individual, with 56 (81%) represented by five or more individuals; median number of individuals per species 10, mean 16.3, and maximum 95. Fifty-one species (74%) were collected from more than one locality; 45 (65%) were collected from three or more localities; median number of localities per species four, mean 6.2, and maximum 35. Forty species (58%) were collected in more than one drainage; 33 (48%) were collected from three or more drainages; median number of drainages per species 2, mean 3.6, and maximum 15.

Point estimates for the SLSD varied between 76 (locMin) and 99 (GMYP) putative species, with 118 unique molecular delimitations over all methods; 95% confidence intervals were largest for locMin at 67-140 species, and lowest for mPTP at 75-83 species. Of the point estimate delimitations, 49% were supported by congruence of all four methods, 60% were supported by three or four methods, and 14% by only one method.

Between 14 (20%; mPTP) and 23 (33%; GMYP) of the species were represented by more than one COI lineage, with between nine (13%; locMin, mPTP) and 13 (19%; GMYP) represented by three or more lineages. Three or more lineages were reported by all methods for *Metynnis luna*, *Mylesinus paraschomburgkii*, *Myloplus asterias*, *Myloplus rhomboidalis*, *Myloplus schomburgkii*, *Myloplus aff. rubripinnis*, and *Mylossoma duriventre*. Of the 13 species delimited by all methods as having more than one lineage, six were within the genus *Myloplus*.

The locMin analysis optimised a divergence threshold of 0.0135 (p-distance) for the dataset with maximum intraspecific divergence varying between 0.023 (*Myloplus rhomboidalis*) and 0.117 (*Myloplus schomburgkii*). The overall mean maximum intraspecific divergence was 0.0068.

Of the 68 *a priori* identified species, 43 (62%) were monophyletic, 20 (29%) were not monophyletic, and six (9%) were singletons. Eighteen species (26%) shared haplotypes with another species, and this most commonly occurred in *Myloplus* (6 spp.) and *Serrasalmus* (5 spp.).

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Jégu, M. (2003). Subfamily Serrasalminae. Check List of the Freshwater Fishes of South and Central America In R.E. Reis, S.O. Kullander, & C.J. Ferraris (Eds) (pp. 185-196). Porto Alegre, RG, Brazil: EDIPUCRS.



Our single locus species delimitation results support a notion that piranha and pacu taxonomic diversity is currently underestimated in the Brazilian Amazon. The four methods achieved a high level of congruence (60% of the lineages were supported by three or more methods), indicating they were recognizing a common signal of diversification, with great majority of these lineages also supported as allopatric and biogeographically distinct populations. The results particularly highlight: (1) the Guiana and Brazilian Shields as regions of underestimated but high ichthyofaunal endemism and diversity; (2) the existence of both between-shield (e.g. *Myloplus schomburgkii*, *M. arnoldi*, and within-shield (*Myleus setiger*, *Mylesinus paraschomburgkii*, *Myloplus rubripinnis/asterias*) diversification patterns in pacus; (3) very recent biogeographic connection between the the Aripuanã (Brazilian Shield) and Guiana Shield rivers; (4) the distinctive lineages of species shared between the Amazon and Orinoco basins (*Mylossoma aureum*, *M. duriventre*, *Piaractus brachypomus*); (5) the evolutionary uniqueness, distinctness, and apparent independent evolution of rheophilic lineages; and (6) the taxonomic difficulties associated with piranhas. Thus, characterization of these faunas by traditional taxonomic methods combined with further effort in sequencing more loci is needed to better understand the implications of these results in an explicit and testable biogeographic framework of Neotropical diversification and community assemblage.

**GMYP:** Fujisawa & Barraclough (2013) doi:10.1093/sysbio/syt033

**bGMYP:** Reid & Carstens (2012) doi:10.1186/1471-2148-12-196

**locMin:** Brown et al. (2012) doi:10.1111/j.1755-0998.2011.03108.x

**mPTP:** Kapli et al. (2017) doi:10.1093/bioinformatics/btx025

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