Neotropical Ichthyology

Original article

https://doi.org/10.1590/1982-0224-2021-0162

Population structuration and chromosomal features homogeneity in *Parodon nasus* (Characiformes: Parodontidae): A comparison between Lower and Upper Paraná River representatives

Correspondence:
Marcelo Ricardo Vicari
vicarimr@uepg.br

Matheus Azambuja[1], Daiane Santana Marcondes[2], Viviane Nogaroto[2], Orlando Moreira-Filho[3] and Marcelo Ricardo Vicari[1]

The ichthyofauna of the La Plata hydrographic basin is divided into Upper and Lower Paraná River systems due to the geographic isolation of the Sete Quedas waterfalls, currently flooded by the lake of the Itaipu dam. In Parodontidae, pairs of species, or groups of cryptic species were described between these systems. Although genetic isolation and speciation have already been proposed in other species in the group, *Parodon nasus* has been maintained as a valid species and distributed throughout the La Plata river basin. In this perspective, specimens of *P. nasus* from four different sampling sites in the Upper and Lower Paraná River systems were compared regarding the karyotypes, molecular analyzes of population biology and species delimitation to investigate their genetic and population isolation in the La Plata river basin. Despite a geographic barrier and the immense geographic distance separating the specimens sampled from the Lower Paraná River system compared to those from the Upper Paraná River, the data obtained showed *P. nasus* as a unique taxon. Thus, unlike other species of Parodontidae that showed diversification when comparing the groups residing in the Lower *versus* Upper Paraná River, *P. nasus* showed a population structure and a karyotypic homogeneity.

Keywords: *COI*, La Plata basin, Populational structure, rDNAs, Sete Quedas waterfalls.

Submitted November 22, 2021
Accepted December 29, 2021 by Guillermo Ortí
Epub March 28, 2022

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

Neotrop. Ichthyol.
vol. 20, no. 1, Maringá 2022

1 Programa de Pós-Graduação em Genética, Universidade Federal do Paraná, Avenida Coronel Francisco H. dos Santos, 100, Jardim das Américas, 81531-990 Curitiba, PR, Brazil. (MA) matheus_azambuja@hotmail.com; (MRV) vicarimr@uepg.br (corresponding author).

2 Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Av. Carlos Cavalcanti, 4748, 84030-900 Ponta Grossa, PR, Brazil. (DSM) daiane-sm1@hotmail.com; (VN) vivianenogaroto@hotmail.com.

3 Departamento de Genética e Evolução, Universidade Federal de São Carlos, Rodovia Washington Luís, km 235, 13565-905 São Carlos, SP, Brazil. omfilho@ufscar.br.

A ictiofauna do sistema hidrográfico La Plata é dividida em alto e baixo rio Paraná devido ao isolamento geográfico dos Saltos das Sete Quedas há 22 milhões de anos, atualmente inundado pelo lago da represa da Usina de Itaipu. Em Parodontidae, espécies pares ou grupos de espécies crípticas foram descritos entre esses sistemas. Contudo, embora o isolamento genético e especiação já tenham sido propostos em outras espécies do grupo, *Parodon nasus* tem sido mantido como espécie válida e distribuída em toda a bacia do rio La Plata. Nessa perspectiva, exemplares de *P. nasus* de quatro diferentes pontos de amostragem nos sistemas do alto e baixo rio Paraná foram comparados quanto ao arranjo dos cariótipos, análises moleculares de biologia populacional e delimitação de espécies, afim de investigar seu isolamento genético e populacional na bacia do rio La Plata. Apesar da barreira geográfica e imensa distância geográfica separando os exemplares amostrados no sistema baixo rio Paraná em comparação àqueles do alto rio Paraná, os dados obtidos demonstraram *P. nasus* como único táxon válido. Dessa forma, diferentemente de outras espécies de Parodontidae que demonstraram diversificação quando comparados grupos pares residentes no baixo e alto rio Paraná, *P. nasus* demonstrou estruturação populacional e homogeneidade cariotípica.

Palavras-chave: Bacia La Plata, COI, Estruturação populacional, rDNAs, Sete Quedas.

INTRODUCTION

Parodontidae is a small family of Neotropical fish with 32 valid species (Fricke *et al*., 2021), grouped into three genera: *Parodon* Valenciennes, 1849, *Saccodon* Kner, 1863 and *Apareiodon* Eigenmann, 1916 (Pavanelli, 2003). They have a wide geographical distribution in South America and Panama rivers, except for some coastal basins in the Atlantic and Patagonia (Pavanelli, Britski, 2003).

The La Plata basin covers an area of $3{,}2.10^6$ Km^2 over five South American countries (Berbery, Barros, 2002). Paraná, Uruguay, La Plata, and Paraguay rivers constitute the main tributaries of the La Plata basin (Berbery, Barros, 2002; Júlio Jr. *et al*., 2009). The Sete Quedas waterfalls (a 114-meter high natural geographic barrier currently submerged due to the Itaipu dam built about 150 km downstream to waterfalls) represented a significant geographic barrier for fish species dispersal in the Paraná River (Agostinho, Zalewski, 1996; Abell *et al*., 2008). Based on the geographic barrier provided by the Sete Quedas waterfalls, the La Plata basin ichthyofauna showed gene flow restriction between these areas, isolating species from the Lower and Upper Paraná River systems (Abell *et al*., 2008; Júlio Jr. *et al*., 2009). The Itaipu dam is currently considered a division point between the Lower and Upper Paraná systems (Júlio Jr. *et al*., 2009). In this region, a fish pass system, called Canal da Piracema, was built to connect downstream and upstream rivers in the Itaipu dam (Makrakis *et al*., 2007). The Canal da Piracema could allow long-distance migratory species to find suitable spawning and nursery areas in the tributaries of Itaipu Reservoir and in the floodplain located upstream

(Agostinho *et al*., 1993; Gomes, Agostinho, 1997). Many endemic species in the Lower Paraná colonized and dispersed to the Upper Paraná after constructing the Itaipu dam and the Sete Quedas waterfalls submersion (Júlio Jr. *et al*., 2009).

Parodon nasus Kner, 1859 inhabits lotic and lentic waters, has migratory reproductive behavior in shoals and spawn with external fertilization (Godoy, 1975). The Cuiabá River (Upper Paraguay River drainage in the Lower Paraná River system) is the type locality for *P. nasus* (Kner, 1859). According to Britski *et al*. (1999), *P. nasus* occurrence would be restricted to rivers belonging to the Paraguay River basin. In opposite, *Parodon tortuosus* Eigenmann & Norris, 1900, nowadays a junior synonym of *P. nasus* (Pavanelli 1999, 2003), had its distribution described for the Upper Paraná River system (Britski, 1972). Analyzing representatives from the Lower and Upper Paraná River, Pavanelli (1999, 2003) did not find robust morphological characters to corroborate *P. tortuosus*, extending the occurrence of *P. nasus* for all rivers that constitute the La Plata Basin.

Parodon nasus and its junior synonym *P. tortuosos* were also cytogenetically investigated (Moreira-Filho *et al*., 1984, 1985; Jesus, Moreira-Filho, 2000; Vicente *et al*., 2001; Centofante *et al*., 2002; Bellafronte *et al*., 2005, 2011; Schemberger *et al*., 2011, 2014, 2016; Ziemniczak *et al*., 2014). Despite some slight chromosome differences, representatives of *P. nasus* from the Lower and Upper Paraná River systems demonstrate a shared karyotype (Bellafronte *et al*., 2005).

In addition to chromosomal and morphological data, the molecular markers are helpful in taxonomy and systematics as they consider nuclear or organellar sequence evolutionary history (Padial *et al*., 2010; Schilick-Steiner *et al*., 2010; Travenzoli *et al*., 2015). These methodologies directly seek variation in the DNA level, allowing phylogenetic and gene flow analysis (Travenzoli *et al*., 2015; Nascimento *et al*., 2018; Santos *et al*., 2019; Traldi *et al*., 2020). Thus, the concept of integrative taxonomy has been used, which uses different sources of information for the delimitation of species in supposedly homogeneous taxa (Padial *et al*., 2010; Ruane, 2015; Gąsiorek *et al*., 2017; Grković *et al*., 2017; Traldi *et al*., 2020). In this context, the study compared karyotypes and genetic parameters of *P. nasus* representatives from four rivers distributed between the Lower and Upper Paraná River system to assess the role of the Sete Quedas barrier on their distribution, population structuring, and speciation.

MATERIAL AND METHODS

Biological samples. Thirty-five *Parodon nasus* specimens (Fig. 1A) from four rivers in the La Plata basin were collected: Cuiabá River – Lower Paraná River; and Mogi-Guaçu River, Passa Cinco River, and Paiol Grande stream – Upper Paraná River (Tab. 1 and Fig. 1B). Specimens were deposited as vouchers in the ichthyological collection at Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (NUP), Universidade Estadual de Maringá, Maringá and Museu Nacional, Rio de Janeiro (MNRJ), Brazil (Tab. 1).

Cytogenetics analyzes. Mitotic chromosomes were obtained from the animals' kidneys, according to Bertollo *et al*. (2015). Heterochromatic regions were identified by the C-banding procedure, according to Sumner (1972), with the final stage of propidium iodide staining (Lui *et al*., 2012). Fluorescence *in situ* hybridization (FISH)

was performed following Pinkel *et al*. (1986), and three sequences of repetitive DNAs were physically mapped: 18S and 5S rDNAs, and satellite DNA *pPh2004*. 18S probe was obtained according to Hatanaka, Galetti Jr. (2004) and labeled with Biotin-16-dUTP (Biotin-Nick Translation Mix; Roche Applied Science). The sequence of the 5S rDNA was obtained and labeled with digoxigenin-11-dUTP (DIG-11-dUTP; Jena Bioscience) by PCR using the genomic DNA of *P. nasus* and the primers 5SA and 5SB (Martins and Galetti, 1999). The *pPh2004* probe was obtained according to Vicente *et al*. (2003) and labeled with digoxigenin-11-dUTP (Dig Nick Translation Mix; Roche Applied Science). FISH was performed under the following conditions: 300 ng of each probe, 50% formamide, 10% dextran sulfate, 2xSSC, 37 °C for 16h. Signal detection was carried out using Streptavidin Alexa Flour 488 (Molecular Probes) and anti-digoxigenin-rhodamine (Roche Applied Science). The chromosomes were stained with DAPI (0.2 µL/mL) present in Vectashield mounting medium (Vector) and analyzed in an epifluorescence microscope (Leica DM 2000) coupled to a DFC3000 G CCD camera (Leica). The chromosomes were classified following the arms ratio rule (Levan *et al*., 1964) and arranged into karyotypes.

Molecular analyzes. Genomic DNAs of nineteen individuals of *P. nasus* from the four different rivers of the La Plata basin were extracted from liver or fin samples following the CTAB (cetyltrimethylammonium bromide) method of Murray, Thompson (1980). DNAs were used to amplify the barcode region of the gene *Cytochrome C Oxidase subunit I* (*COI*) by PCR using the primers Fish F1 and Fish R1 (Ward *et al*., 2005). Reaction mix contained: 1x *Taq* Reaction buffer (200 mM Tris pH 8.4, 500 mM KCl), 1 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM of each primer, 1 U *Taq* DNA polymerase (Invitrogen), and 40 ng of DNA. The following reaction program was used: initial denaturation for 10 minutes at 94 °C, 35 cycles of 94 °C for 1 min, 54.5 °C for 45 sec and 72 °C for 90 sec, and a final extension at 72 °C for 10 min. PCR products were purified with the Illustra GFX PCR DNA and Gel Band Purification (GE Healthcare) and sequenced in an ABI-prism 3500 Genetic Analyzer (Applied Biosystems).

Sequences were checked and corrected in the software Geneious v 7.1.9 (Kearse *et al*., 2012) and stored as voucher in GenBank. The obtained sequences were aligned using the algorithm Clustal W, integrated into Geneious, and used in the populational analysis. The sequences were arranged into groups corresponding to specimens in sampled areas. The genetics distances were calculated in MEGA v 7.0 (Kumar *et al*., 2016), under

TABLE 1 | Collection sites of specimens of *Parodon nasus*, number of individuals analyzed and voucher numbers.

River	Hydrographic system	Location	Specimens	Voucher
Cuiabá River	Lower Paraná River	56°09'59"W 15°34'41"S	8	MNRJ 29787
Mogi-Guacu River	Upper Paraná River	47°22'03"W 21°55'34"S	10	MNRJ 28199
Passa Cinco River	Upper Paraná River	47°46'54"W 22°22'19"S	4	NUP 23215
Paiol Grande stream	Upper Paraná River	45°41'00"W 22°40'34"S	6	MNRJ 28131

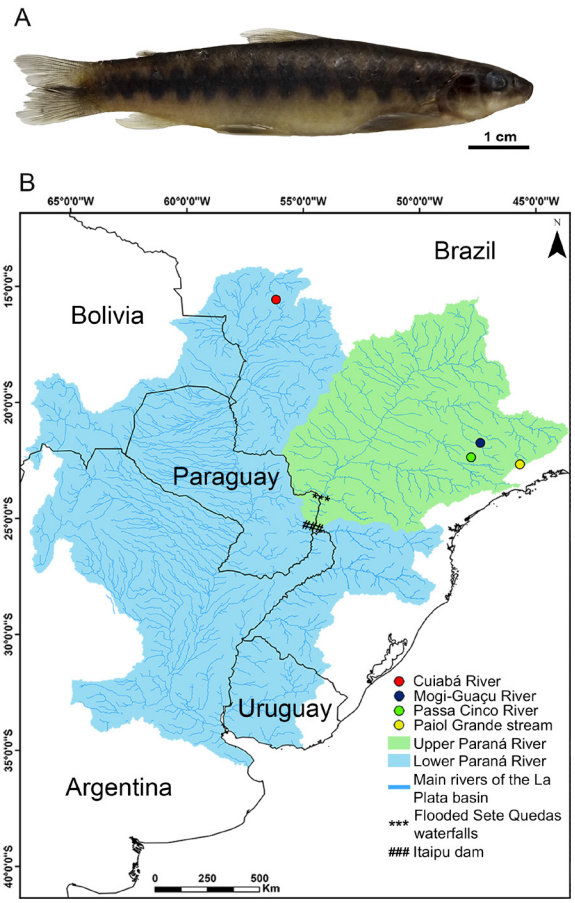

FIGURE 1 | **A.** Adult specimen of *Parodon nasus*. **B.** Partial map of the South America showing the La Plata basin, the principal rivers of the basin, and the collection sites of the *P. nasus* species analyzed.

the Kimura-2-parameters evolution model and 1000 repetitions, considering the four rivers and the Paraná basin structure. The Mantel test was performed in the software Alleles in Space (Miller, 2005). The nucleotide (π) and haplotype (H) diversities were estimated in the software DnaSP v5 (Librado, Rozas, 2009). Population structuring and analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992) were performed by the Arlequin 3.5.2.2 software (Excoffier, Lischer, 2010). Structural analysis was performed by assignments of each individual to the respective populations using Bayesian Analysis of Population Structure – BAPS 6 (Corander *et al*., 2004, 2008). The haplotype network was generated in PopArt 1.7 software (Leigh, Bryant, 2015) through the median-joining criterion (Bandelt *et al*., 1999).

For the phylogenetic analyses and delimitation of species, one *Leporinus piau* Fowler, 1941 *COI* sample (HM405030.1) was used to root the trees. *Leporinus piau COI* sequence was aligned with the *P. nasus* sequences with Clustal W. The final matrix was submitted to jModeltest2 (Posada, 2008) to select the best-fit model of nucleotide evolution to be used in downstream analysis. A Bayesian inference tree was generated in MrBayes 3.2 program (Huelsenbeck, Ronquist, 2001), applying 100.000.000 interactions of Markov Chain Monte Carlo (MCMC), sampling trees every 20.000 generations.

For the species delimitation, two methods were used: (a) General mixed Yule coalescent (GMYC) (Pons *et al*., 2006; Fujisawa, Barraclough, 2013); (b) Automatic Barcode Gap Discovery (ABGD; Puillandre *et al*., 2012). In the GMYC method, an ultrametric gene tree was inferred in Beast v. 2.6.1 (Bouckaert *et al*., 2019) under the Yule model *prior* and Strict Clock. Markov chains included 10.000.000 generations, storing trees every 1.000 generations to obtain 10.001 trees. Tracer v. 1.6 (Rambaut *et al*., 2014) was used to examine the average standard deviation of split frequencies and the convergence of MCMC searches, considering > 200 as an appropriate and effective sample size value. The first 1,000 trees were discarded as burn-in, and the 9,001 trees were summarized in the Maximum Clade Credibility tree (MCC) from the posterior distribution in TreeAnnotator v. 2.6 (Bouckaert *et al*., 2019). The tree was imported into the R software (R Development Core Team, 2013), and delimitation of species was made with the package *SPLITS* (Species' Limits by Threshold Statistics – http://r-forge.r-project.org/projects/splits/) using the single threshold method. For the ABGD analysis, the alignment generated for the sequences was used as an input file in the ABGD web server (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html), the Kimura (K80) TS/TV was selected for distance mode, and the others parameters were kept default.

RESULTS

Cytogenetics analysis. All *P. nasus* analyzed presented 2n = 54 chromosomes, karyotype formulae composed of 48m/sm + 6st and FN = 108. Heteromorphic sex chromosomes were not detected. Heterochromatic blocks were distributed in the centromeric or terminal regions along the chromosomes of specimens analyzed (Figs. 2A–D). A single cluster of 18S rDNA at the q terminal region in the subtelocentric pair 25 was detected (Figs. 3A–D), while the 5S rDNA cluster was observed at the p arm terminal region in pair 25 in the four populations (Figs. 3A–D). Individuals from the Cuiabá River also presented an additional 5S rDNA site at the proximal region in the metacentric pair 17 (Figs. 3A). *pPh2004* DNA satellite sites were located in 4 chromosomal pairs: at the terminal regions of the long arms in chromosome pairs 6, 13, 26, and 27 for the four analyzed populations (Figs. 3A–D – boxes).

Molecular analysis. Nineteen partial *COI* sequences were obtained for *P. nasus* from representatives from four rivers (accession numbers in GenBank: OL584306 – OL584324). All sequences presented a high quality and showed no evidence of indels, deletions and stop codons. The sequences were aligned, and a 631 bp matrix was generated. A total of 7 haplotypes were observed, with nucleotide diversity (π) of 0.00410 and haplotypic (H) of 0.819. The haplotypes were distributed as follow: Haplotype H1 comprises all individuals belonging to the Cuiabá River; haplotype H2 was shared between individuals from the Mogi-Guaçu River and Paiol Grande stream; haplotype H3 was shared by the populations of the Mogi-Guaçu and Passa Cinco rivers, and Paiol Grande stream; haplotype H4 and H7 were exclusive to the Passa Cinco River; and the haplotypes H5 and H6 were exclusive to the population of the Mogi-Guaçu River (Fig. 4A).

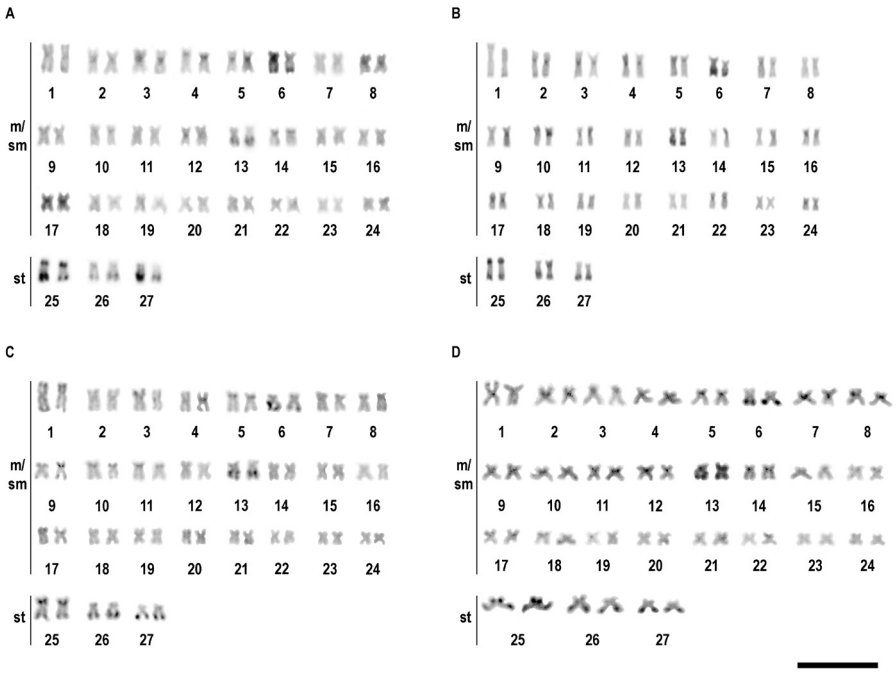

FIGURE 2 | Karyotypes of the four populations of *Parodon nasus* submitted to the C-banding procedure. **A.** Cuiabá River; **B.** Mogi-Guaçu River; **C.** Passa Cinco River; **D.** Paiol Grande stream. Scale bar = 10µm.

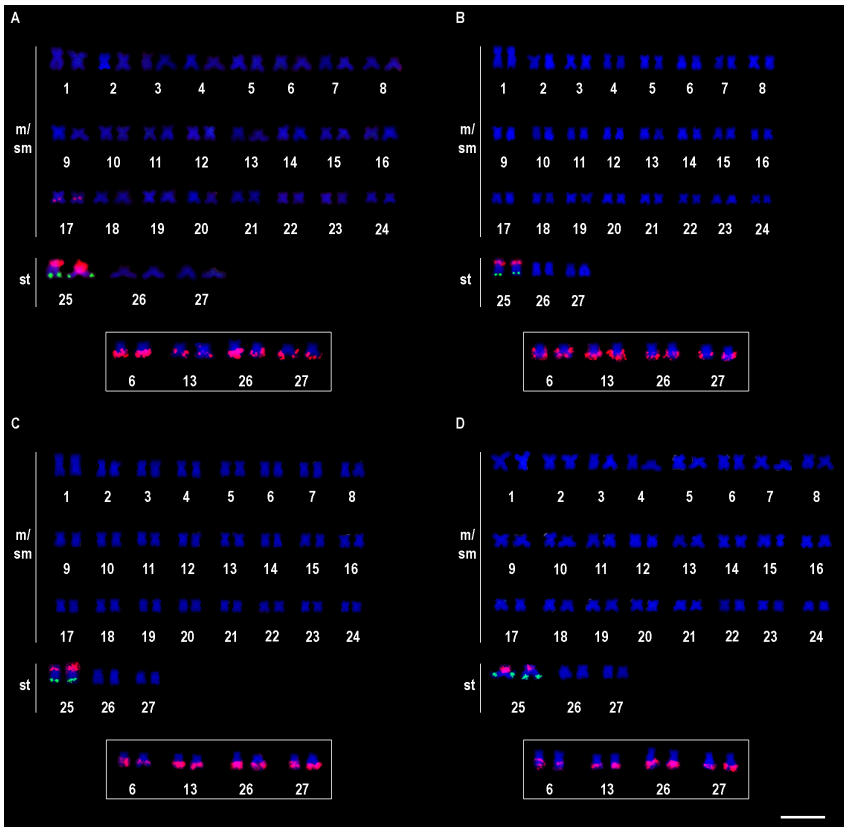

FIGURE 3 | Karyotypes of the four populations of *Parodon nasus* submitted to fluorescence *in situ* hybridization using 18S rDNA (green signal) and 5S rDNA (red signal) probes. Chromosomes with signals for the *pPh2004* probe were highlighted in boxes. **A.** Cuiabá River; **B.** Mogi-Guaçu River; **C.** Passa Cinco River; **D.** Paiol Grande stream. Scale bar = 10µm.

The intraspecific genetic distance for each population ranged from 0 to 0.19%, while the interspecific genetic distance ranged from 0.15 to 0.88% (Tab. 2). When considering the division of the La Plata Basin in Lower and Upper Paraná River, the intraspecific genetic distance in the Lower Paraná was 0% and in the Upper Paraná was 0.16%. In addition, the interspecific genetic distance between Lower and Upper Paraná River samples was 0.79%. The Mantel test demonstrated a robust positive correlation between genetic distance and geographic distance ($r = 0.92$; $p < 0.001$; Fig. **S1**).

For population structure analysis, specimens were gruped in: Lower Paraná (Cuiabá River) and Upper Paraná (Mogi-Guaçu River + Passa Cinco River + Paiol Grande stream). The BAPs result demonstrated the probability of formation of these two clusters ($k = 2$) of 0.99292 (Fig. 4B). The AMOVA analysis showed variance values among groups of 83.57% and within populations of 13.14%, with an FST value = 0.8686 (Tab. 3). The pairwise FSTs values between each population ranged from -0.00515 to 0.93182 and showed the population structure between individuals from the Cuiabá River and those from the other localities (Tab. 4).

The best-fit model of nucleotide evolution inferred using the corrected Akaike information criterion (AICc) for the phylogenetic and species delimitation analyzes was HKY+G (-LNL = 1402.4146). The Bayesian tree showed the individuals belonging

TABLE 2 | Estimates of evolutionary divergence over sequence pairs among groups and standard error in populations of *Parodon nasus*. Bold values in main diagonal show the intraspecific genetic distance. The number of base substitutions per site from averaging over all sequence pairs between groups is shown. Kimura-2-Parameters genetic distance means and standard error. Analyses were conducted using the Kimura 2-parameter model. The analysis involved 19 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 631 positions in the final dataset.

	Cuiabá River	Mogi-Guaçu River	Passa Cinco River	Paiol Grande stream
Cuiabá River	**0.0000 (0.0000)**			
Mogi-Guaçu River	0.0083 (0.0033)	**0.0019 (0.0011)**		
Passa Cinco River	0.0088 (0.0035)	0,0017 (0.0007)	**0.0016 (0.0011)**	
Paiol Grande stream	0.0070 (0.0032)	0.0015 (0.0009)	0.0017 (0.0010)	**0.0010 (0.0009)**

TABLE 3 | Analysis of molecular variance (AMOVA) from the populations of *Parodon nasus*. Two groups were considerate: Cuiabá River; Mogi-Guaçu River + Passa Cinco River + Paiol Grande stream.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among groups	1	16.692	2.16220Va	83.57
Among populations within groups	2	1.471	0.08523Vb	3.29
Within populations	15	5.100	0.34000Vc	13.14
Total	18	23.263	2.58743	
Fixation Indices F_{SC}: 0.20043 F_{ST}: **0.86860** (p<0.001) F_{CT}: 0.83566				

to the Cuiabá River in a clade. In contrast, individuals belonging to the other rivers (Upper Paraná) were distributed in two clades (Fig. 4C). The two species delimitation methods showed identical results. The GMYC analysis suggested the number of two species (outgroup included, confidence interval 2–9 species; the maximum likelihood of null model: 119.1766; the maximum likelihood of GMYC model: 128.8128; likelihood ratio: 19.27235; LR test: 6.532229e-05***; threshold time: -0.0061). The second method, ABGD, resulted in 10 partitions: one partition presented eight species, while the other nine found two species. For partition 5, two species (outgroup included) were observed (prior maximal distance P = 0.007743).

TABLE 4 | Population pairwise FSTs values for the populations of *Parodon nasus* (lower diagonal) and *p-values* (upper diagonal). Bold values were significant ($p < 0.05$).

	Cuiabá River	Mogi-Guaçu River	Passa Cinco River	Paiol Grande stream
Cuiabá River	–	**0.00000+-0.0000**	**0.00901+-0.0091**	**0.00901+-0.0091**
Mogi-Guaçu River	**0.88462**	–	0.85586+-0.0390	0.62162+-0.0317
Passa Cinco River	**0.92147**	-0.00515	–	0.07207+-0.0227
Paiol Grande stream	**0.93182**	0.06250	0.28760	–

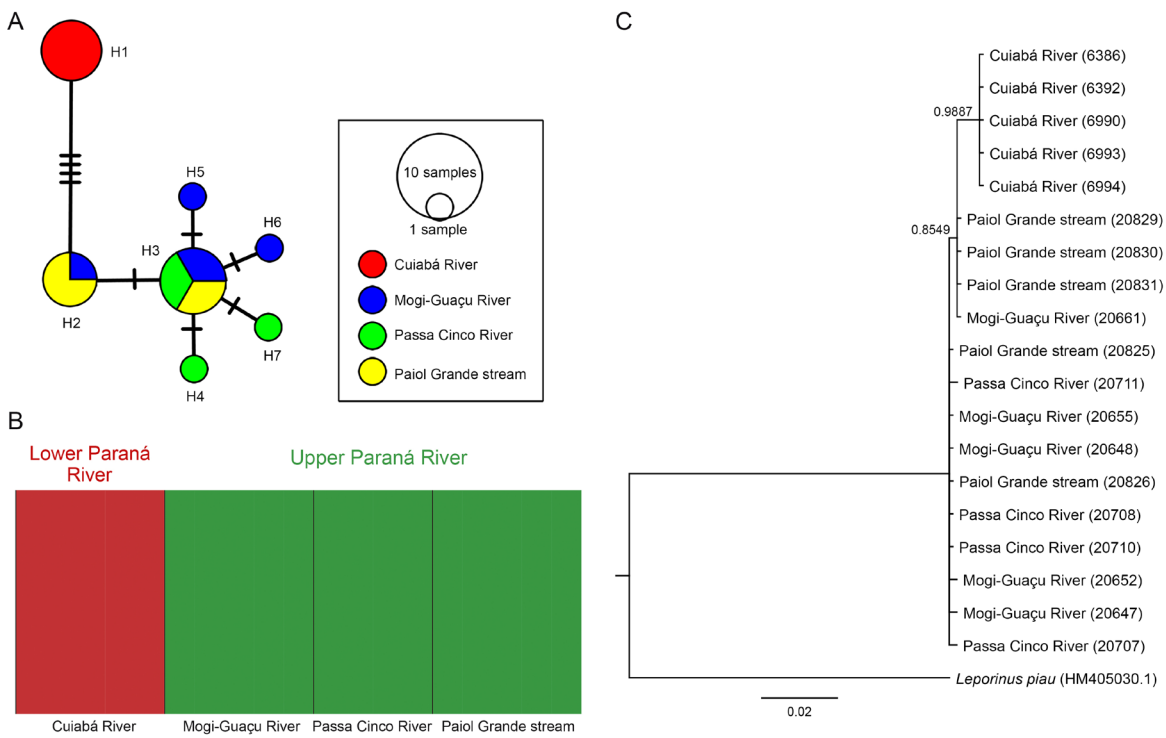

FIGURE 4 | Molecular data of *Parodon nasus* from La Plata basin. **A.** Haplotype network showing the relationship among the sequences. **B.** Structural population inference by BAPs (K = 2) for the four populations showing the division between Upper and Lower Paraná River systems; **C.** Bayesian inference tree showing the phylogenetic relationship of the sequences (numbers on the branches correspond to posterior probability; numbers in parentheses correspond to specimen voucher ID).

DISCUSSION

Numerous valid species in Parodontidae occur in La Plata basin, but most are restricted to the Lower or Upper Paraná River system (Pavanelli, 2003). *Parodon nasus* and *Apareiodon affinis* Steindachner, 1879 are the single valid taxa with broad distribution in Lower and Upper Paraná River systems (Pavanelli, 2003). Due to gene flow restriction between Lower and Upper Paraná River systems, differences in morphological characters, chromosomes, and gene sequences have been reported in *P. nasus* and *A. affinis* (Moreira-Filho *et al*., 1980; Jorge, Moreira-Filho, 2000, 2004; Pavanelli, 2003; Bellafronte *et al*., 2005, 2013; Nascimento *et al*., 2018). Some taxa assignments within Parodontidae are controversial despite chromosomal differences because family members lack reliable diagnostic morphological traits to support accurate phylogenetic analysis (Pavanelli, 2003).

Sequence analysis of the *COI* can help identify species (Hebert *et al*., 2003). Ward (2009) showed that DNA Barcoding is a valuable tool for identifying fish species and proposed the value of 2% of the genetic distance between fish species as a threshold for their separation, a value confirmed by Pereira *et al*. (2013) for Neotropical fish. Still, according to Pereira *et al*. (2011, 2013), this value should only be used as a starting point for investigating divergence between specimens. Other characteristics of the species/group studied, such as their evolutionary history, should be considered before defining a limit for species (Pereira *et al*., 2011). In Parodontidae, integrative studies of DNA Barcoding and chromosomal analysis proved to be effective in delimiting species, even though morphological characters have been overlapped in the taxa (Bellafronte *et al*., 2013; Nascimento *et al*., 2018; Santos *et al*., 2019; Traldi *et al*. 2020). In analyzed *P. nasus* samples, the Sete Quedas barriers did not generate speciation between representatives from Lower and Upper Paraná River systems since the data demonstrated genetic and chromosomal features compatible with a single taxon and population structuring.

The K2P genetic distance among *P. nasus* individuals from Upper and Lower Paraná was below the threshold value of 2%, indicating a single species. In addition, the observed interspecific distance values are less than 10X those found for intraspecific distances. The barcoding gap (the difference between the greatest intraspecific distance and the smallest interspecific distance) greater than 10X is used to separate cryptic species, regardless of the K2P distances less than 2% (Hebert *et al*., 2004). In Parodontidae, *Parodon nasus* X *P. morerai* Ingenito & Buckup, 2005, *Apareiodon piracicabae* Eigenmann, 1907 X *A. vittatus* Garavello, 1977, and *A. machrisi* Travassos, 1957 X *A. cavalcante* Pavanelli & Britski, 2003 demonstrated K2P distances less than 2% (Bellafronte *et al*., 2013; Traldi *et al*., 2020). However, the cytogenetic and morphological characteristics validate these species, possibly indicating recent speciation events between them (Bellafronte *et al*., 2013; Traldi *et al*., 2020). The two species delimitation methods and the Bayesian phylogenetic analysis also demonstrated that the *P. nasus* analyzed samples represent a single species.

All *P. nasus* sampled in this study shared the 2n, karyotype formulae and FN, demonstrating extensive conservation in their karyotype structure between Lower and Upper Paraná River systems. Previous cytogenetic studies showed a similar karyotypic organization among *P. nasus* populations (Moreira-Filho *et al*., 1984, 1985; Jesus, Moreira-Filho, 2000; Vicente *et al*., 2001; Centofante *et al*., 2002; Bellafronte *et al*.,

2005; Ziemniczak *et al*., 2014). The C-banding is an excellent chromosomal marker for identifying sex chromosomes in Parodontidae species since the heterochromatin tends to be located in a sizeable interstitial portion of the W chromosome (Schemberger *et al*., 2011, 2019). In opposite, autosomes show just small pericentromeric and terminal heterochromatin regions in Parodontidae karyotypes (Bellafronte *et al*., 2011; Ziemniczak *et al*., 2014). *Parodon nasus* show a proto sex chromosomes pair (without a sex-specific heteromorphic chromosome region) corresponding to chromosome 13 in the karyotype (Schemberger *et al*., 2011), observed in all analyzed samples in this study. *Parodon nasus* chromosomes show the most prominent heterochromatin regions in the *pPh2004* satellite DNA sites and 45S rDNA cluster, besides that pericentromeric and terminal region of the chromosomes (Centofante *et al*., 2002; Bellafronte *et al*., 2005; Schemberger *et al*., 2011). Despite variations usually found in C-bands, the chromosomal marker was homogeneous among all *P. nasus* specimens analyzed.

In situ localization of the repetitive DNAs has been used to compare Parodontidae karyotypes (Vicente *et al*., 2001; Centofante *et al*., 2002; Vicari *et al*., 2006; Bellafronte *et al*., 2011; Schemberger *et al*., 2011, 2014, 2016; Ziemniczak *et al*., 2014; Nascimento *et al*., 2018). Usually, the mapping of repetitive DNAs shows chromosomal sites diversification between closely related species but tends to be conservative when comparing Parodontidae populations (Bellafronte *et al*., 2005, 2009, 2011; Rosa *et al*., 2006; Schemberger *et al*., 2011). Slight differences in rDNA sites location were demonstrated among *P. nasus* sampled in distinct areas (Vicente *et al*., 2001; Bellafronte *et al*., 2005; Paula *et al*., 2017). This difference is attributed to additional 5S or 45S rDNA sites due to repetitive DNA units transposition and could not represent a substantial genetic difference in the genomic comparison. The mapping of 18S and 5S rDNAs showed a syntenic location for these genes in chromosome 25, and specimens from the Cuiabá River showed an extra site of 5S rDNA in the metacentric pair 17, probably resulting from a transposition event in the karyotype. Despite that, the syntenic condition of rDNAs is considered an apomorphic feature for *P. nasus* (Bellafronte *et al*., 2011), reinforcing their low levels of karyotypic differentiation.

Repetitive DNAs usually generate chromosomal remodeling between populations or closely related species (Cioffi *et al*., 2009; Parise-Maltempi *et al*., 2013; Poltronieri *et al*., 2013; Blanco *et al*., 2017; Crepaldi, Parise-Maltempi, 2020). The distribution and expansion of *pPh2004* satellite DNA had a significant role in chromosomal diversification in Parodontidae (Bellafronte *et al*., 2011; Schemberger *et al*., 2011; Ziemniczak *et al*., 2014). Despite the high *pPh2004* satellite DNA sites dispersion among Parodontidae karyotypes, *P. nasus* from Upper and Lower Paraná River systems have a homogeneous condition in the four samples analyzed. Unlike *P. nasus*, the populations of *A. affinis* distributed along the La Plata basin studied by Nascimento *et al*. (2018) demonstrated a karyotypic and molecular variety among the regions of Lower and Upper Paraná River, despite the absence of morphological differences, suggesting that this group may be a complex of species. Besides karyotype formulas, *pPh2004* satellite DNA sites demonstrate extensive chromosomal remodeling among *A. affinis* sampled in Lower and Upper Paraná River regions (Nascimento *et al*., 2018).

Geographical barriers isolate fish populations, causing intraspecific polymorphisms and genetic divergences among populations over time (Sekine *et al*., 2002). The population

differentiation is expected between Lower and Upper Paraná River representatives caused by the geographical barrier historical of the Sete Quedas waterfalls and currently by the Itaipu dam. *Parodon nasus* from Lower and Upper Paraná River represent two highly structured populations, as demonstrated by the values of pairwise FST and the analyzes of AMOVA and BAPs. The Mantel test show a significant correlation between geographic distance and genetic distance in *P. nasus*. In an isolation-by-distance (IBD) model, genetic distance should be positively correlated with geographical distance (Felsenstein, 1976). It is expected that more abundant and less distant populations have greater gene flow and, therefore, be more genetically similar and have greater genetic diversity relative to smaller, rare and more distant populations (Eckert *et al*., 2008). Thus, the high genetic distance found in *P. nasus* from the Cuiabá River compared to other samples could be reinforced by the long-distance isolation between Lower and Upper Paraná River. Besides that, the haplotype network showed that specimens from the Cuiabá River have a unique haplotype, not shared with specimens from Upper Paraná, and presented an exclusive second pair of chromosomes carrying 5S rDNA cistrons. The haplotype found here for the Cuiabá River is the same observed by Bellafronte *et al*. (2013), reinforcing the low diversity of this population and the lack of haplotype sharing with Upper Paraná.

The population structure between specimens from Lower and Upper Paraná River was accessed and demonstrated for several groups of fish, including species with migratory behavior, through different molecular markers (Sekine *et al*., 2002; Zawadzki *et al*., 2008; Pereira *et al*., 2009; Piálek *et al*., 2012; Nascimento *et al*., 2018; Prado *et al*., 2018). However, it is known that the flooding of Sete Quedas waterfalls, for the construction of the Itaipu hydroelectric plant, allowed the dispersion of species from Lower to Upper Paraná (Agostinho, Júlio Jr., 2002; Júlio Jr. *et al*., 2009). *Apareiodon affinis* was the second most collected species in the Canal da Piracema, occurring mainly in unstructured littoral and lentic waters, but it was classified as a sedentary species with no parental care (SNPC) as a reproductive strategy (Makrakis *et al*., 2007). *Parodon nasus* was not collected in the Canal da Piracema (Makrakis *et al*., 2007). Still, it was only mentioned in the region of influence of the Itaipu reservoir after its formation (Cecílio *et al*., 1997), which may indicate that specimens from the Lower Paraná may have migrated to the Upper Paraná region in the interval between the floods of the Sete Quedas waterfalls and the construction of the Itaipu dam. In the São Francisco River basin, *P. nasus* was observed after the transposition of the Piumhi River (Bellafronte *et al*., 2010). However, it is not clear whether this dispersion was due to the change in the river course or through the flooded areas of the swamp and the effect of this dispersion (Bellafronte *et al*., 2010).

The Sete Quedas waterfalls (currently Itaipu dam) represent an important geographical barrier restricting gene flow in fish populations. This isolation effect has promoted chromosomal e genetic diversification in some Parodontidae species from Lower and Upper Paraná River systems. In *P. nasus* analysis, the similarity of the chromosomal features allied to genetic population parameters indicates a single species broadly distributed in La Plata basin and high population structuration between Lower and Upper Paraná River regions.

ACKNOWLEDGMENTS

This study was supported by the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, MRV grant number: 305142/2019-4), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, MA Finance code 001), Fundação Araucária (Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná, MRV grant number: 09/2017), and FAPESP (Fundacão de Amparo à Pesquisado Estado de São Paulo, MRV grant number: 2015/16661-1).

REFERENCES

- **Abell R, Thieme ML, Revenga C, Bryer M, Kottelat M, Bogutskaya N, Coad B, Mandrak N, Balderas SC, Bussing W, Stiassny MLJ, Skelton P, Allen GR, Unmack P, Naseka A, Ng R, Sindorf N, Robertson J, Armijo E, Higgins JV, Heibel TJ, Wikramanayake E, Olson D, López HL, Reis RE, Lundberg JG, Pérez MHS, Petry P.** Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. BioScience. 2008; 58(5):403–14. https://doi.org/10.1641/B580507
- **Agostinho AA, Zalewski M.** A planície alagável do alto rio Paraná: importância e preservação. Maringá: EDUEM; 1996.
- **Agostinho AA, Vazzoler AEAM, Gomes LC, Okada EK.** Estratificación espacial y comportamiento de *Prochilodus scrofa* em distintas fases del ciclo de vida, em la planície de inundación del alto rio Paraná y embalse de Itaipu, Paraná, Brasil. Rev Hydrobiol Trop. 1993; 26(1):79–90. Available from: http://repositorio.uem.br:8080/jspui/handle/1/5201
- **Agostinho CS, Júlio Jr. HF.** Observation of an invasion of the piranha *Serrasalmus marginatus* Valenciennes, 1847 (Osteichthyes, Serrasalmidae) into the upper Paraná River, Brazil. Acta Sci. 2002; 24(2):391–95. Available from: http://repositorio.uem.br:8080/jspui/handle/1/5245
- **Bandelt HJ, Forster P, Röhl A.** Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol. 1999; 16(1):37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036
- **Bellafronte E, Margarido VP, Moreira-Filho O.** Cytotaxonomy of *Parodon nasus* and *Parodon tortuosus* (Pisces, Characiformes): A case of synonymy confirmed by cytogenetic analyses. Genet Mol Biol. 2005; 28(4):710–16. https://doi.org/10.1590/S1415-47572005000500010
- **Bellafronte E, Vicari MR, Artoni RF, Margarido VP, Moreira-Filho O.** Differentiated ZZ/ZW sex chromosomes in *Apareiodon ibitiensis* (Teleostei, Parodontidae): cytotaxonomy and biogeography. J Fish Biol. 2009; 75(9):2313–25. https://doi.org/10.1111/j.1095-8649.2009.02488.x
- **Bellafronte E, Moreira-Filho O, Vicari MR, Artoni RF, Bertollo LAC, Margarido VP.** Cytogenetic identification of invasive fish species following connections between hydrographic basins. Hydrobiologia. 2010; 649:347–54. https://doi.org/10.1007/s10750-010-0277-9
- **Bellafronte E, Schemberger MO, Moreira-Filho O, Almeida MC, Artoni RF, Margarido VP, Vicari MR.** Chromosomal markers in Parodontidae: an analysis of new and reviewed data with phylogenetic inferences. Rev Fish Biol Fisher. 2011; 21:559–70. https://doi.org/10.1007/s11160-010-9177-3
- **Bellafronte E, Mariguela TC, Pereira LHG, Oliveira C, Moreira-Filho O.** DNA barcode of Parodontidae species from the La Plata river basin – applying new data to clarify Taxonomic problems. Neotrop Ichthyol. 2013; 11(3):497–506. https://doi.org/10.1590/S1679-62252013000300003
- **Berbery EH, Barros VR.** The hydrologic cycle of the La Plata Basin in South America. J hydrometeorol. 2002; 3(6):630–45. https://doi.org/10.1175/1525-7541(2002)003<0630:THCOTL>2.0.CO;2
- **Bertollo LAC, Cioffi MB, Moreira-Filho O.** Direct chromosome preparation from freshwater teleost fishes. In: Ozouf-Costaz C, Pisano E, Foresti F, Almeida Toledo LF, editors. Fish Cytogenetic Techniques (Chondrichthyans and Teleosts). Boca Raton: CRC Press; 2015. p.21–26.

- **Blanco DR, Vicari MR, Lui RL, Traldi JB, Bueno V, Martinez JF, Brandão H, Oyakawa OT, Moreira-Filho O.** Karyotype diversity and evolutionary trends in armored catfish species of the genus *Harttia* (Siluriformes: Loricariidae). Zebrafish. 2017; 14(2):169–76. https://doi.org/10.1089/zeb.2016.1377
- **Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, Maio N, Matschiner M, Mendes FK, Müller NF, Ogilvie HA, Plessis L, Popinga A, Rambaut A, Rasmussen D, Sivero I, Suchard MA, Wu C, Xie D, Zhang C, Stadler T, Drummond AJ.** Beast 2.5: An advanced software platform for Bayesian evolutionary analysis. PLoS Comput Biol. 2019; 15(4):e1006650. https://doi.org/10.1371/journal.pcbi.1006650
- **Britski HA.** Peixes de água doce do Estado de São Paulo: Sistemática. In: Branco SM, org. Poluição e Piscicultura. São Paulo: Faculdade de Saúde Pública da USP e Instituto de Pesca; 1972. p.79–108.
- **Britski HA, Silimon KZ, Lopes BS.** Peixes do Pantanal, Manual de Identificação. Corumbá: Embrapa-SPI; 1999.
- **Cecílio EB, Agostinho AA, Júlio Jr HF, Pavanelli CS.** Colonização ictiofaunística do reservatório de Itaipu e áreas adjacentes. Rev Bras Zool. 1997; 14(1):1–14. https://doi.org/10.1590/S0101-81751997000100001
- **Centofante L, Bertollo LAC, Moreira-Filho O.** A ZZ/ZW sex chromosome system in a new species of the genus *Parodon* (Pisces, Parodontidae). Caryologia. 2002; 55(2):139–50. https://doi.org/10.1080/00087114.2002.10589270
- **Cioffi MB, Martins C, Bertollo LAC.** Comparative chromosome mapping of repetitive sequences. Implications for genomic evolution in the fish, *Hoplias malabaricus*. BMC Genet. 2009; 10:34. https://doi.org/10.1186/1471-2156-10-34
- **Corander J, Waldmann P, Marttinen P, Sillanpää MJ.** BAPS 2: enhanced possibilities for the analysis of genetic population structure. Bioinformatics. 2004; 20(15):2363–69. https://doi.org/10.1093/bioinformatics/bth250
- **Corander J, Marttinen P, Sirén J, Tang J.** Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinformatics. 2008; 9(1):539. https://doi.org/10.1186/1471-2105-9-539
- **Crepaldi C, Parise-Maltempi PP.** Heteromorphic sex chromosomes and their DNA content in Fish: an insight through satellite DNA accumulation in *Megaleporinus elongatus*. Cytogenet Genome Res. 2020; 160:38–46. https://doi.org/10.1159/000506265
- **Eckert GC, Samis KE, Lougheed SC.** Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Mol Ecol. 2008; 17(5):1170–88. https://doi.org/10.1111/j.1365-294X.2007.03659.x
- **Excoffier L, Smouse PE, Quattro JM.** Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 1992; 131(2):479–91.
- **Excoffier L, Lischer HE.** Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010; 10(3):564–67. https://doi.org/10.1111/j.1755-0998.2010.02847.x
- **Felsenstein J.** The theoretical population genetics of variable selection and migration. Annu Rev Genet. 1976; 10:253–80. https://doi.org/10.1146/annurev.ge.10.120176.001345
- **Fricke R, Eschmeyer WN, Van der Laan R.** Eschmeyer's catalog of fishes: genera, species, references [Internet]. San Francisco: California Academy of Science; 2021. Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp
- **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. https://doi.org/10.1093/sysbio/syt033
- **Gąsiorek P, Stec D, Morek W, Michalczyk Ł.** An integrative redescription of *Echinuscus testudo* (Doyère, 1840), the nominal taxon for the class Heterotardigrada (Ecdysozoa: Panarthropoda: Tardigrada). Zool Anz. 2017; 270:107–22. https://doi.org/10.1016/j.jcz.2017.09.006
- **Godoy MP.** Peixes do Brasil. Subordem Characoidei. Bacia do Rio Mogi-Guaçu. Piracicaba: Editora Franciscana; 1975.

- **Gomes LC, Agostinho AA.** Influence of the flooding regime on the nutritional state and juvenile recruitment of the curimba, *Prochilodus lineatus* Steindachner, in the Upper Paraná River, Brazil. Fisheries Manag Ecol. 1997; 4(4):263–74. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-2400.1997.00119.x
- **Grković A, Vujić A, Chroni A, van Steenis J, Đan M, Radenković S.** Taxonomy and systematics of three species of the genus *Eumerus* Meigen, 1822 (Diptera: Syrphidae) new to southeastern Europe. Zool Anz. 2017; 270:176–92. https://doi.org/10.1016/j.jcz.2017.10.007
- **Hatanaka T, Galetti Jr PM.** Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). Genetica. 2004; 122:239–44. https://doi.org/10.1007/s10709-004-2039-y
- **Hebert PDN, Cywinska A, Ball SL, deWaard JR.** Biological identifications through DNA barcodes. Proc R Soc Lond B Biol Sci. 2003; 270(1512):313–21. https://doi.org/10.1098/rspb.2002.2218
- **Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM.** Identification of birds through DNA barcodes. 2004. PLoS Biol. 2004; 2(10):e312. https://doi.org/10.1371/journal.pbio.0020312
- **Huelsenbeck JP, Ronquist F.** MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 2001; 17(8):754–55. https://doi.org/10.1093/bioinformatics/17.8.754
- **Jesus CM, Moreira-Filho O.** Karyotypes of three species of *Parodon* (Teleostei: Parodontidae). Ichthyol Explor Fres. 2000; 11(1):75–80.
- **Jorge LC, Moreira-Filho O.** Cytogenetic studies on *Apareiodon affinis* (Pisces, Characiformes) from Paraná river basin: sex chromosomes and polymorphism. Genetica. 2000; 109:267–73. https://doi.org/10.1023/A:1017522914023
- **Jorge LC, Moreira-Filho O.** Nucleolar organizer regions as markers of chromosomal Polymorphism in *Apareiodon affinis* (Pisces, Parodontidae). Caryologia. 2004; 57(2):195–99. https://doi.org/10.1080/00087114.2004.10589392
- **Júlio Jr. HF, Dei Tós C, Agostinho AA, Pavanelli CS.** A massive invasion of fish species after eliminating a natural barrier in the upper Paraná basin. Neotrop Ichthyol. 2009; 7(4):709–18. https://doi.org/10.1590/S1679-62252009000400021
- **Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A.** Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012; 28(12):1647–49. 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. https://doi.org/10.1093/molbev/msw054
- **Leigh JW, Bryant D.** POPART: full-feature software for haplotype network construction. Methods Ecol Evol. 2015; 6(9):1110–16. https://doi.org/10.1111/2041-210X.12410
- **Levan A, Fredga K, Sandberg AA.** Nomenclature for centromeric position on chromosomes. Hereditas. 1964; 52:201–20. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x
- **Librado P, Rozas J.** DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009; 25(1):1451–52. https://doi.org/10.1093/bioinformatics/btp187
- **Lui RL, Blanco DR, Moreira-Filho O, Margarido VP.** Propidium iodide for making heterochromatin more evident in the C-banding technique. Biotech Histochem. 2012; 87(7):433–38. https://doi.org/10.3109/10520295.2012.696700
- **Makrakis S, Gomes LC, Makrakis MC, Fernandez DR, Pavanelli CS.** The Canal da Piracema at Itaipu Dam as a fish pass system. Neotrop Ichthyol. 2007; 5(2):185–95. https://doi.org/10.1590/S1679-62252007000200013
- **Martins C, Galetti Jr. PM.** Chromosomal localization of 5S rDNA genes in *Leporinus* fish (Anostomidae, Characiformes). Chromosome Res. 1999; 7:363–67. https://doi.org/10.1023/A:1009216030316
- **Miller MP.** Alleles in Space (AIS): computer software for the joint analysis of interindividual spatial and genetic information. J Hered. 2005; 96(6):722–24. https://doi.org/10.1093/jhered/esi119
- **Moreira-Filho O, Bertollo LAC, Galetti Jr. PM.** Evidences for a multiple sex chromosome system with female heterogamety in *Apareiodon affinis* (Pisces, Parodontidae). Caryologia. 1980; 33(1):83–91. https://doi.org/10.1080/00087114.1980.10796821

- **Moreira-Filho O, Bertollo LAC, Galetti Jr. PM.** Structure and variability of nuclear organizer regions in Parodontidae Fish. Can J Genet Cytol. 1984; 26(5):564–68. https://doi.org/10.1139/g84-089
- **Moreira-Filho O, Bertollo LAC, Galetti Jr. PM.** Karyotypic study of some species of family Parodontidae (Pisce–Cypriniformes). Caryologia. 1985; 38(1):47–55. https://doi.org/10.1080/00087114.1985.10797729
- **Murray MG, Thompson WF.** Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 1980; 8(19):4321–26. https://doi.org/10.1093/nar/8.19.4321
- **Nascimento VD, Coelho KA, Nogaroto V, Almeida RB, Ziemniczack K, Centofante L, Pavanelli CS, Torres RA, Moreira-Filho O, Vicari MR.** Do multiple karyomorphs and population genetics of freshwater darter characines (*Apareiodon affinis*) indicate chromosomal speciation?. Zool Anz. 2018; 272:93–103. https://doi.org/10.1016/j.jcz.2017.12.006
- **Padial JM, Miralles A, De la Riva I, Vences M.** The integrative future of taxonomy. Front Zool. 2010; 7(16). https://doi.org/10.1186/1742-9994-7-16
- **Paula AA, Penha HA, Delai VA, Giuliano-Caetano L, Dias AL.** Occurrence of structural polymorphism and supernumerary chromosomes in a population of *Parodon nasus* (Parodontidae). Caryologia. 2017; 70(3):200–05. https://doi.org/10.1080/00087114.2017.1318503
- **Parise-Maltempi PP, Silva EL, Rens W, Dearden F, O'Brien PCM, Trifonov V, Ferguson-Smith MA.** Comparative analyses of sex chromosomes in *Leporinus* species (Teleostei, Characiformes) using chromosome painting. BMC Genet. 2013; 14:60. https://doi.org/10.1186/1471-2156-14-60
- **Pavanelli CS.** Revisão taxonômica da família Parodontidae (Ostariophysi: Characiformes). [PhD Thesis]. São Carlos: Universidade Federal de São Carlos; 1999. Avaliable from: ftp://ftp.nupelia.uem.br/users/Carla/Parodontidae.pdf
- **Pavanelli CS.** Family Parodontidae. In: Reis RE, Kullander SO, Ferraris CJ, eds. Check list of the freshwater fishes of South and Central America. Porto Alegre: EDIPUCRS; 2003. p.46–50.
- **Pavanelli CS, Britski HA.** *Apareiodon* Eigenmann, 1916 (Teleostei, Characiformes), from the Tocantins-Araguaia Basin, with description of three new species. Copeia. 2003; 2:337–48. https://doi.org/10.1643/0045-8511(2003)003[0337:AETCFT]2.0.CO;2
- **Pereira LHG, Foresti F, Oliveira C.** Genetic structure of the migratory catfish *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae) suggests homing behaviour. Ecol Freshw Fish. 2009; 18(2):215–25. https://doi.org/10.1111/j.1600-0633.2008.00338.x
- **Pereira LHG, Pazian MF, Hanner R, Foresti F, Oliveira C.** DNA barcoding reveals hidden diversity in the Neotropical freshwater fish *Piabina argentea* (Characiformes: Characidae) from the Upper Paraná Basin of Brazil. Mitochondr DNA. 2011; 22(sup1):87–96. https://doi.org/10.3109/19401736.2011.588213
- **Pereira LHG, Hanner R, Foresti F, Oliveira C.** Can DNA Barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna?. BMC Genet. 2013; 14:20. https://doi.org/10.1186/1471-2156-14-20
- **Piálek L, Říčan O, Casciotta J, Almirón A, Zrzavý J.** Multilocus phylogeny of *Crenicichla* (Teleostei: Cichlidae), with biogeography of the *C. lacustris* group: species flocks as a model for sympatric speciation in rivers. Mol Phylogenet Evol. 2012; 62(1):46–61. https://doi.org/10.1016/j.ympev.2011.09.006
- **Pinkel D, Straume T, Gray JW.** Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA. 1986; 83:2934–38. https://doi.org/10.1073/pnas.83.9.2934
- **Poltronieri J, Marquioni V, Bertollo LAC, Kejnovsky E, Molina WF, Liehr T, Cioffi MB.** Comparative chromosomal mapping of microsatellites in *Leporinus* species (Characiformes, Anostomidae): Unequal accumulation on the W chromosomes. Cytogenet Genome Res. 2013; 142:40–45. https://doi.org/10.1159/000355908
- **Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP.** Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst Biol. 2006; 55(4):595–609. https://doi.org/10.1080/10635150600852011

- **Posada D.** jModelTest: Phylogenetic Model Averaging. Mol Biol Evol. 2008; 25(7):1253–56. https://doi.org/10.1093/molbev/msn083
- **Prado FD, Fernandez–Cebrián R, Foresti F, Oliveira C, Martinez P, Porto–Foresti F.** Genetic structure and evidence of anthropogenic effects on wild populations of two Neotropical catfishes: baselines for conservation. J Fish Biol. 2018; 92(1):55–72. https://doi.org/10.1111/jfb.13486
- **Puillandre N, Lambert A, Brouillet S, Achaz G.** ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Mol Ecol. 2012; 21:1864–77. https://doi.org/10.1111/j.1365-294X.2011.05239.x
- **R Development Core Team.** R: The R project for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013. Available from: https://www.r-project.org/
- **Rambaut A, Suchard MA, Xie D, Drummond AJ.** Tracer v1.6. 2014. Available from http://beast.bio.ed.ac.uk/Tracer
- **Rosa R, Bellafronte E, Moreira-Filho O, Margarido VP.** Constitutive heterochromatin, 5S and 18S rDNA genes in *Apareiodon* sp. (Characiformes, Parodontidae) with a ZZ/ZW sex chromosome system. Genetica. 2006; 128:159–66. https://doi.org/10.1007/s10709-005-5700-1
- **Ruane S.** Using geometric morphometrics for integrative taxonomy: an examination of head shapes of milksnakes (genus *Lampropeltis*). Zool J Linn Soc. 2015; 174(2):394–413. https://doi.org/10.1111/zoj.12245
- **Santos EO, Deon GA, Almeida RB, Oliveira EA, Nogaroto V, Silva HP, Pavanelli CS, Cestari MM, Bertollo LAC, Moreira-Filho O, Vicari MR.** Cytogenetics and DNA barcode reveal an undescribed *Apareiodon* species (Characiformes: Parodontidae). Genet Mol Biol. 2019; 42(2):365–73. https://doi.org/10.1590/1678-4685-gmb-2018-0066
- **Schemberger MO, Bellafronte E, Nogaroto V, Almeida MC, Schühli GS, Artoni RF, Moreira-Filho O, Vicari MR.** Differentiation of repetitive DNA sites and sex chromosome system reveal closely related group in Parodontidae (Actinopterygii: Characiformes). Genetica. 2011; 139:1499–1508. https://doi.org/10.1007/s10709-012-9649-6
- **Schemberger MO, Oliveira JIN, Nogaroto V, Almeida MC, Artoni RF, Cestari MM, Moreira-Filho O, Vicari MR.** Construction and characterization of a repetitive DNA library in Parodontidae (Actinopterygii: Characiformes): A genomic and evolutionary approach to the degeneration of the W sex chromosome. Zebrafish. 2014; 11(6):518–27. https://doi.org/10.1089/zeb.2014.1013
- **Schemberger MO, Nogaroto V, Almeida MC, Artoni RF, Valente GT, Martins C, Moreira-Filho O, Cestari MM, Vicari MR.** Sequence analyses and chromosomal distribution of the *Tc1/Mariner* element in Parodontidae fish (Teleostei: Characiformes). Gene. 2016; 593:308–14. https://doi.org/10.1016/j.gene.2016.08.034
- **Schemberger MO, Nascimento VD, Coan R, Ramos É, Nogaroto V, Ziemniczak K, Valente GT, Moreira-Filho O, Martins C, Vicari MR.** DNA transposon invasion and microsatellite accumulation guide W chromosome differentiation in a Neotropical fish genome. Chromosoma. 2019; 128:547–60. https://doi.org/10.1007/s00412-019-00721-9
- **Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH.** Integrative taxonomy: a multisource approach to exploring biodiversity. Annu Rev Entomol. 2010; 55:421–38. https://doi.org/10.1146/annurev-ento-112408-085432
- **Sekine ES, Prioli AJ, Prioli SMAP, Júlio Jr. HF.** Genetic differentiation among populations of *Pseudoplatystoma corruscans* (Agassiz, 1829) (Osteichthyes, Pimelodidae) isolated by the Guaíra Falls in the Paraná River. Acta Sci. 2002; 24(2):507–12. Available from: http://repositorio.uem.br:8080/jspui/handle/1/5222
- **Sumner AT.** A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res. 1972; 75(1):304–06. https://doi.org/10.1016/0014-4827(72)90558-7
- **Traldi JB, Vicari MR, Martinez JF, Blanco DR, Lui RL, Azambuja M, Almeida RB, Malimpensa GC, Costa Silva GJ, Oliveira C, Pavanelli CS, Moreira Filho O.** Recent *Apareiodon* species evolutionary divergence (Characiformes: Parodontidae) evidenced by chromosomal and molecular inference. Zool Anz. 2020; 289:166–76. Available from: https://ur.booksc.org/book/84602654/7f6cd2

- **Travenzoli NM, Silva PC, Santos U, Zanuncio JC, Oliveira C, Dergam JA.** Cytogenetic and molecular data demonstrate that the Bryconinae (Ostariophysi, Bryconidae) species from southeastern Brazil form a phylogenetic and phylogeographic unit. PLoS ONE. 2015; 10(9):e0137843. https://doi.org/10.1371/journal.pone.0137843
- **Vicari MR, Moreira-Filho O, Artoni RF, Bertollo LAC.** ZZ/ZW sex chromosome system in an undescribed species of the genus *Apareiodon* (Characiformes, Parodontidae). Cytogenet Genome Res. 2006; 114:163–68. https://doi.org/10.1159/000093333
- **Vicente VE, Jesus CM, Moreira-Filho O.** Chromosomal localization of 5S and 18S rRNA genes in three *Parodon* species (Pisces, Parodontidae). Caryologia. 2001; 54(4):364–69. https://doi.org/10.1080/00087114.2001.10589247
- **Vicente VE, Bertollo LAC, Valentini SR, Moreira-Filho O.** Origin and differentiation of a sex chromosome system in *Parodon hilarii* (Pisces, Parodontidae). Satellite DNA, G- and C-banding. Genetica. 2003; 119:115–20. https://doi.org/10.1023/A:1026082904672
- **Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN.** DNA barcoding Australia's fish species. Philos Trans R Soc Lond B Biol Sci. 2005; 360:1847–57. https://doi.org/10.1098/rstb.2005.1716
- **Ward RD.** DNA barcode divergence among species and genera of birds and fishes. Mol Ecol Resour. 2009; 9(4):1077–85. https://doi.org/10.1111/j.1755-0998.2009.02541.x
- **Zawadzki CH, Renesto E, Peres MD, Paiva S.** Allozyme variation among three populations of the armored catfish *Hypostomus regani* (Ihering, 1905) (Siluriformes, Loricariidae) from the Paraná and Paraguay rivers basins, Brazil. Genet Mol Biol. 2008; 31(3):767–71. https://doi.org/10.1590/S1415-47572008000400025
- **Ziemniczak K, Traldi JB, Nogaroto V, Almeida MC, Artoni RF, Moreira-Filho O, Vicari MR.** In situ localization of (GATA)n and (TTAGGG)n repeated DNAs and W sex chromosome differentiation in Parodontidae (Actinopterygii: Characiformes). Cytogenet Genome Res. 2014; 144(4):325–32. https://doi.org/10.1159/000370297

AUTHORS' CONTRIBUTION

Matheus Azambuja: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-original draft, Writing-review and editing.

Daiane Santana Marcondes: Formal analysis, Investigation.

Viviane Nogaroto: Funding acquisition, Investigation, Project administration, Supervision, Writing-original draft, Writing-review and editing.

Orlando Moreira-Filho: Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Writing-original draft, Writing-review and editing.

Marcelo Ricardo Vicari: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

ETHICAL STATEMENT

Fish were collected with the authorization of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO), Sistema de Autorização e Informação em Biodiversidade (SISBIO, License numbers 10538–3 and 15117–2). The procedures of this study are in agreement with the Ethics Committee of Animal Usage of the Universidade Estadual de Ponta Grossa, Brazil (Protocol: 06/2019).

COMPETING INTERESTS

The authors declare no competing interests.

Neotropical Ichthyology

OPEN ACCESS

Official Journal of the
Sociedade Brasileira de Ictiologia

SBI

HOW TO CITE THIS ARTICLE

- **Azambuja M, Marcondes DS, Nogaroto V, Moreira-Filho O, Vicari MR**. Population structuration and chromosomal features homogeneity in *Parodon nasus* (Characiformes: Parodontidae): A comparison between Lower and Upper Paraná River representatives. Neotrop Ichthyol. 2022; 20(1):e210162. https://doi.org/10.1590/1982-0224-2021-0162