Neotropical Ichthyology

Original article

https://doi.org/10.1590/1982-0224-2021-0005

Molecular species delimitation of the genera *Anodus*, *Argonectes*, *Bivibranchia* and *Micromischodus* (Ostariophysi: Characiformes)

Correspondence:
Acácio Freitas Nogueira
acacio.freitas89@gmail.com

Acácio Freitas Nogueira[1,2,3], Claudio Oliveira[2], Francisco Langeani[4] and Andre Luiz Netto-Ferreira[3]

A good taxonomic assessment of specimens is an essential task to many biological studies and DNA data have provided additional sources of information to assist in the disentanglement of taxonomic problems among living organisms, as has been the case of some taxa of the megadiverse Neotropical ichthyofauna. Here we assessed all valid species in the Neotropical freshwater fish genera *Anodus*, *Argonectes*, *Bivibranchia* and *Micromischodus* of the family Hemiodontidae to establish molecular species boundaries among them. All species delimitation methods defined exactly only one MOTU for *Anodus elongatus*, *Argonectes longiceps*, *A. robertsi*, *Bivibranchia bimaculata*, *B. notata*, *B. velox*, and *Micromischodus sugillatus*, resulting in total congruence between nominal species and MOTUs for these seven taxa. The three species having discordant results across analyses: *Anodus orinocensis*, *Bivibranchia fowleri*, and *Bivibranchia simulata*, matched more than one MOTU *per* species in some methods, meaning that cryptic diversity may exist within these taxa. Overall, this great correspondence among morphological and molecular boundaries for thae species analysed seem to be indicative of a reasonably stable taxonomy within these Hemiodontidae genera.

Keywords: Biodiversity, Cryptic species, DNA barcoding, Hemiodontidae, Taxonomy.

Submitted January 5, 2021
Accepted October 15, 2021
by Alexandre Hilsdorf
Epub December 13, 2021

1 Programa de Pós-Graduação em Zoologia, Instituto de Ciências Biológicas, Universidade Federal do Pará and Museu Paraense Emílio Goeldi, Rua Augusto Corrêa, 01, 66075-110 Belém, PA, Brazil. (AFN) acacio.freitas89@gmail.com (corresponding author).

2 Laboratório de Biologia e Genética de Peixes, Departamento de Biologia Estrutural e Funcional, Instituto de Biociências, Universidade Estadual Paulista, Rua Prof. Dr. Antônio C. W. Zanin, 250, 18618-689 Botucatu, SP, Brazil. (CO) claudio.oliveira@unesp.br.

3 Laboratório de Ictiologia, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, 91501-970 Porto Alegre, RS, Brazil. (ALNF) alnferreira@gmail.com.

4 Departamento de Ciências Biológicas, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista, Rua Cristóvão Colombo, 2265, 15054-000 São José do Rio Preto, SP, Brazil. (FL) francisco.langeani@unesp.br.

Online version ISSN 1982-0224
Print version ISSN 1679-6225

Neotrop. Ichthyol.
vol. 19, no. 4, Maringá 2021

Uma avaliação taxonômica adequada dos espécimes é uma tarefa essencial para muitos estudos biológicos, e os dados de DNA têm fornecido fontes adicionais de informações para auxiliar no desemaranhamento de muitos grupos taxonômicos, como tem sido o caso de alguns táxons da megadiversa ictiofauna Neotropical. Aqui examinamos todas as espécies válidas dos gêneros de peixes Neotropicais de água doce *Anodus*, *Argonectes*, *Bivibranchia* e *Micromischodus* (família Hemiodontidae) para estabelecer limites moleculares entre elas. Todos os métodos de delimitação definiram exatamente apenas uma MOTU para *Anodus elongatus*, *Argonectes longiceps*, *A. robertsi*, *Bivibranchia bimaculata*, *B. notata*, *B. velox* e *Micromischodus sugillatus*, resultando em congruência total entre espécies nominais e MOTUs para estes sete táxons. As três espécies com resultados divergentes entre as análises, a saber, *Anodus orinocensis*, *Bivibranchia fowleri* e *Bivibranchia simulata*, corresponderam a mais de um MOTU por espécie em alguns métodos, mostrando que pode existir uma diversidade críptica dentro desses taxa. No geral, esta grande correspondência entre os limites morfológicos e moleculares para as espécies analisadas parece indicar uma taxonomia relativamente estável para esses gêneros de Hemiodontidae.

Palavras-chave: Biodiversidade, DNA barcoding, Espécies crípticas, Hemiodontidae, Taxonomia.

INTRODUCTION

South America houses the most diverse freshwater fish fauna on Earth, with about 5,160 spp. (Reis *et al*., 2016; Dagosta, de Pinna, 2019). However, the taxonomy of this megadiverse ichthyofauna remains unresolved as many species yet wait for description (Birindelli, Sidlauskas, 2018; Albert *et al*., 2020), whereas other described species contain taxonomic uncertainties, such as species complexes and cryptic biodiversity (Pereira *et al*., 2013; Guimarães *et al*., 2018; García-Melo *et al*., 2019) that hamper proposition of accurate boundaries among closely related species. Indeed, proper species delimitation is a crucial prerequisite to many biological disciplines, such as ecology, population genetics, and conservation, where incorrect identification may lead to a cascade of errors with negative consequences for scientific progress as well as for biodiversity and human welfare (Bortolus, 2008). To circumvent these challenges, DNA information has been used in biodiversity research to take into account the genetic diversity in the identification and discovery of taxa, especially new species (Godfray, 2007).

The first global molecular identification tool proposed for animals was the DNA barcoding, which employs DNA sequences of the mitochondrial gene cytochrome c oxidase I (COI) to create "COI profiles" or barcodes for a particular taxon (Hebert *et al*., 2003). In the classical framework, such COI profiles are delineated by a particular sequence or a tight cluster of very similar sequences visualized in a Neighbour-joining phenogram constructed under the Kimura 2–parameters model (Hebert *et al*., 2003; Ward *et al*., 2005). DNA barcoding successfully delimits species if their representative sequences have intraspecific distance (among them) lower than interspecific distances

(among different clusters) (Ratnasingham, Hebert, 2013). For fish and other animals, a fixed threshold of 2% has been proposed to delimitate species (Ward, 2009), however modern use of DNA sequences in alpha taxonomy relies on algorithms for *de novo* operational taxonomic units (OTU) – picking approaches that cluster sequences into molecular operational taxonomic units (MOTU) or lineages, which may or may not be assigned to morphological species or putative new species (Goldstein, DeSalle, 2011). Some methods are not distance-based, like the DNA barcoding, but theoretically based on the phylogenetic species concept (PSC) and use phylogenetic trees to yield PSC-based results (Zhang *et al*., 2013).

In the Neotropical freshwater fish fauna, especially within the order Characiformes, molecular sequences, particularly DNA barcoding, have provided a valuable source of information in helping biologists to address many taxonomic questions concerning cryptic biodiversity and species boundaries (Machado *et al*., 2017; Melo *et al*., 2018; Arruda *et al*., 2019; Serrano *et al*., 2019; Ramirez *et al*., 2020), and integrative analyses combining molecular and morphological data have been recently used to describe new species (Agudelo-Zamora *et al*., 2020; Mateussi *et al*., 2020). However, in some taxa, taxonomic uncertainties persist, especially in groups where the taxonomic delimitation of species has followed the traditional, morphological approach, and has not been scrutinized by molecular tools, and this can be the case of the characiform family Hemiodontidae.

Hemiodontids are swift swimmers with fusiform and streamlined bodies that occur in most rivers and basins of northern South America to the east of the Andes, such as Amazon, Orinoco, Tocantins and Paraná-Paraguay basins, and in rivers of the Guiana Shield and Northeastern Brazil (Langeani, 2003). Most of its members can be distinguished externally from other Characiformes by the possession of a round (midlateral) spot on the flank, an adipose eyelid well-developed covering the entire eye with a narrow opening over the pupil; a suprapectoral sulcus or axillary depression; and nine to 11 branched pelvic-fin rays (Langeani, 1998). Among the five genera in the family, only *Hemiodus* Müller, 1842 was investigated with molecular data, providing a molecular species delineation for 19 of its 23 valid species based on barcoding (COI gene) sequences (Nogueira *et al*., 2020). The other four genera, namely *Anodus* Cuvier, 1829, *Argonectes* Böhlke & Myers, 1956, *Bivibranchia* Eigenmann, 1912, and *Micromischodus* Roberts, 1971 lack molecular studies exploring their species boundaries.

Those four genera lacking molecular studies include ten species (two for *Anodus* and *Argonectes*, each, five for *Bivibranchia* and one for *Micromischodus*), without any new species being described in the last twenty years (Fricke *et al*., 2021). These species can be well-distinguished from their congeners by traditional morphological characters (Langeani, 1998). Although the literature seems to indicate little taxonomic problems in these genera (Langeani, 1996), cryptic species, defined as two or more species lacking morphological distinguishability between each other but discernible in other traits such as in genetic, can be hidden even into species without apparent taxonomic problems, as reported for some species of *Hemiodus* (Nogueira *et al*., 2020). Therefore, considering the aforementioned lack of molecular taxonomic scrutiny for *Anodus*, *Argonectes*, *Bivibranchia*, and *Micromischodus*, the aim of the present study is to test the accuracy of morphospecies in those genera via DNA barcoding of automatic species delimitations approaches (ABGD, GMYC, and PTP).

MATERIAL AND METHODS

Sampling and sequencing. To build our data set, we sequenced the cytochrome c oxidase I (COI) gene for 41 specimens of *Anodus*, *Argonectes*, *Bivibranchia*, and *Micromischodus*, our focal genera, and downloaded nine other sequences from GenBank (Tab. 1). Downloaded sequences included three from our focal species, four from the related genus *Hemiodus* and two from other Characiformes families to root the trees (Tab. 1). All voucher specimens sampled in this study are deposited in museum collections. Institutional abbreviations follow Sabaj (2019), except GEPEMA – Grupo de Estudos de Peixes do Médio Araguaia, from UFMT – Universidade Federal de Mato Grosso. Newly generated and downloaded sequences have their GenBank accession numbers at Tab. 1. Vouchers were morphologically identified following original descriptions and taxonomic keys (Langeani, 1996) whenever possible.

Total DNA was extracted from tissue samples preserved in 95% ethanol according to Wizard® Genomic DNA Purification Kit (Promega) manufacturer's instructions and partial COI sequences were PCR amplified using the primers FishF1 and FishR1 (Ward *et al*., 2005). PCR reactions were performed in a total volume of 12.5 µl, putting 1.25 µl buffer (10X), 0.5 µl MgCl2 (50 mM), 0.5 µl dNTP (10 mM), 0.25 µl from each primer (200 ng / ml), 0.2 µl Taq DNA polymerase enzyme (5 U / µl), 1 µl DNA (200 ng / µl) and adding double-distillated water to complete final volume. The amplification program consisted of an initial denaturation (94 °C for 5 min) followed by 25 cycles (94 °C for 45 s, 54 °C for 45 s and 68 °C for 1 min) then a final extension of 68 °C for 7 min. PCRs products were visualized in 1% agarose gel electrophoresis and after confirmation purified with ExoSap-IT® following the manufacturer's instructions. Purified PCRs proceeded to sequencing reaction with dye terminator nucleotides (BigDye™ Terminator v.3.1 Cycle Sequencing Ready Reaction Kit, Applied Biosystems), and the products purified through EDTA + sodium acetate mix and ethanol precipitation. We then sequenced dye-tagged samples in an automatic sequencer ABI 3130-Genetic Analyser (Applied Biosystems®).

Alignment and species delimitation analyses. Forward and reverse strands of each specimen were assembled and edited to form a single consensus sequence in Geneious 8.05 (Kearse *et al*., 2012). The final matrix of 50 sequences were aligned using the MUSCLE algorithm (Edgar, 2004) in MEGA 7.0 (Kumar *et al*., 2016) under default parameters and the alignment inspected by eye to detect possible misalignments, like internal gaps. We selected the best-fitting nucleotide substitution model in MEGA 7.0 (Kumar *et al*., 2016). For the single-locus species delimitation we used three approaches: the Automatic Barcode Gap Discovery – ABGD (Puillandre *et al*., 2012), the Generalized Mixed Yule Coalescent method – GMYC (Pons *et al*., 2006; Fujisawa, Barraclough, 2013) and the Poisson Tree Processes model – PTP (Zhang *et al*., 2013). ABGD was implemented with the MUSCLE aligned matrix as the input file in the ABGD web server (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) adopting Kimura (k80) model = 2.0, X (relative gap width) = 1.1 and keeping other parameters as default values (Pmin = 0.001, Pmax = 0.1; Steps 10; Nb bins = 20). For the GMYC analysis, an ultrametric tree was generated in BEAST v1.8 (Drummond *et al*., 2012) running two independent MCMC runs of 40 million generations sampling a tree every

4,000, assuming Birth-Death Process, uncorrelated relaxed lognormal clock model, HKY+G+I model (best model selected) and keeping other parameters as default values. Posterior distributions (ESS > 200) were examined in Tracer v1.6 (Rambaut *et al*., 2018). The two runs were combined with LogCombiner v1.8.4 (Rambaut, Drummond, 2016a) and then summarized in TreeAnnotator v1.8.4 (Rambaut, Drummond, 2016b) with 25% of the trees discarded as burn-in. GMYC was performed in the package 'SPLITS' for R program using the single threshold method. For PTP delimitation, we constructed a maximum likelihood (ML) tree in RAxML v8.2.10 (Stamatakis, 2014) running 20 ML searches to find the best tree with GTR-GAMMA model and keeping other parameters as default values. Topological robustness was verified using bootstrap algorithm (Felsenstein, 1985) through autoMR-based bootstopping criterion, which stops the searching when enough replicates have been achieved. The bootstrap results were reconciled with the best ML tree using draw bipartition. The reconciled draw bipartition tree was used as the input file in the bPTP web server (https://species.h-its.org/ptp) to implement 500,000 MCMC generations (thinning = 500) and keeping other parameters at default values. Additionally, the species in the four genera under analysis that were put together into exclusive cluster or monophyletic group by the phylogenetic analyses used had their intraspecific and interspecific genetic distance calculated and displayed in a pairwise group distance matrix. Genetic distances were calculated in MEGA 7 (Kumar *et al*., 2016) and corrected by the best model available, keeping other parameters at default settings.

TABLE 1 | Geographic and catalogue museum information of the voucher specimens utilized in this study. *Downloaded from GenBank; **Approximate coordinates.

Species	Vouchers	Locality	Collection site	GenBank number
*Anodus elongatus**	LBP 2006–14235	Brazil, Acre River	10°03'0.6"S 67°50'51.4"W	MT948959
Anodus elongatus	LBP 22551–87826	Brazil, Solimões River	04°19'28"S 69°57'36.7"W	MW377177
Anodus elongatus	MCP 52228	Brazil, Amazonas River	02°14'7.2"S 54°48'13"W	MW377178
*Anodus orinocensis**	GEPEMA 5216	Brazil, Araguaia Basin	15°53'24"S 52°14'25.1"W**	KF568969
Anodus orinocensis	LBP 2210–15615	Venezuela, Orinoco River	07°30'50.9"N 66°09'19.8"W	MW377179
Anodus orinocensis	INPA–ICT 050194–P22861	Brazil, Trombetas River	01°26'26"S 56°46'31"W	MW377180
Anodus orinocensis	INPA–ICT 049168–P28384	Brazil, Japurá River	01°43'24"S 69°08'24"W	MW377181
Argonectes longiceps	LBP 15040–61672	Brazil, Tapajós River	04°42'55.1"S 56°26'25.3"W	MW377182
Argonectes longiceps	LBP 16124–66840	Brazil, Tapajós River	04°33'09.7"S 56°17'59.6"W	MW377183
Argonectes longiceps	LBP 20500–80631	Brazil, Jari River	00°42'57"S 52°29'42.2"W	MW377184
Argonectes longiceps	MZUEL 10215–82	Brazil, Uatumã River	00°53'16"S 59°34'28.4"W	MW377185
Argonectes longiceps	MZUEL 14988–A1127	Brazil, Negro River	02°46'45.3"S 60°46'57.2"W	MW377186
Argonectes robertsi	ANSP 198706–t3087	Brazil, Xingu Basin	03°49'10.2"S 52°40'31.6"W	MW377187
Argonectes robertsi	LBP 1804–13167	Brazil, Araguaia River	15°32'00"S 52°12'00"W	MW377188
Argonectes robertsi	LBP 15818–64896	Brazil, Xingu Basin	12°31'55.7"S 52°20'29.8"W	MW377189
Argonectes robertsi	LBP 19043–75540	Brazil, Tocantins Basin	12°37'31.9"S 47°52'59.8"W	MW377190
Argonectes robertsi	MZUSP 96605–3510	Brazil, Teles Pires River	10°13'14"S 54°58'02"W	MW377191
Bivibranchia bimaculata	ANSP 189149–6861	Suriname, Lawa River	03°19'31"N 54°03'48"W	MW377192
Bivibranchia bimaculata	MHNG 2716.1 SU08–795	Suriname, Tapanahony River	03°21'57.6"N 55°25'55.6"W	MW377193
Bivibranchia bimaculata	MHNG 2757.097 GFSU14e–1559	French Guiana, Maroni River	02°51'30.9"N 53°58'38.2"W	MW377194

TABLE 1 | (Continued)

Species	Vouchers	Locality	Collection site	GenBank number
Bivibranchia bimaculata	ROM 097907–T18687	Suriname, Marowijne River	04°59'26.8"N 54°26'25"W	MW377195
Bivibranchia bimaculata	ROM 097924–T18736	Suriname, Marowijne River	04°39'13.9"N 54°25'54.3"W	MW377196
Bivibranchia bimaculata	ROM 100867–T19949	Suriname, Marowijne River	05°03'42.6"N 54°25'13.8"W	MW377197
Bivibranchia fowleri	LBP 6893–33235	Brazil, Negro River	00°08'09.4"S 67°05'03.4"W	MW377198
Bivibranchia fowleri	LBP 15937–65648	Brazil, Xingu Basin	13°29'41.8"S 53°04'57.7"W	MW377199
Bivibranchia fowleri	MCP 46116	Brazil, Jauaperi River	00°52'27"N 59°39'49"W	MW377200
*Bivibranchia fowleri**	MCP 51634	Brazil. Tapajós River	02°53'27.2"S 55°10'31.1"W	MT948960
Bivibranchia fowleri	ROM 094314–T09446	Venezuela, Orinoco River	04°04'43.3"N 66°51'30.6"W	MW377201
Bivibranchia fowleri	ROM 097421–T20350	Guyana, Mazaruni River	06°13'20.3"N 60°09'03.5"W	MW377202
Bivibranchia notata	LBP 24822–89219	Brazil, Teles Pires River	08°22'02.2"S 57°40'10.2"W	MW377203
Bivibranchia notata	LBP 24822–89222	Brazil, Teles Pires River	08°22'02.2"S 57°40'10.2"W	MW377204
Bivibranchia notata	LBP 24822–89224	Brazil, Teles Pires River	08°22'02.3"S 57°40'10.2"W	MW377205
Bivibranchia simulata	MHNG 2753.099 GFSU14–1117	Suriname, Nickerie River	04°51'07.5"N 56°47'12.1"W	MW377206
Bivibranchia simulata	ROM 098760–T19383	Suriname, Brokopondo River	04°55'20.1"N 55°07'37.8"W	MW377207
Bivibranchia velox	LBP 1582–11727	Brazil, Araguaia River	15°54'18.1"S 52°19'24.2"W	MW377208
Bivibranchia velox	LBP 5757–28123	Brazil, Araguaia River	15°53'31.5"S 52°15'02"W	MW377209
Bivibranchia velox	LBP 19134–77160	Brazil, Tocantins Basin	12°37'31.9"S 47°52'59.8"W	MW377210
*Hemiodus bimaculatus**	LBP 8016–37708	Brazil, Arinos River	14°08'21.6"S 56°04'19.5"W	MT948986
*Hemiodus huraulti**	LBP 15048–61700	Brazil, Tapajós River	04°37'28"S 56°23'18"W	MT948993
*Hemiodus semitaeniatus**	LBP 26860–65654	Brazil, Culuene River	13°29'41.8"S 53°04'57.7"W	MT949028
*Hemiodus unimaculatus**	LBP 2314–15858	Venezuela, Rio Orinoco	05°53'29.9"N 67°24'14.1"W	MT949066
Micromischodus sugillatus	DEPRJ 8696–2	Brazil, Oriximiná city	01°29'29.2"S 56°20'05.9"W	MW377211
Micromischodus sugillatus	DEPRJ 8698–1	Brazil, Oriximiná city	01°29'29.2"S 56°20'5.9"W	MW377212
Micromischodus sugillatus	DEPRJ 8698–3	Brazil, Oriximiná city	01°29'29.2"S 56°20'05.9"W	MW377213
Micromischodus sugillatus	INPA 37204–P17919	Brazil, Jatapu River	02°10'31.4"S 58°10'26"W	MW377214
Micromischodus sugillatus	LBP 12867–53427	Brazil, Tapajós River	05°05'10"S 56°52'02.7"W	MW377215
Micromischodus sugillatus	LBP 13852–57320	Brazil, Tapajós River	05°06'10.4"S 56°51'31.4"W	MW377216
Micromischodus sugillatus	MCP 52467	Brazil, Arapiuns River	02°31'22"S 55°12'51.5"W	MW377217
*Parodon nasus**	LBP 20440	–	–	GU701588
*Serrasalmus maculatus**	LBP 26723	–	–	GU701512

RESULTS

We obtained partial COI sequences for all valid species into the four analysed genera, resulting in a total of 44 sequences, of which three represent *Anodus elongatus* Agassiz, 1829; four *Anodus orinocensis* (Steindachner, 1887); five *Argonectes longiceps* (Kner, 1858); five *Argonectes robertsi* Langeani, 1999; six *Bivibranchia bimaculata* Vari, 1985; six *B. fowleri* (Steindachner, 1908); three *B. notata* Vari & Goulding, 1985; two *B. simulata* Géry, Planquette & Le Bail, 1991; three *B. velox* (Eigenmann & Myers, 1927); and seven *Micromischodus sugillatus* Roberts, 1971. Collection sites for the specimens analysed herein are shown in Fig. 1. The alignment of 50 COI sequences (ingroup plus outgroups) yielded a total of 650 positions in the final matrix with 228 variable sites and 203 parsimony informative sites. Nucleotide frequency was 23.3% adenine, 28.4% cytosine, 18.5% guanine and 29.8% thymine. Average values of intra- and interspecific

genetic distance for the ten species analysed are shown in Tab. 2. Genetic distances were calculated by the Tamura Nei (TN93) model, the second best ranked because HKY is not available for computing genetic distance in MEGA. Average intraspecific genetic distance (bold number) ranged from 0.001 within *Bivibranchia bimaculata*, *B. notata*, *B. velox* and *Micromischodus sugillatus* to 0.039 within *B. fowleri*. For interspecific distance, the lowest average value was 0.055 between the two species of *Argonectes* and the highest was 0.215 between *Bivibranchia fowleri* and *Micromischodus sugillatus*.

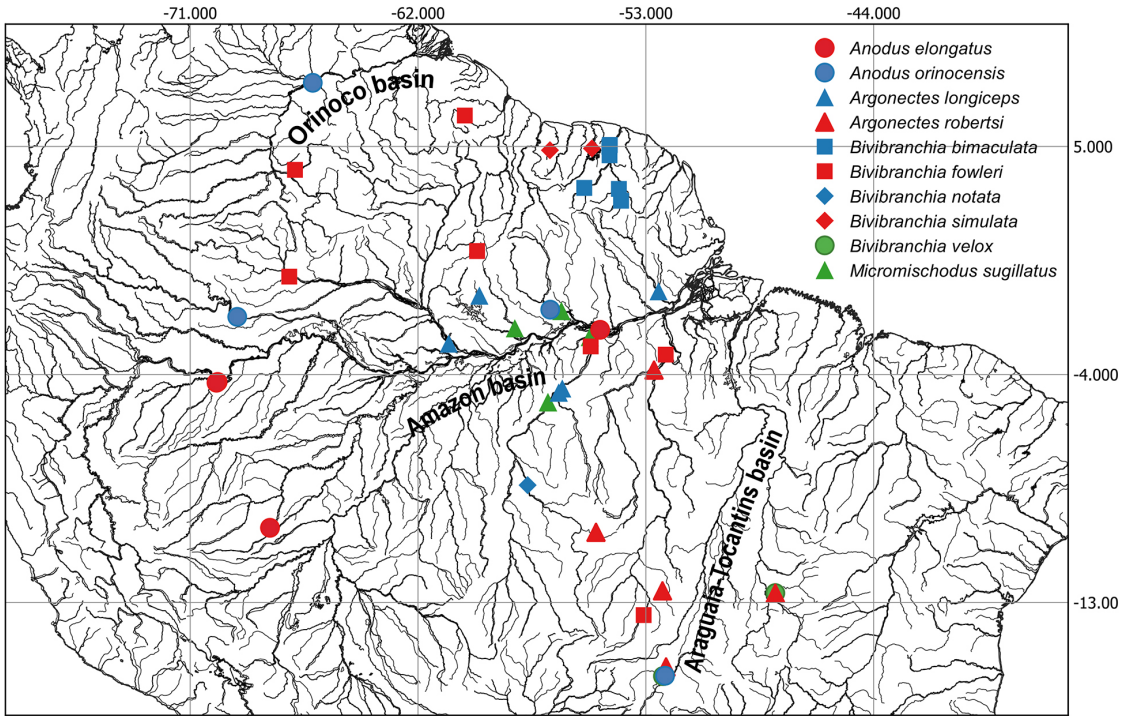

FIGURE 1 | Map of northern South America showing collection sites of Hemiodontidae specimens analysed in this study, per species.

TABLE 2 | Matrix of pairwise TN93 genetic distance among species of Hemiodontidae with interspecific distances below the diagonal of bold numbers, the standard error estimates of interspecific distances above this diagonal and bold numbers representing intraspecific genetic distance.

		1	2	3	4	5	6	7	8	9	10
1	*Anodus elongatus*	**0.003**	0.018	0.020	0.020	0.021	0.020	0.021	0.023	0.022	0.017
2	*Anodus orinocensis*	0.154	**0.038**	0.017	0.018	0.017	0.020	0.020	0.018	0.019	0.016
3	*Argonectes longiceps*	0.168	0.148	**0.003**	0.010	0.018	0.022	0.017	0.019	0.020	0.018
4	*Argonectes robertsi*	0.171	0.160	0.055	**0.007**	0.017	0.020	0.017	0.019	0.019	0.019
5	*Bivibranchia bimaculata*	0.183	0.144	0.140	0.135	**0.001**	0.020	0.017	0.015	0.018	0.020
6	*Bivibranchia fowleri*	0.188	0.192	0.202	0.181	0.162	**0.039**	0.020	0.017	0.016	0.022
7	*Bivibranchia notata*	0.202	0.190	0.158	0.162	0.151	0.184	**0.001**	0.018	0.019	0.020
8	*Bivibranchia simulata*	0.202	0.166	0.165	0.164	0.124	0.163	0.150	**0.032**	0.016	0.020
9	*Bivibranchia velox*	0.194	0.186	0.172	0.167	0.147	0.155	0.173	0.138	**0.001**	0.022
10	*Micromischodus sugillatus*	0.142	0.143	0.155	0.162	0.183	0.215	0.180	0.173	0.211	**0.001**

In the molecular delimitation analyses, the maximum clade credibility tree generated by the Bayesian inference was used to display the results in Fig. 2. The ABGD method resulted in nine partitions ranging from 9 to 18 groups or MOTUs for the ten species. The most frequent result was the number of 12 MOTUs, found in three partitions (Pmax range = 0.0046 to 0.0129). This result is also realistic and conservative considering the total number of valid species. The maximum likelihood (ML) result of GMYC delimited 14 ML entities that are all delimited lineages including single specimen MOTUs. The likelihood of the GMYC model (= 299.9232) is higher than the likelihood of the null model (= 267.2621), indicating that GMYC results are reliable. The ML solution of the PTP delineated 14 MOTUs. All delimitation results described above do not include the outgroups.

GMYC and PTP defined the same MOTUs, meaning total congruence between both tree-based methods, indeed the trees used as inputs for these methods, which are ML and Bayesian trees, are very similar in topology. Species matched by just one MOTU in all delimitation strategies were *Anodus elongatus*, *Argonectes longiceps*, *Argonectes robertsi*, *Bivibranchia bimaculata*, *B. notata*, *B. velox*, and *Micromischodus sugillatus*. About divergence across methods, only ABGD established one MOTU for *Anodus orinocensis* whereas PTP and GMYC defined the sole specimen from the Orinoco River (LBP 15615), the type locality of the species, as one MOTU and the other three specimens, coming from the Amazon (INPA) and Araguaia-Tocantins (GEPEMA) basins, as another MOTU. The two samples of *Bivibranchia simulata* constituted a single MOTU in the ABGD results but were divided in two by the tree-based methods, constituting two single specimen MOTUs, distributed into two geographically close but independent water bodies, the Brokopondo and Nickerie Rivers.

DISCUSSION

As all delimitation approaches showed absolutely concordance in delimiting *Anodus elongatus*, *Argonectes longiceps*, *Argonectes robertsi*, *Bivibranchia bimaculata*, *B. notata*, *B. velox*, and *Micromischodus sugillatus*, with total correspondence between nominal species and MOTUs, these delimitation results should probably be correct (Dellicour, Flot, 2018). The conservativeness of the AGBD and the similarity between GMYC and PTP obtained herein were also observed in the species delimitation of other characiform fishes, like in the subfamily Stevardiinae (genera *Bryconamericus* Eigenmann, 1907, *Hemibrycon* Günther, 1864, *Knodus* Eigenmann, 1911, and *Eretmobrycon* Fink, 1976), where ABGD yields a more conservative delimitation, close to the number of morphological species, while GMYC and PTP yield more splits and similar number of MOTUs (García-Melo *et al*., 2019); and in the genus *Megaleporinus* Ramirez, Birindelli & Galetti, 2017, where the same 18 MOTUs were obtained by GMYC and PTP, whereas ABGD recovered, in six partitions, from 16 to 27 MOTUs (Ramirez *et al*., 2017). On the other hand, is noteworthy that the three individuals of *Bivibranchia notata*, a species occurring in the rivers Tapajós, Trombetas and Tocantins, from an unique place in the Teles Pires River (a Tapajós tributary) could cause biases in the delimitation process since intraspecific genetic variation tends to increase with increases in geographical scale (Bergsten *et al*., 2012), but congruence among results across different strategies are good indicative that

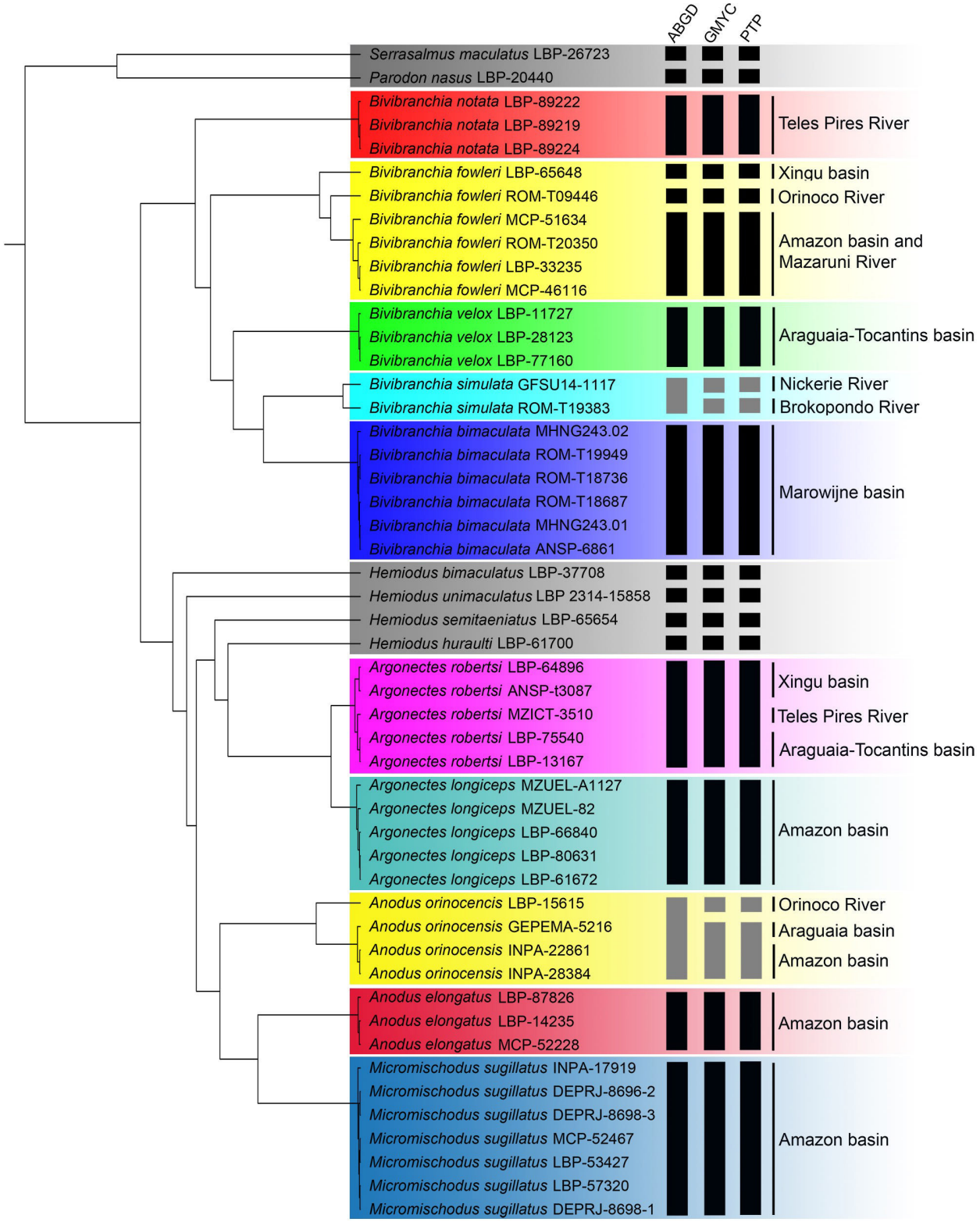

FIGURE 2 | Bayesian tree of the specimens showing the MOTUs delimited by ABGD, PTP, and GMYC methods. Congruence across methods is represented by black rectangles and discordance between them is represented by grey rectangles.

main assumptions of them were not violated, reducing the potential bias of the present result (Carstens *et al*., 2013). A similar situation of limited geographical sampling is the case of *Bivibranchia velox*, which occurs in the Xingu and Araguaia-Tocantins basins, but was sampled only in the Araguaia River.

The four samples of *Anodus orinocensis* were scattered over a huge area in South America with collection sites spanning large distances from each other but encompassing the three basins in which the species occurs, Amazon, Orinoco and Araguaia-Tocantins (see Fig. 1). This sparse geographical sampling can negatively impact the effectiveness of the delimitation methods because intermediated populations may not be considered, violating the assumption that geographic sampling is comprehensive with most or all populations, biogeographic regions, and contact zones being represented (Mason *et al*., 2020). Violated assumptions may lead to errors in delimitation outcomes that we can notice in discordant results across different delimitation methods (Carstens *et al*., 2013). The prominent type of error in ABGD is overlumping, whereas the main type of error in tree-based approaches is oversplitting (Dellicour, Flot, 2018). Those arguments seem to meet what we found in *Anodus orinocensis* with possible overlumping in ABGD or oversplitting in GMYC and PTP. In such cases of uncertainties, a conservative inference is preferable, for in most context it is better to fail to delimit species than to falsely delimit entities that do not represent true independent lineages (Carstens *et al*., 2013). Therefore we suggest *Anodus orinocensis* having two structured populations, as GMYC has been criticized for assuming that species comprise a single unstructured population and can mistakenly delimit isolated population as separate species, especially if linking populations have not been sampled (Fujisawa, Barraclough, 2013).

Bivibranchia fowleri was the most split species with three MOTUs delimited in all outcomes. Its sampling scenario encompasses most of its geographical distribution (see Langeani, 2003) with representatives lacking only from the Tocantins and Madeira Rivers. Broad sampling is especially good for widespread species, like *Bivibranchia fowleri*, because it would maximize the chance of including intermediate (population) diversity and thus generating more accurate results (Mason *et al*., 2020). Also, species sampled throughout its geographical range will reveal greater genetic variation than if the variation was estimated from a single smaller region (Bergsten *et al*., 2012), which can explain the greatest intraspecific genetic distance found in *Bivibranchia fowleri*, 3.9%, probably because more linking populations were sampled. After checked the vouchers, we did not find morphological differences that justify the description of new species, and we propose that *Bivibranchia fowleri* may contain cryptic species, regarding COI sequences, or structured population, similar to what was proposed for *Pygocentrus nattereri* Kner, 1858 (Mateussi *et al*., 2019). Such cryptic speciation may have evolved by recent divergence in which cryptic species are sister taxa or members of a species complex with short divergence times and morphological traits evolving slowly under neutral evolution or might be constrained by stabilizing selection and represent early stages of morphological stasis (Fišer *et al*., 2018; Struck *et al*., 2018).

Bivibranchia simulata is the least sampling taxon or the rarest species with only two individuals sampled, but it is among the hemiodontids with one of the smallest distribution range (Langeani, 2003) and DNA barcoding delimit more accurately species with smaller geographical scale (Bergsten *et al*., 2012). Unfortunately this scenario for *Bivibranchia simulata* is the reality of many DNA studies in biodiversity

where rarity and small geographical ranges for many species are due to logistics of fieldwork (Ahrens *et al*., 2016). Comparing the delineation strategies, ABGD is known for performing slightly worse than tree-based methods on simulated data sets using a sampling scheme with a few abundant species and many rare ones (Dellicour, Flot, 2018), the case found here for *Bivibranchia simulata*. Indeed, rarely sampled species are not problematic *per se* for GMYC and PTP approaches (Ahrens *et al*., 2016; Dellicour, Flot, 2018). A solution to compensate or overcome the limitations of low sample size (rare species and singletons) is adding closely related species or clades ("clade-wise addition") to the rare taxon (Ahrens *et al*., 2016), and *Bivibranchia simulata* meets this solution because it is surrounded by all their closely related clades, that are all other valid species within *Bivibranchia*. Nevertheless, in face of the conflicting results and low sampling we tend to the conservative way, likewise that for *Anodus orinocensis*, and propose that the two single specimen MOTUs delimited in *Bivibranchia simulata* by the tree-based approaches most likely constitute genetically-structured lineages like has been proposed in the characiforms genera *Brycon* Müller & Troschel, 1844 (Arruda *et al*., 2019) and *Pygocentrus* Müller & Troschel, 1844 (Mateussi *et al*., 2019). It is worthy to mention that Géry *et al*. (1991) have already noticed morphological variation among some other subsets of *B. simulata* populations, which were described by them as distinct subspecies: *B. s. simulata* from French Guyana, in the Oyapoque River, and *B. s. surinamensis* from Suriname, in the Coppename, Nickerie, and Suriname Rivers. These two subspecies were later considered synonyms by Langeani (2003), taking the same conservative decision as ours here.

Unlike the results of Nogueira *et al*. (2020) that strongly suggested cryptic and undescribed species for *Hemiodus*, the three species herein that matched more than one MOTUs *per* nominal species did not have congruent results across all methods and most probably be cases of recent divergence in which MOTUs are exhibiting different degrees of genetic divergence, but not high enough to be detected by all methods. Nevertheless, those MOTUs hidden into the three taxa could be better highlighted by using other data sets and analyses, maybe multi-locus analyses (Piggott *et al*., 2011), or by adding more individuals from distant localities (Mason *et al*., 2020). Despite those limitations, the present survey is the broadest molecular study ever done for Hemiodontidae including all valid species in the focal genera without any singleton (*i.e*., MOTUs represented by just one individual) while the study of Nogueira *et al*. (2020) did not include all valid *Hemiodus* species and three species were represented by singletons. Also, the results obtained here reveal a broad and strong congruence between current valid species and MOTUs, with seven of ten species being matched by exactly one MOTU, equally delimited by all strategies. This correspondence of 70% between nominal species and mitochondrial lineages may highlight a relatively stable taxonomy for these four genera of Hemiodontidae.

ACKNOWLEDGMENTS

We are grateful to the following researchers and institutions for donations of tissue samples: Mark Sabaj and Mariangeles Arce from ANSP Fish Collection (iXingu Project & NSF DEB–1257813); Érica Caramaschi and Bruno Eleres from DEPRJ (UFRJ);

Camila Ribas from INPA (Avian Collection); Carlos Lucena from MCP (PUC-RS); José Birindelli from MZUEL; Aléssio Datovo and Osvaldo Oyakawa from MZUSP; Raphael Covain from MHNG; Mary Burridge and Erling Holm from ROM-Ichthyology. We also thank UNESP to provide tissue samples (LBP) and *Brycon* server (LBP-IBB; FAPESP proc. 2014/26508.3) where were executed the phylogenetic analyses. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – finance code 001 (Acácio Nogueira). Francisco Langeani receives financial support by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, proc. 305756/2018–4). Claudio Oliveira receives financial support from Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP grants 2018/20610-1, 2016/09204–6, 2014/26508–3 and Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq proc. 306054/2006–0.

REFERENCES

- **Agudelo-Zamora HD, Tavera J, Murillo YD, Ortega-Lara A.** The unknown diversity of the genus *Characidium* (Characiformes: Crenuchidae) in the Chocó biogeographic region, Colombian Andes: Two new species supported by morphological and molecular data. J Fish Biol. 2020; 97(6):1662–75. doi: https://doi.org/10.1111/jfb.14527.
- **Ahrens D, Fujisawa T, Krammer HJ, Eberle J, Fabrizi S, Vogler AP.** Rarity and incomplete sampling in DNA-based species delimitation. Syst Biol. 2016; 65(3):478–94. doi: https://doi.org/10.1093/sysbio/syw002.
- **Albert JS, Tagliacollo VA, Dagosta F.** Diversification of Neotropical freshwater fishes. Annu Rev Ecol Evol Syst. 2020; 51(1). doi: https://doi.org/10.1146/annurev-ecolsys-011620-031032.
- **Arruda PSS, Ferreira DC, Oliveira C, Venere PC.** DNA barcoding reveals high levels of divergence among mitochondrial lineages of *Brycon* (Characiformes, Bryconidae). Genes (Basel). 2019; 10(9):5–07. doi: https://doi.org/10.3390/genes10090639.
- **Bergsten J, Bilton DT, Fujisawa T, Elliott M, Monaghan MT, Balke M *et al*.** The effect of geographical scale of sampling on DNA barcoding. Syst Biol. 2012; 61(5):851–69. doi: https://doi.org/10.1093/sysbio/sys037.
- **Birindelli JLO, Sidlauskas BL.** Preface: How far has Neotropical Ichthyology progressed in twenty years? Neotrop Ichthyol. 2018; 16(3):1–8. doi: https://doi.org/10.1590/1982-0224-20180128.
- **Bonani Mateussi NT, Melo BF, Oliveira C.** Molecular delimitation and taxonomic revision of the wimple piranha *Catoprion* (Characiformes: Serrasalmidae) with the description of a new species. J Fish Biol. 2020; 97(3):668–85. doi: https://doi.org/10.1111/jfb.14417.
- **Bortolus A.** Error cascades in the biological sciences: The unwanted consequences of using bad taxonomy in ecology. Ambio. 2008; 37(2):114–18. doi: https://doi.org/10.1579/0044-7447(2008)37[114:ECITBS]2.0.CO;2.
- **Carstens BC, Pelletier TA, Reid NM, Satler JD.** How to fail at species delimitation. Mol Ecol. 2013; 22(17):4369–83. doi: https://doi.org/10.1111/mec.12413.
- **Dagosta FCP, de Pinna M.** The fishes of the Amazon: distribution and biogeographical patterns, with a comprehensive list of species. Bull Am Museum Nat Hist. 2019(431):1–163.
- **Dellicour S, Flot JF.** The hitchhiker's guide to single-locus species delimitation. Mol Ecol Resour. 2018; 18(6):1234–46. doi: https://doi.org/10.1111/1755-0998.12908.
- **Drummond AJ, Suchard MA, Xie D, Rambaut A.** Bayesian Phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol. 2012; 29(8):1969–73. doi: https://doi.org/10.1093/molbev/mss075.
- **Edgar RC.** MUSCLE: A multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics. 2004; 5:1–19. doi: https://doi.org/10.1186/1471-2105-5-113.

- **Felsenstein J.** Confidence limits on phylogenies: an approach using the bootstrap. Evoltution. 1985; 39:783–91.
- **Fišer C, Robinson CT, Malard F.** Cryptic species as a window into the paradigm shift of the species concept. Mol Ecol. 2018; 27(3):613–35. doi: https://doi.org/10.1111/mec.14486.
- **Fricke R, Eschmeyer WN, van der Laan R.** Eschmeyer's catalog of fishes: genera, species, references 2021. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (accessed January 2, 2021).
- **Fujisawa T, Barraclough TG.** Delimiting species using single-locus data and the generalized mixed Yule coalescent approach: a revised method and evaluation on simulated data sets. Syst Biol. 2013; 62(5):707–24. doi: https://doi.org/10.1093/sysbio/syt033.
- **García-Melo JE, Oliveira C, Da Costa Silva GJ, Ochoa-Orrego LE, Garcia Pereira LH, Maldonado-Ocampo JA.** Species delimitation of Neotropical Characins (Stevardiinae): Implications for taxonomy of complex groups. PLoS One. 2019; 14(6):1–22. doi: https://doi.org/10.1371/journal.pone.0216786.
- **Géry J, Planquette P, Le Bail PY.** Faune characoïde (poissons ostariophysaires) de l'Oyapock, l'Approuague et la rivière de Kaw (Guyane Française). Cybium. 1991: 15(1): 1-69.
- **Godfray HCJ.** Linnaeus in the information age. Nature. 2007; 446(7133):259–60. doi: https://doi.org/10.1038/446259a.
- **Goldstein PZ, DeSalle R.** Integrating DNA barcode data and taxonomic practice: Determination, discovery, and description. BioEssays. 2011; 33(2):135–47. doi: https://doi.org/10.1002/bies.201000036.
- **Guimarães KLA, de Sousa MPA, Ribeiro FRV, Porto JIR, Rodrigues LRR.** DNA barcoding of fish fauna from low order streams of Tapajós River basin. PLoS One. 2018; 13(12):1–16. doi: https://doi.org/10.1371/journal.pone.0209430.
- **Hebert PDN, Cywinska A, Ball SL, DeWaard JR.** Biological identifications through DNA barcodes. Proc R Soc B Biol Sci. 2003; 270(1512):313–21. doi: https://doi.org/10.1098/rspb.2002.2218.
- **Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S *et al*.** Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012; 28(12):1647–49. doi: https://doi.org/10.1093/bioinformatics/bts199.
- **Kumar S, Stecher G, Tamura K.** MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. Mol Biol Evol. 2016; 33(7):1870–74. doi: https://doi.org/10.1093/molbev/msw054.
- **Langeani F.** Family Hemiodontidae. In: Reis RE, Kullander SO, Ferraris CJ, editors. Check List Freshw. Fishes South Cent. Am. Porto Alegre: Edipucrs; 2003. p.742.
- **Langeani F.** Phylogenetic study of the Hemiodontidae (Ostariophysi: Characiformes). In: Malabarba LR, Reis RE, Vari RP, Lucena ZM, Lucena C, editors. Phylogeny Classif. Neotrop. Fishes. Porto Alegre: Edipucrs; 1998. p.145–60.
- **Langeani F.** Estudo filogenético e revisão taxonômica da família Hemiodontidae Boulenger, 1904 (*sensu* Roberts, 1974) (Ostariophysi, Characiformes). [PhD Thesis]. São Paulo: Universidade de São Paulo; 1996.
- **Machado CB, Ishizuka TK, Freitas PD, Valiati VH, Galetti PM.** DNA barcoding reveals taxonomic uncertainty in *Salminus* (Characiformes). Syst Biodivers. 2017; 15(4):372–82. doi: https://doi.org/10.1080/14772000.2016.1254390.
- **Mason NA, Fletcher NK, Gill BA, Funk WC, Zamudio KR.** Coalescent-based species delimitation is sensitive to geographic sampling and isolation by distance. Syst Biodivers. 2020; 18(3):269–80. doi: https://doi.org/10.1080/14772000.2020.1730475.
- **Mateussi NTB, Melo BF, Foresti F, Oliveira C.** Molecular data reveal multiple lineages in piranhas of the genus *Pygocentrus* (Teleostei, Characiformes). Genes (Basel). 2019; 10(5):371. doi: https://doi.org/10.3390/genes10050371.
- **Melo BF, Dorini BF, Foresti F, Oliveira C.** Little divergence among mitochondrial lineages of *Prochilodus* (Teleostei, Characiformes). Front Genet. 2018; 9(APR):1–09. doi: https://doi.org/10.3389/fgene.2018.00107.

- **Nogueira AF, Oliveira C, Langeani F, Netto-Ferreira AL.** Overlooked biodiversity of mitochondrial lineages in *Hemiodus* (Ostariophysi, Characiformes). Zool Scr. 2020(December):1–15. doi: https://doi.org/10.1111/zsc.12469.
- **Pereira LHG, Hanner R, Foresti F, Oliveira C.** Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? BMC Genet. 2013; 14(1):20. doi: https://doi.org/10.1186/1471-2156-14-20.
- **Piggott MP, Chao NL, Beheregaray LB.** Three fishes in one: Cryptic species in an Amazonian floodplain forest specialist. Biol J Linn Soc. 2011; 102(2):391–403. doi: https://doi.org/10.1111/j.1095-8312.2010.01571.x.
- **Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S *et al*.** Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst Biol. 2006; 55(4):595–609. doi: https://doi.org/10.1080/10635150600852011.
- **Puillandre N, Lambert A, Brouillet S, Achaz G.** ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Mol Ecol. 2012; 21:1864–77. doi: https://doi.org/10.1111/j.1365-294X.2011.05239.x.
- **Rambaut A, Drummond AJ.** LogCombiner v1.8.4 2016a.
- **Rambaut A, Drummond AJ.** TreeAnnotator v1.8.4 2016b.
- **Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA.** Posterior summarization in Bayesian hylogenetics using Tracer 1.7. Syst Biol. 2018; 67(5):901–4. doi: https://doi.org/10.1093/sysbio/syy032.
- **Ramirez JL, Birindelli JL, Carvalho DC, Affonso PRAM, Venere PC, Ortega H *et al*.** Revealing hidden diversity of the underestimated neotropical ichthyofauna: DNA barcoding in the recently described genus *Megaleporinus* (Characiformes: Anostomidae). Front Genet. 2017; 8(OCT):1–11. doi: https://doi.org/10.3389/fgene.2017.00149.
- **Ramirez JL, Santos CA, Machado CB, Oliveira AK, Garavello JC, Britski HA *et al*.** Molecular phylogeny and species delimitation of the genus *Schizodon* (Characiformes, Anostomidae). Mol Phylogenet Evol. 2020; 153(August):106959. doi: https://doi.org/10.1016/j.ympev.2020.106959.
- **Ratnasingham S, Hebert PDN.** A DNA-based registry for all animal species: The Barcode Index Number (BIN) System. PLoS One. 2013; 8(7). doi: https://doi.org/10.1371/journal.pone.0066213.
- **Reis R, Albert J, Di Dario F, Mincarone M, Petry P, Rocha L.** Fish biodiversity and conservation in South America. J Fish Biol. 2016; 89(1):12–47. doi: https://doi.org/10.1111/jfb.13016.
- **Serrano ÉA, Melo BF, Freitas-Souza D, Oliveira MLM, Utsunomia R, Oliveira C *et al*.** Species delimitation in Neotropical fishes of the genus *Characidium* (Teleostei, Characiformes). Zool Scr. 2019; 48(1):69–80. doi: https://doi.org/10.1111/zsc.12318.
- **Stamatakis A.** RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014; 30(9):1312–13. doi: https://doi.org/10.1093/bioinformatics/btu033.
- **Struck TH, Feder JL, Bendiksby M, Birkeland S, Cerca J, Gusarov VI *et al*.** Finding evolutionary processes hidden in ryptic species. Trends Ecol Evol. 2018; 33(3):153–63. doi: https://doi.org/10.1016/j.tree.2017.11.007.
- **Ward RD.** DNA barcode divergence among species and genera of birds and fishes. Mol Ecol Resour. 2009; 9(4):1077–85. doi: https://doi.org/10.1111/j.1755-0998.2009.02541.x.
- **Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN.** DNA barcoding Australia's fish species. Philos Trans R Soc B Biol Sci. 2005; 360(1462):1847–57. doi: https://doi.org/10.1098/rstb.2005.1716.
- **Zhang J, Kapli P, Pavlidis P, Stamatakis A.** A general species delimitation method with applications to phylogenetic placements. Bioinformatics. 2013; 29(22):2869–76. doi: https://doi.org/10.1093/bioinformatics/btt499.

AUTHORS' CONTRIBUTION

Acácio Freitas Nogueira: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-original draft.

Claudio Oliveira: Conceptualization, Funding acquisition, Project administration, Resources, Supervision.

Francisco Langeani: Conceptualization, Project administration, Supervision, Visualization, Writing-original draft.

Andre Luiz Netto-Ferreira: Conceptualization, Investigation, Project administration, Resources, Supervision, Validation, Writing-original draft.

ETHICAL STATEMENT

Not applicable.

COMPETING INTERESTS

The authors declare no competing interests.

HOW TO CITE THIS ARTICLE

- **Nogueira AF, Oliveira C, Langeani F, Netto-Ferreira AL**. Molecular species delimitation of the genera *Anodus*, *Argonectes*, *Bivibranchia* and *Micromischodus* (Ostariophysi: Characiformes). Neotrop Ichthyol. 2021; 19(4):e210005. https://doi.org/10.1590/1982-0224-2021-0005

Neotropical Ichthyology

OPEN ACCESS

Official Journal of the
Sociedade Brasileira de Ictiologia
SBI