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Molecular phylogenetics provides a novel hypothesis of chromosome evolution in Neotropical fishes of the genus *Potamorhina* (Teleostei, Curimatidae)

Beatriz F. Dorini | **Luís R. Ribeiro-Silva** | **Fausto Foresti** | **Claudio Oliveira** | **Bruno F. Melo**

Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, IBB/UNESP, Botucatu, São Paulo, Brazil

Correspondence
Bruno F. Melo, Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, SP, Brazil.
Email: brunfmelo@gmail.com

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Abstract

Potamorhina includes the largest species in the Neotropical fish family Curimatidae. They perform long-distance migrations in large schools and represent relative importance for regional fisheries in South American lowlands. A morphology-based phylogenetic study recognized five species and proposed interspecific phylogenetic relationships mostly based on osteology, squamation, and morphology of the gasbladder. Subsequent cytogenetic studies revealed extreme variability in diploid numbers and other cytomolecular structures and hypothesized multiple events of chromosome rearrangements with centric fissions followed by reversed fusions. However, neither the taxonomic revision and phylogeny nor the cytogenetic hypothesis of chromosome evolution in *Potamorhina* was tested using molecular phylogenetic approaches. Here, we use mitochondrial and nuclear DNA sequences to delimit species of *Potamorhina* with an extensive sampling across the Amazon basin and use phylogenetic methods to test prior hypothesis of multiple events of chromosome rearrangements during the evolution of the genus. Phylogenetic and species delimitation methods clearly support the presence of five species but reveal novel interspecific relationships allowing a reinterpretation of the morphological characters relative to the number of vertebrae, caudal peduncle pigmentation, and modifications in the gasbladder chambers. With the new phylogenetic arrangement, we propose a novel hypothesis of occurrence of a single chromosome fission in the lineage of *P. latior* followed by an extraordinary event that involved more than 20 chromosome-pair fissions during the evolution of the ancestor of *P. altamazonica* and *P. squamoralevis*. This novel hypothesis represents a simpler and more conceivable explanation for the achievement of these elevated chromosome numbers during the evolution of *Potamorhina*.

KEYWORDS

Characiformes, cytogenetics, morphology, Ostariophysi, systematics

Contributing authors: Beatriz F. Dorini (beadorini@gmail.com), Luís R. Ribeiro-Silva (rickbiologia@gmail.com), Fausto Foresti (fforesti@ibb.unesp.br), Claudio Oliveira (claudio@ibb.unesp.br), Bruno F. Melo (brunfmelo@gmail.com)

1 | INTRODUCTION

Curimatidae harbor 115 fish species that are extremely abundant in freshwater environments from coastal rivers of Costa Rica to southern La Plata estuary in Argentina (Fricke, Eschmeyer, & Fong, 2019; Vari, 2003) and contains eight extant genera: *Curimatopsis*, *Potamorhina*, *Curimata*, *Psectrogaster*, *Pseudocurimata*, *Curimatella*, *Cyphocharax*, and *Steindachnerina* (Vari, 1989). Every genus was subject of comprehensive taxonomic revisions and phylogenies based on morphological data (Dillman, Sidlauskas, & Vari, 2016; Vari, 2003), and the relationships were recently tested by molecular data that revealed new phylogenetic arrangements (Melo, Sidlauskas, et al., 2018). In addition, a previous molecular study of *Curimatopsis* has challenged the current species diversity in the family by revealing several undescribed and cryptic species that was thought to contain only five species for more than 30 years (Melo, Ochoa, Vari, & Oliveira, 2016), five of them latter described (Dutra, Melo, & Netto-Ferreira, 2018; Melo & Oliveira, 2017; Melo, Vari, & Oliveira, 2016). That evidence raised the possibility that comparable studies with remaining curimatids would reveal similar patterns of hidden species diversity.

A genus demanding molecular investigation is *Potamorhina* including the largest members of the family (*P. altamazonica* reaches 27 cm standard length; Vari, 2003) that congregate in large schools to perform long-distance migrations in lowland Amazon and Orinoco rivers (Smith, 1981) and that also occur in the Lago Maracaibo and the Paraguay basin. They also represent an important regional food source with three Amazonian species dominating fish landings in different portions of the Amazon basin (Fernandes, 1997; Garcia, Tello, Vargas, & Duponchelle, 2009). The taxonomic revision associated with the morphology-based phylogeny of *Potamorhina* (Vari, 1984) recognized five species: *P. altamazonica* (Cope, 1878) from the Amazon and Orinoco basins, *P. laticeps* (Valenciennes, 1849) from the Lago Maracaibo, *P. latior* (Spix & Agassiz, 1829) and *P. pristigaster* (Steindachner, 1876), both from the Amazon basin, and *P. squamoralevis* (Braga & Azpelicueta, 1983) from the Rio Paraguay and lower Rio Paraná. That study also provided the first hypothesis of interspecific relationships (Figure 1) mostly based on osteology, morphology of the gasbladder, squamation, and pigmentation (Vari, 1984).

Although anostomoid fishes present a highly conserved chromosome formulae of 2*n* = 54 (Feldberg, Porto, & Bertollo, 1992; Oliveira, Almeida-Toledo, Foresti, Britski, & Toledo-Filho, 1988; Sampaio et al., 2016), species of *Potamorhina* have been models to study the chromosomal evolution due to extreme disparities in the diploid and fundamental numbers, positions of heterochomatin, ribosomal genes, and other physical DNA regions (Brassesco, Pastori, Roncati, & Fenocchio, 2004; Feldberg, Porto, Nakayama, & Bertollo, 1993; Pinheiro et al., 2016). For example, diploid numbers range from 2*n* = 54 in *P. pristigaster* to 2*n* = 102 in *P. squamoralevis* (Feldberg et al., 1993). These extreme variations led authors to hypothesize multiple events of chromosome rearrangements including occurrences of centric fissions and subsequent fusions (reversions) during the evolution of *Potamorhina* (Figure 1) (Feldberg et al., 1993; Pinheiro et al., 2016). However, this hypothesis was never tested based on molecular phylogenetic approaches.

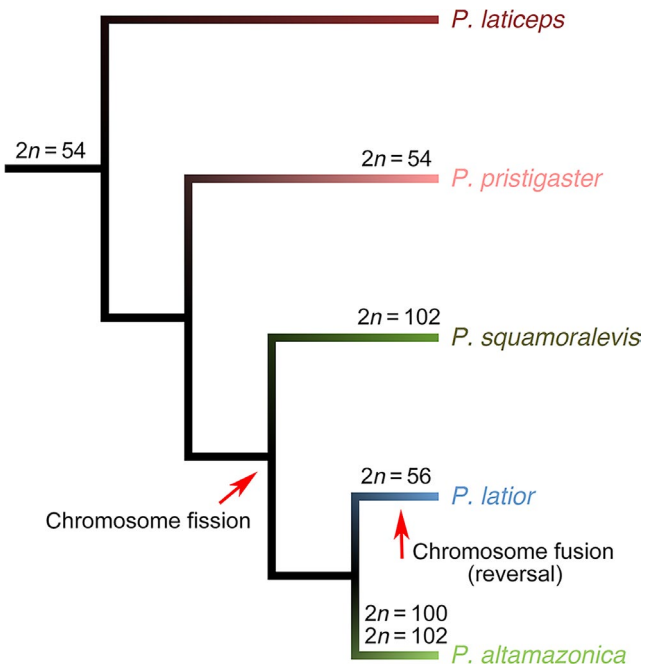

FIGURE 1 Phylogenetic relationships among *Potamorhina* species based on morphological characters (modified from Vari, 1984) and prior hypothesis of chromosome evolution (Feldberg et al., 1993; Pinheiro et al., 2016)

Here, we used mitochondrial and nuclear DNA sequences and multiple species delimitation methods to test Vari's (1984) hypothesis of the presence of five species in *Potamorhina* with an extensive sampling across the Orinoco, Amazon, and Paraguay basins and used phylogenetic methods to test Feldberg et al.'s (1993) hypothesis of multiple events of chromosome rearrangements.

2 | MATERIAL AND METHODS

2.1 | Sampling and sequencing

We included 46 specimens of all five species of *Potamorhina* and one sample of *Psectrogaster amazonica* or *Psectrogaster rhomboides* to root the trees. Only species of *Psectrogaster* were used as outgroups because the focus of the study was restricted to species of *Potamorhina*. We attempted to include samples from the entire distribution of each species as much as possible, for example, in the case of *P. altamazonica* (Juruá, Madeira, Orinoco, Purus, and Solimões) and *P. latior* (Branco, Japurá, Juruá, Madre de Diós, Madeira, Purus, Solimões, Trombetas, and Xingu) from the Amazon basin (Figure 2). Vouchers were fixed in 95% ethanol or 10% formalin and permanently stored in 70% ethanol. After species identifications following the taxonomic revision (Vari, 1984), vouchers and tissues were deposited in the Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista, Botucatu, Brazil (LBP), Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA),

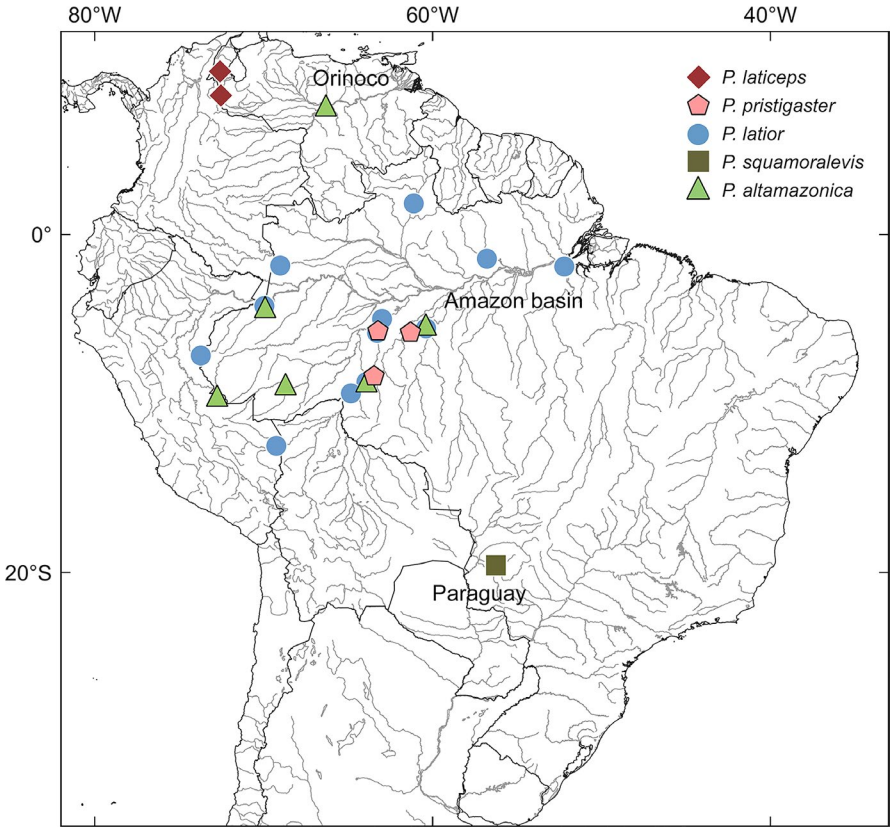

FIGURE 2 South American map showing the distribution of the analyzed specimens of *Potamorhina*

Universidade Federal de Rondônia, Porto Velho, Brazil (UNIR), Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos, Lima, Peru (MUSM), and the Academy of Natural Sciences of Drexel University, Piladelphia, USA (ANSP) (Table 1). Fishes were collected according to Brazilian environmental laws through SISBIO/MMA permit no. 3245 and procedures for collection, maintenance, and analyses followed the international guidelines for animal experiments through CEEAA IBB/UNESP protocol no. 304.

Total DNA was extracted from muscle tissues preserved in 95% ethanol with a DNeasy Tissue kit (Qiagen Inc.) according to the manufacturer's instructions. We used the mitochondrial gene *cytochrome c oxidase subunit I* (*COI*) that contains substantial polymorphism within curimatids (Melo, Ochoa, et al., 2016; Melo, Sidlauskas, et al., 2018) to perform species delimitation methods and also used the nuclear *myosin heavy chain 6* (*Myh6*) gene, along with the multilocus matrix to test the position of *Potamorhina pristigaster*, since the phylogenetic study of the family Curimatidae did not include such species (Melo, Sidlauskas, et al., 2018). Partial sequences of *COI* were amplified by polymerase chain reaction (PCR) using the primers L6252-Asn and H7271 described for characiforms (Melo, Benine, Mariguela, & Oliveira, 2011), and partial sequences of *Myh6* were amplified with primers F329 and A3R1 described for actinopterygians (Li, Ortí, Zhang, & Lu, 2007). We used 12.5 μl as a total volume containing 9.075 μl of double-distilled water, 1.25 μl 5x buffer, 0.375 μl $MgCl_2$ (50 mM), 0.25 μl dNTP mix at 8 mM, 0.25 μl of each primer at 10 μM, 0.05 μl Platinum Taq DNA polymerase enzyme (Invitrogen), and 1.0 μl genomic DNA (10–50 ng). The PCR consisted of an initial denaturation (4 min at 95°C) followed by 30 cycles of chain denaturation (30 s at 95°C), primer hybridization (30–60 s at 54°C for *COI* and 45 s at 52°C for *Myh6*), and nucleotide extension (30–60 s at 72°C). After the visualization of the fragments using 1% agarose gel, we performed the sequencing reaction using dye terminators (BigDye™ Terminator v 3.1 Cycle Sequencing Ready Reaction Kit, Applied Biosystems) purified again through ethanol precipitation. Products were then loaded onto an automatic sequencer ABI 3130-Genetic Analyzer (Applied Biosystems).

2.2 | Phylogenetic and species delimitation analyses

Geneious v8.05 (Kearse et al., 2012) was used to assemble and edit abi sequences, to generate consensus sequences for each individual, and to align them in Muscle v5.3.38 (Edgar, 2004) using default parameters. The two matrices (*COI* and *Myh6*) were visualized and edited to minimize missing data and posteriorly checked for the presence of putative stopcodons. The occurrence of substitution saturation was estimated through the index of substitution saturation in asymmetrical (Iss.cAsym) and symmetrical (Iss.cSym) topologies in Dambe v5.3.38 (Xia, 2013). The best-fit model of nucleotide evolution was obtained in MEGA v7.0 (Kumar, Stecher, & Tamura, 2016) under the BIC criterion. Sequences were posteriorly deposited to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with the accession numbers MN551002–MN551052.

Sequences of *COI* were binned into species groups following the taxonomic identification and the overall mean distance, intraspecific distances, and interspecific distances and their

TABLE 1 Taxon, vouchers, locality information, and GenBank accession numbers of the analyzed specimens of *Potamorhina*

Taxon	Voucher	Specimen	Geographic origin	*COI*	*Myh6*
P. altamazonica	LBP 2571	17020	Rio Purus, Boca do Acre, Amazonas, Brazil	–	HQ288971
P. altamazonica	LBP 3056	19162	Rio Orinoco, Caicara del Orinoco, Bolivar, Venezuela	–	MN551035
P. altamazonica	LBP 22323	86456	Rio Solimões, Tabatinga, Amazonas, Brazil	MN551020	–
P. altamazonica	LBP 22323	86457	Rio Solimões, Tabatinga, Amazonas, Brazil	MN551021	MN551039
P. altamazonica	LBP 22323	86458	Rio Solimões, Tabatinga, Amazonas, Brazil	–	MN551040
P. altamazonica	LBP 22323	86459	Rio Solimões, Tabatinga, Amazonas, Brazil	MN551007	–
P. altamazonica	LBP 22323	86460	Rio Solimões, Tabatinga, Amazonas, Brazil	MN551008	–
P. altamazonica	UNIR uncat	67081	Rio Madeira, Porto Velho, Rondônia, Brazil	MN551002	–
P. altamazonica	UNIR uncat	67083	Rio Madeira, Rondônia, Brazil	–	MN551037
P. altamazonica	UNIR uncat	67084	Rio Madeira, Rondônia, Brazil	MN551003	–
P. altamazonica	UNIR uncat	67085	Rio Madeira, Rondônia, Brazil	–	–
P. altamazonica	MUSM 33756	AP41	Rio Juruá, Breu, Ucayali, Peru	MN551031	MN551050
P. altamazonica	MUSM 39528	AP122	Rio Purus, Peru	–	MN551052
P. laticeps	LBP 6133	29516	Lago Maracaibo, Machiques de Perijá, Zulia, Venezuela	MH537301	MH537516
P. laticeps	CZUT 19174	T8217	Río Catatumbo, Maracaibo, El Zulia, Norte de Santander, Colombia	MN551033	–
P. latior	INPA 43285	T2222	Rio Xingu, Porto de Moz, Pará, Brazil	MN551032	–
P. latior	LBP 4252	22717	Rio Juruá, Cruzeiro do Sul, Acre, Brazil	MH537302	MH537507
P. latior	LBP 14931	61544	Rio Branco, Caracaraí, Roraima, Brazil	MH537303	MH537555
P. latior	LBP 22324	86461	Rio Solimões, Tabatinga, Amazonas, Brazil	MN551009	–
P. latior	LBP 22324	86462	Rio Solimões, Tabatinga, Amazonas, Brazil	MN551010	MN551041
P. latior	LBP 22324	86463	Rio Solimões, Tabatinga, Amazonas, Brazil	–	MN551042
P. latior	LBP 22324	86464	Rio Solimões, Tabatinga, Amazonas, Brazil	MN551011	MN551043
P. latior	LBP 22324	86465	Rio Solimões, Tabatinga, Amazonas, Brazil	MN551012	MN551044
P. latior	INPA 41660	88402	Rio Purus, Tapauá, Amazonas, Brazil	MN551013	–
P. latior	INPA 41660	88403	Rio Purus, Tapauá, Amazonas, Brazil	MN551022	MN551045
P. latior	INPA 41660	88404	Rio Purus, Tapauá, Amazonas, Brazil	MN551014	–
P. latior	INPA 41660	88424	Rio Purus, Tapauá, Amazonas, Brazil	MN551025	–
P. latior	INPA 50202	88407	Rio Trombetas, Oriximiná, Pará, Brazil	MN551023	MN551046
P. latior	INPA 50211	88408	Rio Trombetas, Oriximiná, Pará, Brazil	MN551024	–
P. latior	INPA 43285	88412	Rio Xingu, Porto de Moz, Pará, Brazil	–	MN551047
P. latior	INPA 41916	88429	Rio Purus, Tapauá, Amazonas, Brazil	MN551028	–
P. latior	INPA 48972	88436	Rio Japurá, Japurá, Amazonas, Brazil	MN551015	MN551049
P. latior	INPA 49132	88437	Rio Japurá, Japurá, Amazonas, Brazil	MN551029	–
P. latior	INPA 49132	88438	Rio Japurá, Japurá, Amazonas, Brazil	MN551030	–
P. latior	MUSM 37186	M6	Rio Madre de Dios/Madeira, Tambopata, Madre de Dios, Peru	MN551016	MN551051
P. latior	UNIR uncat	67085	Rio Madeira, Rondônia, Brazil	MN551017	–
P. latior	UNIR uncat	67086	Rio Aripuanã/Madeira, Rondônia, Brazil	MN551004	–
P. latior	UNIR uncat	67087	Rio Madeira, Rondônia, Brazil	MN551018	–
P. latior	UNIR uncat	67090	Rio Madeira, Rondônia, Brazil	MN551005	MN551038
P. pristigaster	INPA 41839	88425	Rio Purus, Tapauá, Amazonas, Brazil	MN551026	–
P. pristigaster	INPA 41839	88426	Rio Purus, Tapauá, Amazonas, Brazil	MN551027	–
P. pristigaster	INPA 41839	88427	Rio Purus, Tapauá, Amazonas, Brazil	–	MN551048
P. pristigaster	UNIR 2045	67092	Rio Madeira, Manicoré, Amazonas, Brazil	MN551019	–
P. pristigaster	UNIR 9600	67094	Rio Madeira, Cuniã, Rondônia, Brazil	MN551006	–
P. squamoralevis	LBP 3768	22067	Rio Paraguay, Aquidauana, Mato Grosso do Sul, Brazil	MH537304	MH537503
P. squamoralevis	LBP 3768	22070	Rio Paraguay, Aquidauana, Mato Grosso do Sul, Brazil	MH537305	MH537504
Psectrogaster amazonica	LBP 19054	75535	Rio Tocantins, Paranã, Tocantins, Brazil	MN551034	–
P. rhomboides	LBP 5533	27204	Rio Parnaíba, Balsas, Maranhão, Brazil	–	MN551036

TABLE 2 Pairwise K2P genetic distance among distinct lineages of *Potamorhina* (below diagonal) and values of standard error (above diagonal). Diagonal bold numbers represent intraspecific K2P genetic distances

	P. laticeps	*P. pristigaster*	*P. latior*	*P. squamoralevis*	*P. altamazonica*
P. laticeps	**0.019**	0.075	0.069	0.063	0.070
P. pristigaster	0.235	**0.014**	0.048	0.040	0.038
P. latior	0.226	0.140	**0.005**	0.019	0.024
P. squamoralevis	0.193	0.109	0.045	**0.015**	0.007
P. altamazonica	0.215	0.100	0.049	0.007	**0.000**

respective standard deviation values were calculated using Kimura-2-parameters (K2P) model (as selected by MEGA) with 1,000 bootstrap pseudoreplicates in MEGA v7.0 (Kumar et al., 2016). Distances were only estimated for the *COI* matrix as a standard in barcoding and species delimitation studies (Hebert, Cywinska, Ball, & DeWaard, 2003). A consensus neighbor-joining tree (NJ) with 1,000 bootstrap pseudoreplicates was generated for both *COI* and *Myh6* matrices in MEGA v7.0. Then, a maximum likelihood (ML) approach was performed for both matrices using RAxML-HPC on XSEDE v8.2.12 (Stamatakis, 2014) implemented on Cipres (Miller, Pfeiffer, & Schwartz, 2010) using the GTRGAMMA model (Stamatakis, Hoover, & Rougemont, 2008) with other parameters at default. One thousand bootstrap pseudoreplicates tested the obtained relationships.

For species delimitation analyses using the *COI* dataset, we applied the Bayesian Poisson Tree Process (bPTP) (Zhang, Kapli, Pavlidis, & Stamatakis, 2013) using the best ML tree generated by RAxML, 500,000 generations (thinning = 500), and other parameters at default in the bPTP webserver (https://species.h-its.org). Then, we applied the Automatic Barcode Gap Discovery (ABGD) (Puillandre, Lambert, Brouillet, & Achaz, 2012) using an input FASTA file into the ABGD webserver (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html), intraspecific priors ranging from 0.001 to 0.019 (determined by a previous distance analyses in MEGA), a relative gap 2.0, and K2P model. Finally, we concatenated the two datasets and aligned the two-loci matrix into the large six-loci matrix with 216 taxa used in the phylogeny of the Curimatidae (Melo, Sidlauskas, et al., 2018) in order to compare phylogenetic relationships and test the position of *P. pristigaster* that was absent in that analysis. We then realigned the matrices with Muscle v5.3.38 (Edgar, 2004) using default parameters, excluded duplicated sequences, and ran a ML tree using the same RAxML procedures described above. *Brycon pesu* was used to root this curimatid tree.

3 | RESULTS

The final *COI* matrix of *Potamorhina* includes 38 terminals with 594 bp and 154 variable sites (25.9%) (Alignment S1). The *Myh6* matrix includes 24 terminals, 738 bp, and 62 variable sites (8.4%) (Alignment S2). These two matrices were aligned into the expanded 216-taxa curimatid dataset (Melo, Sidlauskas, et al., 2018) that resulted in 255 terminals, 5,358 bp, and 2,147 variable sites (40%) (Alignment S3). No saturation signal was detected in any matrix. In the *COI* dataset, the overall K2P mean distance was 0.0070 ± 0.016, and intraspecific distances ranged from 0.000 ± 0.000 within *P. squamoralevis* from Rio Paraguay to 0.019 ± 0.014 within *P. laticeps* from Lago Maracaibo and Rio Catatumbo. Genetic distance values among species were very high in 9/10 pairwise comparisons (i.e., 90%) appearing with more than 0.04 of genetic distance (i.e., >4%). The lowest pairwise K2P genetic distance was between *P. altamazonica* and *P. squamoralevis* (0.007 ± 0.007), and the highest distance was between *P. laticeps* and *P. pristigaster* (0.235 ± 0.075). Both intra- and interspecific genetic distances are included in Table 2.

The schematic ML tree (Figure 3) denotes the interspecific phylogenetic relationships in *Potamorhina* with a novel hypothesis of morphological and chromosome evolution. Our combined results show that *P. pristigaster* (Amazon basin) is sister to the trans-Andean *P. laticeps* (Lago Maracaibo), with this clade sister to the three-species clade composed of *P. latior* (Amazon basin) as the sister to *P. altamazonica* (Amazon basin) and *P. squamoralevis* (Paraguay basin). All ML and NJ trees of the mitochondrial and nuclear datasets (Figures S1–S4) presented topologies supporting the monophyly of most *Potamorhina* species, with exception of the placement of *P. squamoralevis* inside *P. altamazonica* in the *Myh6* trees (Figures S2 and S4). However, the extended multilocus phylogenetic tree (Figure S5) overwhelmingly supports the monophyly of each species (including *P. altamazonica* and *P. squamoralevis*) and the same position of *Potamorhina* as sister to *Curimata* (Melo, Sidlauskas, et al., 2018) with high bootstrap support. Long branches separate each of the species except the clade *P. altamazonica* and *P. squamoralevis* that have smaller genetic variation. The specimens of *P. latior* and *P. altamazonica* from distant localities in the Amazon basin appeared nested within large clusters without an evident drainage-based structure (Figure 4). All matrices and topologies are available as Alignments S1–S3 and Figures S1–S5.

The two species delimitation methods (bPTP and ABGD) returned similar results supporting the presence of five species of *Potamorhina*. Support values of bPTP are relatively high for *P. laticeps* (0.925), *P. pristigaster* (0.879), *P. altamazonica* (0.642), moderate for *P. latior* (0.556), and lower for *P. squamoralevis* (0.422). The ML solution of bPTP delimited the five species (plus the outgroup), splitting *P. altamazonica* of *P. squamoralevis*, the two species that possess the lowest genetic distance values in the group. The ABGD

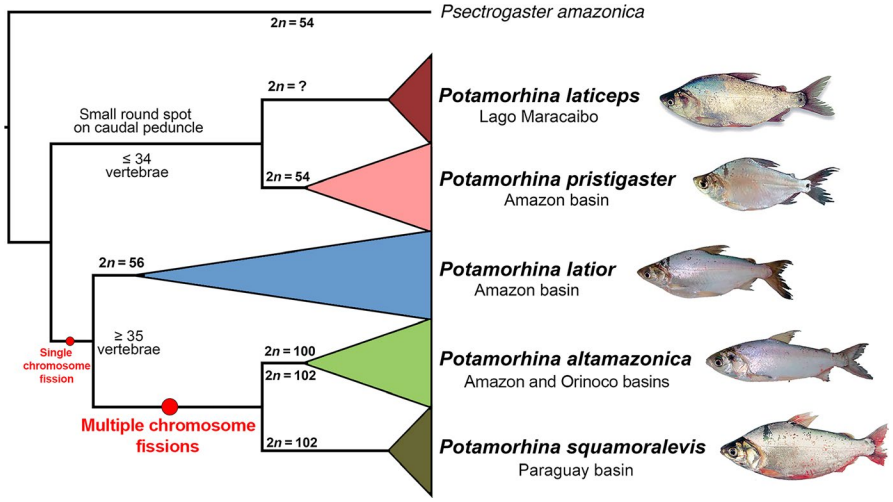

FIGURE 3 Collapsed maximum likelihood tree of *Potamorhina* showing chromosome formulae and diagnostic morphological features for each clade. Red circles highlight our hypothesis for a single fission followed by a major event of chromosome fissions

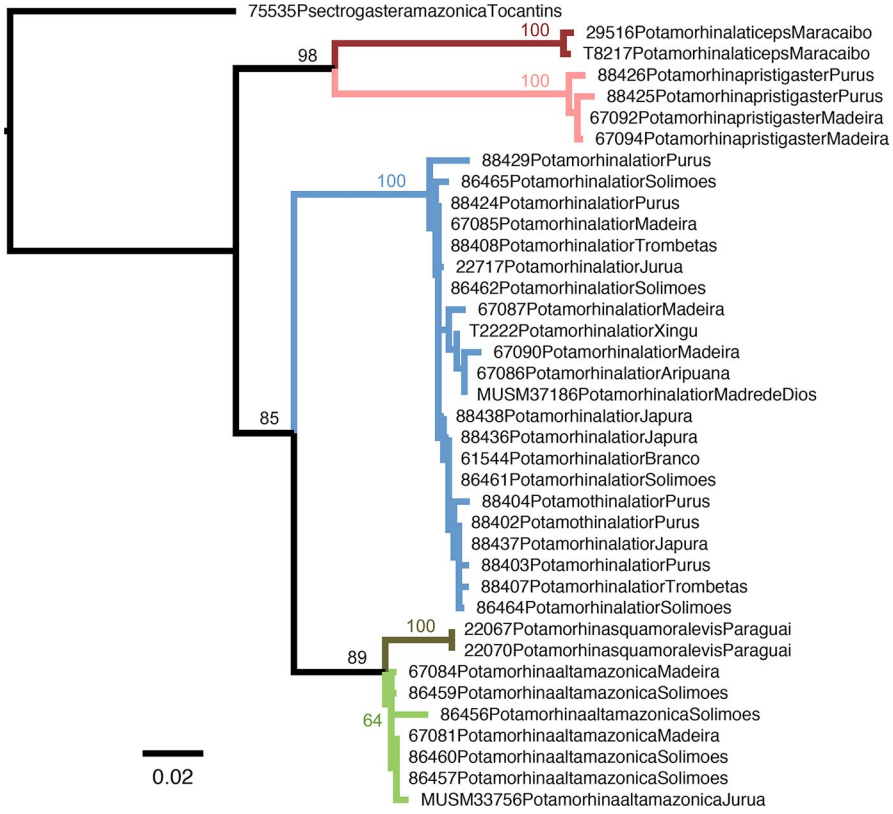

FIGURE 4 Maximum likelihood tree of the *Potamorhina* species based on partial sequences of the *cytochrome c oxidase subunit I* gene. Numbers near nodes represent bootstrap support for each species

returned 10 partitions: Five partitions indicating seven species (prior maximal distance p = 0.001–0.003), two partitions indicating six species (p = 0.005–0.007), and three partitions indicating five species (p = 0.009–0.019). Considering the average of intraspecific distances being 0.010 (Table 2), the ABGD resulted in five species with *P. altamazonica* and *P. squamoralevis* nested in a single species. Even though the ABGD delimiting *P. altamazonica* and *P. squamoralevis* as a single species, the combined results of the phylogenetic and delimitation methods suggest that they should be recognized as distinct species. Factors in favor of this statement are the reciprocal monophyly based on the multilocus dataset, the bPTP analysis, morphological diagnoses, and allopatric distribution.

4 | DISCUSSION

4.1 | Phylogeny and morphological characters

Results indicate a phylogenetic arrangement with *Potamorhina laticeps* sister to *P. pristigaster*, and this clade sister to the clade with *P. latior* as the sister species of the subclade *P. altamazonica* plus *P. squamoralevis*. The pattern matches the molecular phylogeny of the family (Melo, Sidlauskas, et al., 2018) that proposed the "*P. laticeps* clade" and "*P. latior* clade"; here, we refine this structure by adding *P. pristigaster* to the "*P. laticeps* clade". Molecular-based interspecific relationships slightly differ of those hypothesized by the morphological phylogeny

(see Figure 1) (Vari, 1984), which allow us to provide reinterpretations of the evolution of morphological characters. First, the sister relationship between *P. laticeps* and *P. pristigaster* implies the inapplicability of the character #7 (presence of 33 or more vertebrae) that once united *P. pristigaster* to *P. altamazonica*, *P. latior*, and *P. squamoralevis*, but rather strengthens the character #8 (presence of 35 or more vertebrae) that unites *P. altamazonica*, *P. latior*, and *P. squamoralevis* (Vari, 1984). Furthermore, the absence of a pigmented spot on midlateral surface of caudal peduncle (character #10) unites *P. altamazonica*, *P. latior*, and *P. squamoralevis* (Vari, 1984), while *P. laticeps* and *P. pristigaster* have the plesiomorphic condition: presence of such pigmented spot. Vari (1984) proposed that this character is a plesiomorphy for Curimatidae due its wide occurrence in various congeners including *Curimatopsis*, the group sister to all remaining curimatids (Melo, Sidlauskas, et al., 2018; Vari, 1989). We follow Vari's conclusion and additionally point out that this character is very useful to diagnose *P. laticeps* and *P. pristigaster* (*P. laticeps* clade) from remaining congeners.

Gasbladder modifications represent three synapomorphies supporting the clade composed by *Potamorhina altamazonica* and *P. latior* (Vari, 1984). These are the presence of elaborated anterior diverticula in the anterior chamber of the gasbladder (character #13), the presence of numerous lateral outpocketings in the anterior chamber (character #14), and the overlap of these outpocketings over the anterior chamber of the gasbladder (character #15) (Vari, 1984). However, the molecular phylogeny (Melo, Sidlauskas, et al., 2018) and our phylogenetic reconstructions evidenced another arrangement in which *P. latior* is sister to the clade with *P. altamazonica* and *P. squamoralevis* (Figure 3). This novel hypothesis, therefore, suggests that the gasbladder modifications either emerged in the ancestor of *P. altamazonica*, *P. latior*, and *P. squamoralevis* with subsequent loss in *P. squamoralevis*, or appeared independently in *P. altamazonica* and *P. latior*.

In terms of species diversity, the molecular data and species delimitation analyses support Vari's taxonomic revision that recognized five species of *Potamorhina*, which are promptly identified by the degree of flatteness in the prepelvic and postpelvic regions, number of scales and vertebrae, and possession of a dark round spot in the caudal peduncle (Vari, 1984). Although *Curimatopsis* surprinsingly presented an underestimated cryptic/undescribed diversity (Melo, Ochoa, et al., 2016), with five species described subsequently (Dutra et al., 2018; Melo, Ochoa, et al., 2016; Melo & Oliveira, 2017), we could not find either undescribed or cryptic species in *Potamorhina*, even including samples from distant and remote regions of the Amazon basin, such as *P. altamazonica* and *P. latior* collected in the Branco, Japurá, Juruá, Madre de Dios, Madeira, Purus, Solimões, Trombetas, and Xingu rivers of the Amazon basin in Brazil and Peru, or even *P. altamazonica* from the Orinoco in Venezuela (Figure 2). Similarly reported for migratory species of *Prochilodus* (Melo, Dorini, Foresti, & Oliveira, 2018), long-distance migrations typical for species of *Potamorhina* (Smith, 1981) and non-existent for species of *Curimatopsis* probably maximize the intense gene flow among populations throughout lowlands of the Amazon basin. As such, further studies with other non-migratory curimatid genera (e.g., *Cyphocharax* and *Steindachnerina*) might reveal distinct results.

4.2 | A simpler hypothesis for chromosome evolution in *Potamorhina*

The extreme variability in chromosome number and structure ($2n$ = 54 to $2n$ = 102) along with other cytogenetic markers have been the central basis for Feldberg et al.'s hypothesis of multiple centric fissions in chromosomes during the evolution of *Potamorhina* (Figure 1) (Ferdberg et al., 1993). At that time, these cytogenetic findings were placed in the phylogenetic context following the available morphological hypothesis (Vari, 1984), which led them to hypothesize multiple chromosome fissions in the ancestor of *P. altamazonica*, *P. squamoralevis*, and *P. latior* with a subsequent reversion (i.e., chromosome fusions) in *P. latior* (Pinheiro et al., 2016). However, the recent molecular phylogeny (Melo, Sidlauskas, et al., 2018) and the interspecific relationships presented here (Figure 3) allow us to propose an alternative hypothesis for the chromosome evolution in *Potamorhina*, which is much simpler than previously thought.

First, the molecular phylogenetic evidence suggests that the $2n$ = 54 formula is plesiomorphic in Curimatidae, and maintained in *P. pristigaster* and probably *P. laticeps*, for which cytogenetic data are still unavailable. Subsequent fission in one pair of chromosome likely occurred during the evolution of the ancestor of the clade including *P. latior*, *P. altamazonica*, and *P. squamoralevis*, and the configuration of $2n$ = 56 chromosomes appeared and persisted in *P. latior*. Finally, an extraordinary event fissioned more than 20 chromosome pairs in the ancestor of *P. altamazonica* and *P. squamoralevis* resulting in $2n$ = 100 or more chromosomes. This scenario, after the evidence from the molecular phylogeny and the inclusion of *P. pristigaster* into that phylogeny, represents a simpler and more conceivable hypothesis for the acquirement of these elevated chromosome numbers that are very unusual among diploid teleosts (Arai, 2011).

Some questions still remain to be explored in *Potamorhina*. The disjunct occurrence of *P. altamazonica* in both Amazon and Orinoco basin (Vari, 1984) and the fact that these populations present different chromosome numbers (Feldberg et al., 1993; Nirchio, Rossi, Foresti & Oliveira, 2014) are intriguing and raise doubts as to whether they represent one or two species. Further research might include more samples of *P. altamazonica* from the Orinoco basin, which only one specimen was included in this study. Additionally, new cytogenetic data for *P. laticeps* are fundamental to confirm the presence of the plesiomorphic condition of $2n$ = 54 found in *P. pristigaster* (Feldberg et al., 1993), the sister species as suggested by the phylogenetic analyses. Following *Curimatopsis* (Melo, Ochoa, et al., 2016), this is the second study aimed to investigate the molecular diversity across the family Curimatidae and studies in progress will provide further genetic and evolutionary information for the remaining six genera.

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ORCID

Fausto Foresti https://orcid.org/0000-0002-0862-0445
Claudio Oliveira https://orcid.org/0000-0002-4143-7212
Bruno F. Melo https://orcid.org/0000-0002-0499-567X

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Maximum likelihood tree of the *Potamorhina* species based on partial sequences of the *cytochrome c oxidase subunit I* gene. Numbers near nodes represent bootstrap support.

Figure S2. Maximum likelihood tree of the *Potamorhina* species based on partial sequences of the *myosin heavy chain 6* gene. Numbers near nodes represent bootstrap support.

Figure S3. Neighbor-joining tree of the *Potamorhina* species based on partial sequences of the *cytochrome c oxidase subunit I*. Numbers near nodes represent bootstrap support.

Figure S4. Neighbor-joining tree of the *Potamorhina* species based on partial sequences of the *myosin heavy chain 6* gene. Numbers near nodes represent bootstrap support.

Figure S5. Maximum likelihood tree of the *Potamorhina* species based on the multilocus matrix. Numbers near nodes represent bootstrap support.

Alignment S1. *COI* matrix of *Potamorhina*.

Alignment S2. *Myh6* matrix of *Potamorhina*.

Alignment S3. Multilocus matrix of Curimatidae aligned with the two matrices of *Potamorhina* generated herein.

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