Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Denise Felicetti, Chrystian Aparecido Grillo Haerter, Lucas Baumgärtner, Leonardo Marcel Paiz, Daniel Rodrigues Blanco, Eliana Feldberg, Vladimir Pavan Margarido, Maelin da Silva, Roberto Laridondo Lui (2023). Cytogenetic analysis of sympatric *Trachelyopterus* Valenciennes 1840 (Siluriformes, Auchenipteridae) species reveals highly conserved karyotypes despite the geographic distance. *Caryologia* 76(2): 41-50. doi: 10.36253/caryologia-2284

Received: August 16, 2023

Accepted: October 24, 2023

Published: December 31, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Cytogenetic analysis of sympatric *Trachelyopterus* Valenciennes 1840 (Siluriformes, Auchenipteridae) species reveals highly conserved karyotypes despite the geographic distance

Denise Felicetti[1], Chrystian Aparecido Grillo Haerter[2], Lucas Baumgärtner[1], Leonardo Marcel Paiz[1], Daniel Rodrigues Blanco[3], Eliana Feldberg[2], Vladimir Pavan Margarido[1], Maelin da Silva[4], Roberto Laridondo Lui[1,*]

[1] *Centro de Ciências Biológicas e da Saúde, Universidade Estadual do Oeste do Paraná, Cascavel, Paraná, Brazil*
[2] *Coordenação da Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Manaus, Brasil*
[3] *Universidade Tecnológica Federal do Paraná, Santa Helena, Brasil*
[4] *Universidade Estadual de Ponta Grossa, Ponta Grossa, Brasil*
*Corresponding author. E-mail: roberto.lui@unioeste.br

Abstract. *Trachelyopterus* Valenciennes 1840 species exhibit striking morphological and cytogenetic similarities, leading to persistent taxonomic challenges. This research focuses on *Trachelyopterus galeatus* Linnaeus 1766 and *Trachelyopterus porosus* Eigenmann & Eigenmann 1888, both widely distributed throughout South America and often sympatric, facilitating cytogenetic comparisons. These taxonomic entities are noteworthy for their extensive geographical ranges within the genus. We examined two populations of *T. galeatus* and *T. porosus* collected from sympatric sites in the Amazon and Pantanal regions. Both species had the same diploid number and simple Ag-NORs. The 18S rDNA sites were found in only one subtelocentric chromosome pair. Meanwhile, the 5S rDNA sites were found on two distinct chromosomal pairs, with differences in the chromosomal morphology and site position among the species, constituting the most efficient chromosomal marker to distinguish them. The 5S rDNA pattern differed between species but remained consistent between populations of the same species. Minor differences were observed between the *T. galeatus* populations, probably related to chromosomal rearrangements. In contrast, despite the considerable geographical distance, no cytogenetic differences were detected among the *T. porosus* populations. Overall, the congruence between cytogenetic and morphological characteristics, combined with our findings from sympatric samples and existing data from geographically separated populations of *Trachelyopterus*, indicates that the cytogenetic is a promising tool for species differentiation and for delving into the cytotaxonomic and evolutionary aspects of Auchenipteridae.

Keywords: "*Parauchenipterus*", sympatric species, neotropical fish species, cytotaxonomy, chromosomal markers, taxonomic challenges, biogeography.

INTRODUCTION

Siluriformes comprises 39 families and 499 genera, representing a significant component of the world's freshwater fish diversity, with over 4,000 valid species (Fricke et al. 2023). Within the neotropical region, it stands as the second-largest group of fish, accounting for approximately 40% of the total Brazilian fish species (de Pinna 1998; Ferraris 2007). Among the families of Siluriformes, Auchenipteridae, commonly referred to as the driftwood catfishes, is an endemic group to the Neotropical region, encompassing 25 genera and 128 species. Auchenipteridae is subdivided into two subfamilies: Centromochlinae with seven valid genera, and Auchenipterinae, comprosing 18 valid genera (Fricke et al. 2023), including *Trachelyopterus* Valenciennes 1840, the focus of this paper. *Trachelyopterus* species are widely distributed throughout South America, occurring in the Paraná-Paraguay, Amazon, Orinoco, Guiana and São Francisco River basins, trans-Andean and Brazilian coast basins. Among *Trachelyopterus* species, *Trachelyopterus galeatus* Linnaeus 1766 and *Trachelyopterus porosus* Eigenmann & Eigenmann 1888 exhibit the most extensive geographical distribution within the genus. *Trachelyopterus galeatus* can be found across most hydrographic basins of South America, whereas *T. porosus* inhabit the Paraná-Paraguay, Amazon and French Guiana basins.

The genus *Trachelyopterus* has been a subject of controversy for over two centuries, characterized by ongoing discussions and taxonomic reviews, largely driven by the morphological similarities among its species (Akama 2004). While some authors have affirmed its validity as a distinct genus (Mees 1974; Curran 1989; Royero 1999; Akama 2004; Birindelli 2010), others have treated it as synonymous with other genera, such as *Parauchenipterus* (Ferraris 1988, 2003), *Auchenipterus* Valenciennes, 1840 (Günther 1864) and *Trachycorystes* Bleeker 1858 (Eigenmann and Eigenmann 1888, 1890; Regan 1911; Miranda-Ribeiro 1911; Britski 1972). In this context, cytogenetic studies can provide new information that can contribute to the taxonomy and enhance discussions concerning the evolutionary and biogeographic aspects of *Trachelyopterus* species.

Currently, seven genera of Auchenipteridae catfishes have been cytogenetically analyzed (see Santos et al. 2021). The diploid number of 58 chromosomes is constant for this family, except for *Ageneiosus* Lacépède 1803, *Centromochlus* Kner 1858 and *Tympanopleura* Eigenmann 1912, in which some species exhibited a diploid number reduction (e.g., Fenocchio and Bertollo 1992; Lui et al. 2013b; Kowalski et al. 2020). In *Trachelyopterus*, cytogenetic analyses include four valid species and two suggested new ones (*Trachelyopterus* aff. *galeatus* and *Trachelyopterus* aff. *coriaceus*). All of them had the same diploid number (58), with small karyotypic and fundamental number differences (Tab. 1). *Trachelyopterus galeatus* is the most studied species of the genus, and recently, a cytogenetic study suggested that a population of *T. galeatus* from the Araguaia River basin may constitute a new species (Santos et al. 2021). In contrast to *T. galeatus*, *T. porosus* has three cytogenetic studies related to only one population, which focused on the evolution of B chromosomes and chromosomal markers through fluorescence in situ hybridization (Felicetti et al. 2021; Haerter et al. 2022, 2023).

Interestingly, *T. galeatus* and *T. porosus* have few morphological differences and are often found in sympatry, creating a propitious scenario to compare cytogenetic data with morphological identification as well as trace evolutionary patterns among geographically isolated populations. Thus, using classic and molecular cytogenetic tools, we aimed to compare two sympatric populations of *T. galeatus* and *T. porosus* from the Amazon River and Paraguay River basins, seeking cytogenetic differences that can contribute to better identification of them and also discussing chromosomal evolutionary patterns.

MATERIAL AND METHODS

The sympatric populations of *Trachelyopterus porosus* and *Trachelyopterus galeatus* were collected from two hydrographic basins of South America: (1) in the Catalão Lake, Amazonas River basin, near Manaus 03°09'47"S and 59°54'29"W, northern South America; (2) and in the Miranda River, municipality of Corumbá 19°34'37.80"S and 57°01'07.08"W, Paraguay River basin (Permanent license SISBIO 49379-1). In the Catalão Lake, we collected 14 specimens of *T. porosus* (4 males and 10 females) and 13 specimens of *T. galeatus* (6 males and 7 females). In the Paraguay River Basin, we collected 13 specimens of *T. porosus* (6 males and 7 females) and 12 specimens of *T. galeatus* (6 males 6 females). They were deposited in the Zoology Museum at the Universidade Estadual de Londrina (MZUEL 18212 for *T. porosus* and MZUEL 18213 for *T. galeatus*) and in the Zoological Collection at the Instituto Nacional de Pesquisas da Amazônia (INPA 57939 for *T. galeatus* and INPA 57940 for *T. porosus*).

Classic and molecular cytogenetic

Anterior kidney cells were used to obtain the mitotic chromosome suspension (Bertollo et al. 1978). The

Table 1. Cytogenetic data available for *Trachelyopterus*. FN: Fundamental number; 2n: diploid number; Res.: Reservoir; AM: Amazonas; GO: Goiás; PR: Paraná; MS: Mato Grosso do Sul; MG: Minas Gerais; RN: Rio Grande do Norte; MT: Mato Grosso; Ref.: References; m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric; p: short arm; q: long arm; i interstitial; t: terminal; References: 1- Santos et al. (2021); 2- Felicetti et al. (2021); 3- Ravedutti and Júlio (2001); 4- Lui et al. (2010); 5- Araujo and Molina (2013); 6- Lui et al. (2021); 7- Haerter et al. (2022 ; 8- Haerter et al. (2023); 9- Lui et al. (2009). *The citation of the species name has changed over the years, and can be found in the two formats mentioned.

Species	Locality	FN	2n	Karyotype formula	AgNORs/18S rDNA	5S rDNA	Histone H3	Histone H4	U2 snRNA	SSR $(GATA)_n$	Ref
Trachelyopterus coriaceus	Araguaia River, Araguaia-Tocantins river basin - GO	108	58	20m+18sm+12st+8a	pair 23, p, st	pair 3, p, m / pair 16, q, sm	pair 23, p, st	pair 23, p, st	pair 28, p ,a	scattered	1, 7, 8
Trachelyopterus aff. *galeatus* (*cited as *Parauchenipterus galeatus*)	Araguaia River, Araguaia-Tocantins river basin - GO	108	58	20m+18sm+12st+8a	pair 24, p, st	pair 3, p, m	pair 24, p, st/ pair 25, p, st	pair 24, p, st/ pair 25, p, st	pair 26, q/p, a	scattered	1*, 7, 8
Trachelyopterus galeatus (*cited as *Parauchenipterus galeatus*)	Catalão Lake, Amazonas River basin - AM	106	58	20m+12sm+18st+8a	pair 20, p, st	pair 14, p, sm/ pair 17, q, sm	pair 20, p, st/ pair 21, p, st	pair 20, p, st/ pair 21, p, st	pair 28, p, a	scattered	2, 7, 8
	Miranda River, Paraguay River basin - PY	108	58	24m+12sm+14st+8a	pair 24, p, st	pair 14, p, sm/ pair 17, q, sm	-	-	-	-	2
	Paraná River, Paraná River basin - PR	98	58	22m+12sm+6st+18a	pair 23, p, a	-	-	-	-	-	3*
	Paraná River, Paraná River basin - MS	108	58	24m+18sm+8st+8a	pair 25, p, st	pair 16, p, sm / pair 17, q, sm	-	-	-	-	4*
	Piumhi River, Paraná River basin - MG	108	58	20m+16sm+14st+8a	pair 24, p, st	pair 15, p, sm / pair 16, q, sm	-	-	-	-	4*
	Lagoa da Prata – São Francisco River basin - MG	108	58	22m+16sm+12st+8a	pair 23, p, st	pair 16, p, sm / pair 17, q, sm	-	-	-	-	4*, 6, 9
	Pium River, Parnamirim - RN	108	58	24m+16sm+10st+8a	p, sm	-	-	-	-	-	5*
Trachelyopterus porosus	Catalão Lake, Amazonas River basin - AM	106	58	22m+16sm+10st+10a	pair 23, p, st	pair 3, p, m/ pair 4, p, m	pair 23, p, st/ pair 24, p, st	pair 23, p, st/ pair 24, p, st	pair 26, p, a	scattered	2, 7, 8
	Miranda River, Paraguay River basin - PY	106	58	22m+16sm+10st+10a	pair 23, p, st	pair 3, p, m/ pair 4, p, m	-	-	-	-	2
Trachelyopterus aff. *coriaceus* (*cited as *Trachelyopterus* sp.)	Arrombado lagoon, Bento Gomes River basin- MT	108	58	22m+20sm+8st+8a	pair 22, p, st	pair 16, p, sm/ pair 18, q, sm	pair 23, p, st	pair 23, p, st	pair 27, p, a	scattered	6*, 7, 8
Trachelyopterus striatulus (*cited as *Parauchenipterus striatulus*)	Verde lagoon, Doce River basin - MG	106	58	18m+20sm+10st+10a	par 23, p, st	pair 10, p, sm/ pair 13, p, sm/ pair 15, q, sm	pair 18, p, sm/ pair 23, p, st	pair 18, p, sm/ pair 23, p, st	pair 28, p, a	scattered	1*, 7, 8

samples were treated with a 0.02% colchicine solution (1 mL/100g of body weight) for 30-40 minutes before euthanizing the animal by clove oil overdose (Griffiths 2000) (according to the ethics committee on animal experimentation and practical classes at Unioeste: 09/13 - CEEAAP / Unioeste) to remove tissues for cytogenetic and molecular analyses. The chromosome morphology was classified according to Levan et al. (1964). The heterochromatin distribution pattern was determined according to Sumner (1972), with changes in the staining process proposed by Lui et al. (2012). The nucleolar organizer regions (Ag-NOR) were detected by silver nitrate impregnation (Howell and Black 1980).

Fluorescent *in situ* hybridization (FISH) was carried out according to Pinkel et al. (1986) with modifications suggested by Margarido and Moreira-Filho (2008). The 5S rDNA probes were obtained from Mini-prep of *Megaleporinus elongatus* Valenciennes 1850 (Martins et al. 2000) and the 18S rDNA probes were obtained from Mini-prep of *Prochilodus argenteus* Spix and Agassiz 1829 (Hatanaka and Galetti Jr, 2004). The 5S probes were labeled by nick translation with digoxigenin-11-dUTP (Dig 11 Nick Translation Mix - Roche), according to the manufacturer's instructions, and detected with anti-digoxigenin rhodamine (Roche Diagnostics). The 18S rDNA probes were labeled with biotin-16-dUTP (Biotin 16 Nick Translation Mix - Roche), according to the manufacturer's instructions, and detected using anti-biotin-avidin (Roche Diagnostics). The FISH stringency was 77% for both the 5S and 18S rDNA probes (200 ng of each probe, 50% formamide, 10% dextran sulfate, 2xSSC, pH 7.0-7.2, at 37°C overnight). All images were captured by the DP Controller 3.2.1.276 software using the Olympus DP71 digital camera connected to the BX61 epifluorescence microscope (Olympus America Inc., Center Valley, PA, United States of America).

RESULTS

Trachelyopterus porosus from the Amazon River basin had 2n=58 chromosomes for both sexes (4 males and 10 females), with 22 metacentric, 16 submetacentric, 10 subtelocentric, 10 acrocentric and a fundamental number (NF) of 106 (Fig. 1a). Among the 14 specimens, eight individuals (3 males and 5 females) had 1-3 small and metacentric B chromosomes (Bs). The Bs had intraindividual and interindividual numerical variation. The C-banding revealed heterochromatin in the terminal position of almost all complement A chromosomes (Fig. 1b). The silver nitrate impregnation showed simple NOR in the terminal position of the short arm of the pair 23 (Fig. 1b), which was confirmed by FISH with the 18S rDNA probes (Fig. 2). FISH with the 5S rDNA probes revealed sites on the short arm of the pair 3 and 4, both metacentric (Fig. 2).

Trachelyopterus porosus from the Paraguay River basin had 2n=58 chromosomes for both sexes (6 males and 7 females), with 22 metacentric, 16 submetacentric, 10 subtelocentric, 10 acrocentric, and NF=108 (Fig. 1c). The C-banding revealed heterochromatin in the terminal position of almost all complement A chromosomes (Fig. 1d). The silver nitrate impregnation showed simple NOR in the terminal position of the short arm of the pair 23 (Fig. 1d), which was confirmed by FISH with the 18S rDNA probes (Fig. 2). FISH with the 5S rDNA probes revealed sites on the short arm of the pair 3 and 4, both metacentric (Fig. 2).

Trachelyopterus galeatus from the Amazon River basin had 2n=58 chromosomes for both sexes (6 males and 7 females), with 20 metacentric, 12 submetacentric, 18 subtelocentric, 8 acrocentric, and NF=106 (Fig. 1e). Among the 13 specimens, six (1 male and 5 females) had 1-3 small and metacentric Bs. The Bs had intraindividual and interindividual numerical variation. The C-banding revealed heterochromatin in the terminal position of almost all complement A chromosomes (Fig. 1f). The silver nitrate impregnation showed simple NOR in the terminal position of the short arm of the pair 20 (Fig. 1f), which was confirmed by FISH with rDNA 18S probes (Fig. 2). FISH with the 5S rDNA probes revealed sites on the short arm of the pair 14 and on the long arm of the pair 16, both submetacentric Fig. 2).

Trachelyopterus galeatus from the Paraguay River basin had 2n=58 chromosomes for both sexes (6 males and 6 females), with 24 metacentric, 12 submetacentric, 14 subtelocentric, 8 acrocentric, and NF=108 (Fig. 1g). Among the 12 specimens, one female had 1-2 small and metacentric Bs. The Bs had intraindividual and interindividual numerical variation. The C-banding revealed heterochromatin in the terminal position of almost all complement A chromosomes (Fig. 1h). The silver nitrate impregnation showed simple NOR in the terminal position of the short arm of the pair 24 (Fig. 1h), which was confirmed by FISH with the 18S rDNA probes (Fig. 2). FISH with the 5S rDNA probes revealed sites on the short arm of the pair 14 and on the long arm of the pair 17, both submetacentric (Fig. 2).

DISCUSSION

The diploid number of 58 chromosomes is a recurrent pattern in Auchenipteridae species (Ravedutti and

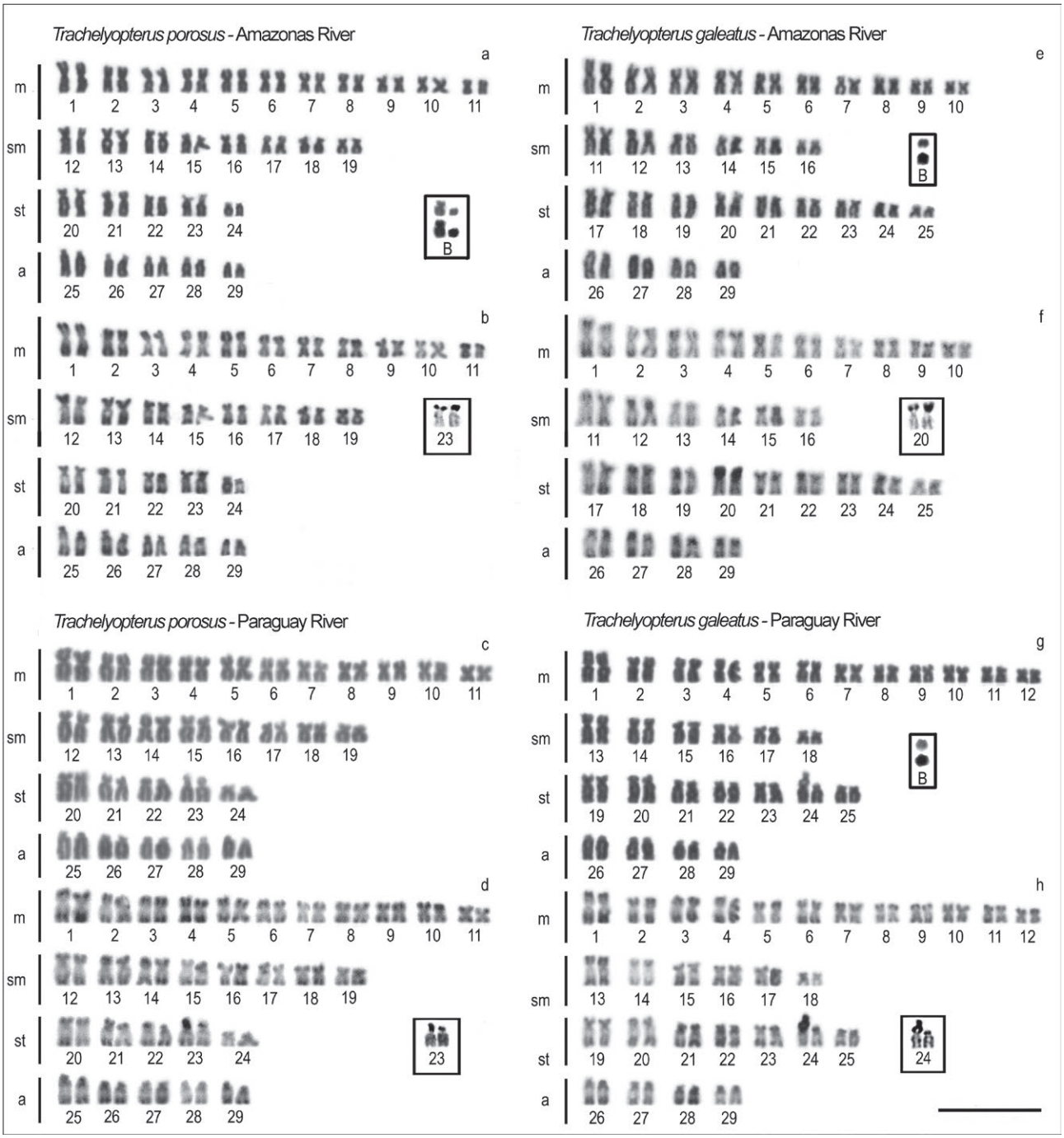

Figure 1. Karyotypes of *Trachelyopterus porosus* (a, c) and *Trachelyopterus galeatus* (e, g) stained with Giemsa and sequentially C-banded (b, d, f and h, respectively). The boxes correspond to the Ag-NORs and the B chromosomes of their respective populations. Bar = 10μm.

Júlio Jr 2001; Fenocchio et al. 2008; Lui et al. 2009, 2010, 2013a, 2013b, 2015, 2021; Santos et al. 2021), indicating that it is a conserved aspect of the family. Historically, deviations from this pattern were only reported for *Ageneiosus inermis* Linnaeus 1766 and *Tympanopleura atronasus* Eigenmann and Eigenmann 1888 with a 2n = 56 (Fenocchio and Bertollo 1992; Lui et al. 2013b), a potential consequence of chromosomal fusions that appear to be a basal event in the diversification of the genus *Ageneiosus* (Lui et al. 2013b). However, Kowalski

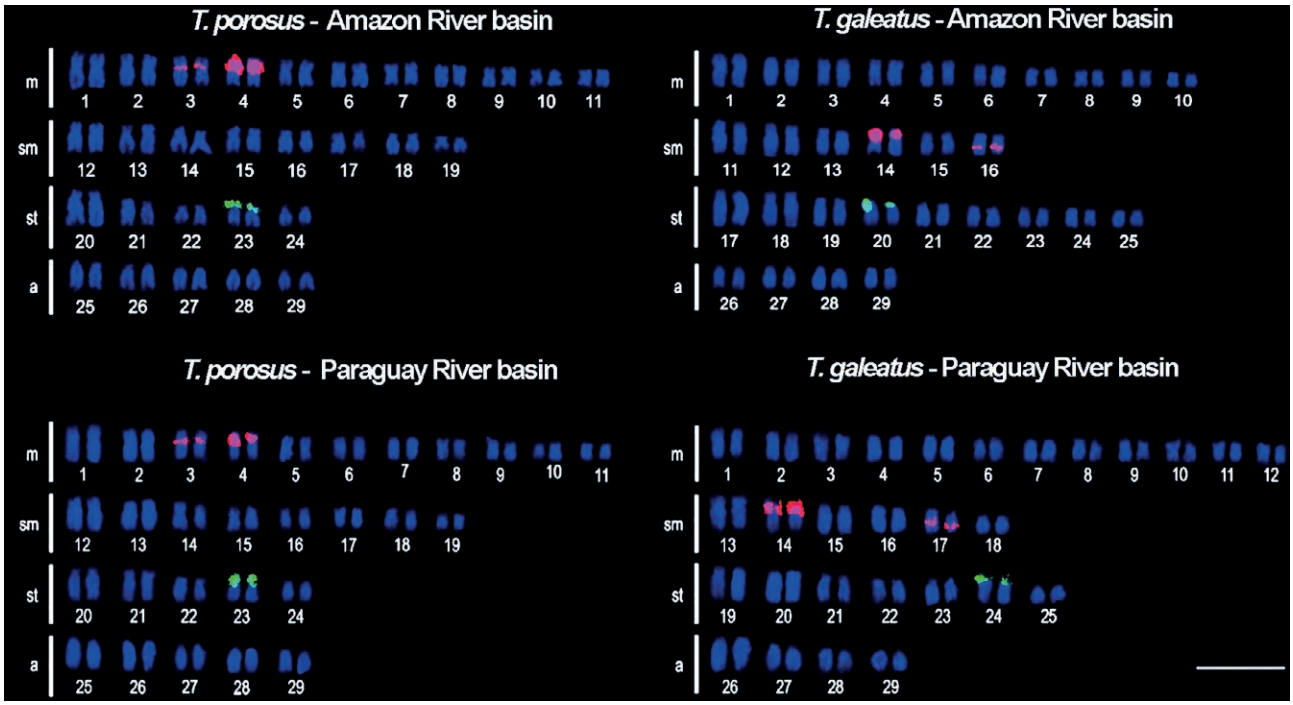

Figure 2. Karyotypes hybridized with 5S rDNA (Rhodamine, red) and 18S rDNA probes (FITC, green) in *Trachelyopterus porosus* from the Amazon River and Paraguay River and *Trachelyopterus galeatus* from the Amazon River and Paraguay River. Bar = 10 µm.

et al. (2020) recently introduced a new exception to this prevailing diploid number pattern in Auchenipteridae, *Centromochlus heckelii* De Filippi 1853 with 46 chromosomes, further emphasizing the role of chromosome rearrangements in the family diversification.

In the closest group, Doradidae, a variable diploid number can be observed (2n=56, 2n=58 and 2n=66). For a considerable period, 2n=58 was regarded as the plesiomorphic state for the family, given its prevalence among most analyzed species (Eler et al. 2007; Milhomem et al. 2008; Baumgärtner et al. 2016; Takagui et al. 2017, 2019). However, seeking to ascertain the ancestral diploid number within the nodes of the Doradidae family, Takagui et al. (2021) concluded that the determination of the plesiomorphic condition remains elusive, as both 56 and 58 chromosomes are equally parsimonious states. In Auchenipteridae, there are still too few species cytogenetically studied to reconstruct the ancestral diploid number. Therefore, even though 58 chromosomes are the most recurrent diploid number, it is also premature to determine whether it is a plesiomorphic trait or not.

Karyotypic formula variations are frequently reported among *Trachelyopterus galeatus* populations (Tab. 1), and our study corroborates this trend. However, similar to most studies, *T. galeatus* from the Amazon basin also showed only four pairs of acrocentric chromosomes—a recurrent characteristic of this species. Thus far, the only exceptions are *T. galeatus* from Puerto Rico in the Paraná River, which had nine acrocentric pairs (e.g., Ravedutti and Júlio Jr 2001) and *T. galeatus* from the Araguaia River, a suggested new species with five pairs of acrocentric chromosomes (Santos et al. 2021). The karyotypic differences between these species populations may be the result of geographic isolation. It is important to highlight that *T. galeatus* is widely distributed throughout South America (Akama 2004). Similar cytogenetic differences have been reported in other groups of neotropical fish, such as *Astyanax* Baird and Girard 1854 (Peres et al. 2009; Tenório et al. 2013; Piscor et al. 2017) and *Rhamdia* Bleeker 1858 (Stivari and Martins-Santos 2004; Martinez et al. 2011). On the other hand, both *T. porosus* populations presented the same karyotype formula, C-band pattern, Ag-NORs, 18S and 5S rDNA sites. Compared to *T. galeatus*, only small differences could be observed, primarily related to the karyotype formula and 5S rDNA sites.

In both species, *T. galeatus* and *T. porosus*, the heterochromatin was found in the terminal regions of most chromosomes. The C-band pattern aligns with findings from other cytogenetic studies in Auchenipteridae, such as in *A. inermis*, *Tympanopleura atronasus* (cited as *Ageneiosus atronases*), *Glanidium ribeiroi* Haseman 1911 and *T. galeatus* (Fenocchio and Bertollo 1992; Ravedutti and Júlio Jr 2001; Fenocchio et al. 2008; Lui et al. 2009,

2010, 2013b, 2015), which suggests that it is a shared feature within the family. Only small differences in the heterochromatin pattern can be seen in Auchenipteridae catfishes: *A. inermis* exhibited strongly marked heterochromatic blocks (Lui et al. 2013b); some chromosomes of *Tatia jaracatia* Pavanelli and Bifi 2009 showed centromeric heterochromatic blocks, and *Tatia neivai* Ihering 1930, which had an interstitial heterochromatic block in a submetacentric pair (Lui et al. 2013a). Similar to most studied Auchenipteridae species, no differences in the heterochromatin distribution patterns for both species were found; therefore, it does not seem to be a reliable marker for distinguishing *Trachelyopterus* species.

Simple NORs (silver nitrate staining and FISH with 18S rDNA probes) were found in the terminal position of a subtelocentric chromosome pair in both species. Currently, only *C. heckelii* was reported with multiple NORs (Kowalski et al. 2020), whereas all other cytogenetically analyzed Auchenipteridae species had simple NORs, with differences only in the position (terminal and interstitial), which may be a consequence of non-Robertsonian rearrangements, such as paracentric and/or pericentric inversions. In both species, *T. galeatus* and *T. porosus*, the 18S rDNA pattern is similar to other *Trachelyopterus* species. It can be found in subtelocentric chromosomes, acrocentric chromosomes (Ravedutti and Júlio Jr. 2001) or even in submetacentric pairs (Araújo and Molina 2013). However, it is worth noting that variations in chromosome measurement employed by different researchers could introduce a minor margin of error. These variations may stem from considerations such as the inclusion or exclusion of secondary constrictions as part of chromosomal arms, as well as discrepancies in chromosome condensation levels, which could contribute to subtle differences in karyotype organization.

In both species, *T. galeatus* and *T. porosus*, the 5S rDNA sites were detected on two chromosome pairs. Although this 5S rDNA pattern is prevalent in most Auchenipteridae species (see Lui *et al.* 2021), it should not be unequivocally viewed as a plesiomorphic or a conserved trait. In fact, it is the most variable chromosomal marker within the family (Santos et al. 2021), ranging from only one chromosome pair with the ribosomal sequence, as found in *Glanidium ribeiroi*, *Ageneiosus inermis* (Lui et al. 2013b, 2015), *T. galeatus* from the Araguaia River basin (Santos *et al.* 2021) and in *Auchenipterus nuchalis* Spix 1829 (Machado et al. 2021) to three chromosome sites in *T. striatulus* Steindachner 1876 (Lui et al. 2021) and *T. neivai* (Lui et al. 2013a), four chromosome sites in *T. jaracatia* (Lui et al. 2013a) or even seven chromosome carriers in *Entomocorus radiosus* Reis and Borges 2006 (Machado et al. 2021). This substantial variation positions the 5S rDNA marker as one of the most promising tools for species differentiation and for delving into the cytotaxonomic and evolutionary aspects of Auchenipteridae thus far.

ACKNOWLEDMENTS

We thank to Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for the authorization to collect the animals (Permit 49379-1), Instituto Nacional de Pesquisas da Amazônia (INPA) for logistical support and for providing laboratory technicians who helped with the sample collections in the Amazon rainforest, and the Universidade Estadual de Londrina (UEL) researchers and students for their support in the sample collection in the Pantanal.

DATA AVAILABILITY

The chromosomal data that support the findings of this study are fully available within the article and additional information are available from the corresponding author, Roberto Laridondo Lui.

DATA DEPOSITION

They specimens used in this study were deposited in the Zoology Museum at Universidade Estadual de Londrina (MZUEL 18212 for *T. porosus* and MZUEL 18213 for *T. galeatus*) and in the Zoological Collection at the Instituto Nacional de Pesquisas da Amazônia (INPA 57939 for *T. galeatus* and INPA 57940 for *T. porosus*).

GEOLOCATION INFORMATION

The sympatric populations of *Trachelyopterus porosus* and *Trachelyopterus galeatus* were collected from two hydrographic basins of South America: (1) in the Catalão Lake, Amazonas River basin, near Manaus 03°09'47"S and 59°54'29"W, northern South America; (2) and in the Miranda River, municipality of Corumbá 19°34'37.80"S and 57°01'07.08"W, Paraguay River basin.

STATEMENT OF ETHICS

Fish collections were authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, Permit number 49379- 1), and the experimental proce-

dures were approved by the Ethics Committee on Animal Experimentation and Practical Classes at Unioeste (09/13-CEEAAP/Unioeste).

FUNDING

This work was supported by the Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Paraná (Public Call 09/2016); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Universal, Proc. 446660/2014-4); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Universidade Estadual do Oeste do Paraná (Unioeste); Postgraduate Program in Conservação e Manejo de Recursos Naturais (Unioeste-PPRN); and Instituto Nacional de Pesquisas da Amazônia (INPA).

REFERENCES

Akama A. 2004. Sistemática dos gêneros *Parauchenipterus* Bleeker 1862 e *Trachelyopterus* Valenciennes, 1840 (Siluriformes, Auchenipteridae) [dissertation]. São Paulo (SP): Universidade de São Paulo.

April J, Mayden RL, Hanner RH, Bernatchez L. 2011. Genetic calibration of species diversity among North America's freshwater fishes. Proc Natl Acad Sci USA. 108(26):10602–10607. doi: 10.1073/pnas.1016437108.

Araújo WC, Molina WF. 2013. Citótipo exclusivo para *Parauchenipterus galeatus* (Siluriformes, Auchenipteridae) na Bacia do Atlântico NE Oriental do Brasil: Indicações de um complexo de espécies. Biota Amazôn. 3(2):33–39. doi: 10.18561/2179-5746/biotaamazonia.v3n2p33-39.

Baumgärtner L, Paiz LM, Margarido VP, Portela-Castro ALB. 2016. Cytogenetics of the thorny catfish *Trachydoras paraguayensis* (Eigenmann e Ward, 1907), (Siluriformes, Doradidae): Evidence of pericentric inversions and chromosomal fusion. Cytogenet Genome Res. 149(3):201–206. doi: 10.1159/000448126 .

Bertollo LAC, Takahashi CS, Moreira-Filho O. 1978. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Revista Brasileira de Genética. 1:103–120.

Birindelli, JLO. 2010. Relações filogenéticas da superfamília Doradoidea (Ostariophysi, Siluriformes) [dissertation]. São Paulo (SP): Universidade de São Paulo.

Britski HÁ 1972. Sistemática e evolução dos Auchenipteridae e Ageneiosidae (Teleostei, Siluriformes) [dissertation]. São Paulo (SP): Universidade de São Paulo.

Castro Paz FP, da Silva Batista JB, Porto JIR. 2014. DNA barcodes of rosy tetras and allied species (Characiformes: Characidae: Hyphessobrycon) from the Brazilian Amazon Basin. PloS One. 9(5): e98603. doi: 10.1371/journal.pone.0098603.

Curran DJ. 1989. Phylogenetic relationships among the Catfish Genera of the Family Auchenipteridae (Teleostei: Siluroidea). Copeia. 1989(2):408–419. https://doi.org/doi:10.2307/1445438.

de Pinna M. 1998. Phylogenetic relationships of Neotropical Siluriformes (Teleostei: Ostariophysi): historical overview and synthesis of hypotheses. In: Malabarba LR, Reis RE, Vari RP, Lucena ZM, Lucena CAS, editors. Phylogeny and Classification of Neotropical Fishes. Porto Alegre: Edipucrs; pp. 279–330.

Eigenmann CH, Eigenmann RS. 1888. Preliminary notes on South American Nematognathi. Proc Calif Aca. Sci. 1:119–172.

Eigenmann CH, Eigenmann RS. 1890. A revision of the South American Nematognathi, or catfishes. Occas pap Calif Acad Sci. 1:1–508.

Eler ES, Dergam JA, Vênere PC, Paiva LC, Miranda GA, Oliveira AA. 2007. The karyotypes of the thorny catfishes *Wertheimeria maculata* Steindachner, 1877 and *Hassar wilderi* Kindle, 1895 (Siluriformes, Doradidae) and their relevance in doradids chromosomal evolution. Genetica. 130(1):99–103. doi: 10.1007/s10709-006-0023-4.

Felicetti D, Haerter CAG, Baumgärtner L, Paiz L, Takagui FH, Margarido VP, Blanco DR, Feldberg E, Silva M, Lui RL. 2021. A new variant B chromosome in Auchenipteridae: The role of $(GATA)_n$ and $(TTAGGG)_n$ sequences in understanding the evolution of supernumeraries in *Trachelyopterus*. Cytogenet Genome Res. 161:70–81. doi: 10.1159/000513107.

Fenocchio A, Bertollo LAC. 1992. Karyotype, C-bands and NORs of the neotropical Siluriform fish *Ageneiosus brevifilis* and *Ageneiosus atronases* (Ageneiosidae). Cytobios. 72(288):19–22.

Fenocchio AS, Dias AL, Margarido VP, Swarça AC. 2008. Molecular cytogenetic characterization of *Glanidium ribeiroi* (Siluriformes) endemic to the Iguaçu River, Brazil. Chromosome Sci. 11:61–66.

Ferraris Jr CJ. 1988. The Auchenipteridae: putative monophyly and systematics, with a classification of the neotropical Doradoid catfishes (Ostariophysi: Siluriformes) [unpublished dissertation]. New York(NY): City University of New York.

Ferraris Jr CJ. 2003. Family Auchenipteridae. In: Reis RE, Kullander SO, Ferraris Jr CJ, editors. Check List of Freshwater Fishes of South and Central America. Porto Alegre: Edipucrs; pp. 742.

Ferraris Jr CJ. 2007. Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of Siluriform primary types. Zootaxa. 1418:1–628. doi: 10.11646/zootaxa.1418.1.1.

Frézal L, Leblois R. 2008. Four years of DNA barcoding: current advances and prospects. *Infec Genet Evol.* 8(5):727–736. doi: 10.1016/j.meegid.2008.05.005.

Fricke R, Eschmeyer WN, Van Der Laan R. 2021. Eschmeyer's Catalog of Fishes: Genera, Species, References. San Francisco(SF): The California Academy of Sciences; [accessed 2023 sep 23]. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

Griffiths SP. 2000. The use of clove oil as an anaesthetic and method or sampling intertidal rockpool fishes. J Fish Biol. 57(6):1453–1464. doi: 10.1111/j.1095-8649.2000.tb02224.x.

Günther A. 1864. Catalogue of the fishes in the British Museum. Catalogue of the Physostomi, containing the families Siluridae, Characinidae, Haplochitonidae, Sternoptychidae, Scopelidae, Stomiatidae in the collection of the British Museum. 5nd ed. Londres (LND): Printed by order of the trustees.

Haerter CAG, Blanco DR, Traldi JB, Feldberg E, Margarido VP, Lui RL, 2023. Are scattered microsatellites weak chromosomal markers? Guided mapping reveals new insights into *Trachelyopterus* (Siluriformes: Auchenipteridae) diversity. PLoS ONE. 18(6):e0285388. doi: 10.1371/journal.pone.0285388

Haerter CAG, Margarido VP, Blanco DR, Traldi JB, Feldberg E, Lui RL. (2022). Contributions to *Trachelyopterus* (Siluriformes: Auchenipteridae) species diagnosis by cytotaxonomic autapomorphies: from U2 snRNA chromosome polymorphism to rDNA and histone gene synteny. Org Div Evol. 22: 1021-1036. doi: 10.1007/s13127-022-00560-0

Hatanaka T, Galetti Jr, PM. 2004. Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). Genetica. 122(3):239244. doi: 10.1007/s10709-004-2039-y.

Howell WM, Black DA. 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia. 36(8):1014–1015. doi: 10.1007/BF01953855.

Kowalski S, Paiz LM, Silva M, Machado AS, Feldberg E, Traldi JB, Margarido VP, Lui RL. 2020. Chromosomal analysis of *Centromochlus heckelii* (Siluriformes: Auchenipteridae), with a contribution to *Centromochlus* definition. Neotrop Ichthyol. 18: e200009. doi: 10.1590/1982-0224-2020-0009.

Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. 52:201–220. doi: 10.1111/j.1601-5223.1964.tb01953.x.

Lui RL, Blanco DR, Margarido VP, Moreira-Filho O. 2009. First description of B chromosomes in the family Auchenipteridae, *Parauchenipterus galeatus* (Siluriformes) of the São Francisco River Basin (MG, Brazil). Micron. 40:552–559. doi: 10.1016/j.micron.2009.03.004.

Lui RL, Blanco DR, Margarido VP, Moreira-Filho O. 2010. Chromosome characterization and biogeographic relations among three populations of the driftwood catfish *Parauchenipterus galaetus* (Linnaeus, 1766) (Siluriformes: Auchenipteridae) in Brazil. Biol J Linn Soc. 99:648–656. doi: 10.1111/j.1095-8312.2009.01389.x.

Lui RL, Blanco DR, Moreira-Filho O, Margarido VP. 2012. Propidium iodide for making heterochromatin more evident in the C-banding technique. *Biotech Histochem.* 87(7):433–438. doi: 10.3109/10520295.2012.696700.

Lui RL, Blanco DR, Margarido VP, Troy WP, Moreira-Filho O. 2013a. Comparative chromosomal analysis and evolutionary considerations concerning two species of genus *Tatia* (Siluriformes, Auchenipteridae). Comp Cytogenet. 7(1):63–71. doi: 10.3897/CompCytogen.v7i1.4368.

Lui RL, Blanco DR, Martinez JF, Margarido VP, Venere PC, Moreira-Filho O. 2013b. The role of chromosomal fusion in the karyotypic evolution of the genus *Ageneiosus* (Siluriformes, Auchenipteridae). Neotrop Ichthyol. 11:327–334. doi: 10.1590/S1679-62252013005000004.

Lui RL, Blanco DR, Traldi JB, Margarido VP, Moreira-Filho O. 2015. Karyotypic variation of *Glanidium ribeiroi* Haseman, 1911 (Siluriformes, Auchenipteridae) along the Iguazu River basin. Braz J Biol: 75(4):215–221. doi: 10.1590/1519-6984.10714.

Lui RL, Traldi JB, Blanco DR, Margarido VP, Mariotto S, Centofante L, Artoni RF, Moreira-Filho O. 2021. Possible common origin of B chromosomes in Neotropical Fish (Siluriformes, Auchenipteridae) reinforced by repetitive DNA mapping**.** Braz Arch Biol Technol. 64:e21190494. doi: 10.1590/1678-4324-2021190494.

Machado AS, Kowalski S, Paiz LM, Margarido VP, Blanco DR, Venere PC, Mariotto S, Centofante L, Moreira-Filho O, Lui RL. 2021. Comparative cytogenetic analysis between species of *Auchenipterus* and *Entomocorus* (Siluriformes, Auchenipteridae). Caryologia. 74(2):89–101. doi: 10.36253/caryologia-1058.

Margarido VP, Moreira-Filho O. 2008. Karyotypic differentiation through chromosome fusion and number reduction in *Imparfinis hollandi* (Ostariophysi, Heptapteridae). Genetic Mol Biol. 31:235–238. doi: 10.1590/S1415-47572008000200012.

Martinez JF, Lui RL, Blanco DR, Traldi JB, Silva LF, Venere PC, Souza IL, Moreira-Filho O. 2011. Comparative cytogenetics of three populations from the *Rhamdia quelen* species complex (Siluriformes, Heptapteridae) in two Brazilian hydrographic Basins. Caryologia. 64:121–128. doi: 10.1080/00087114.2011.10589772.

Martins C, Wasko AP, Oliveira C, Wright JM. 2000. Nucleotide sequence of 5S rDNA and localization of the ribosomal RNA genes to metaphase chromosomes of the Tilapiine cichlid fish, *Oreochromis niloticus*. Hereditas. 133:39–46. doi: 10.1111/j.1601-5223.2000.00039.x.

Mees GF. 1974. The Auchenipteridae and Pimelodidae of Suriname (Pisces, Nematognathi). Zool Verh. 132: 12–256.

Milhomem SSR, Souza ACP, Nascimento AL, Carvalho Jr JR, Feldberg E, Pieczarka JC, Nagamachi CY. 2008. Cytogenetic studies in fishes of the genera *Hassar*, *Platydoras* and *Opsodoras* (Doradidae, Siluriformes) from Jarí and Xingú Rivers, Brazil. Genet Mol Biol. 31:256–260. doi: 10.1590/S1415-47572008000200017.

Miranda-Ribeiro A. 1911. Fauna brasiliensis. Peixes IV(a). Eleutherobranchios Aspirophoros. Arch Mus Nac Rio de J. 16:1–504.

Nelson JS, Grande TR, Wilson MVH. 2006. Fishes of the World. 5nd ed. New Jersey(NJ): John Wiley and Sons.

Peres WAM, Buckup PA, Kantek DLZ, Bertollo LAC, Moreira-Filho O. 2009. Chromosomal evidence of downstream dispersal of *Astyanax fasciatus* (Characiformes, Characidae) associated with River shed interconnection. Genetica. 137(3):305–311. doi: 10.1007/s10709-009-9389-4.

Pinkel D, Straume T, Gray J. 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA. 83(9):2934–2938. doi: 10.1073/pnas.83.9.2934.

Piscor D, Centofante L. Parise-Maltempi PP. 2017. Distinct classical and molecular cytogenetics of *Astyanax marionae* and *A. fasciatus* (Characiformes: Characidae): a comparative study of the organization of heterochromatin and repetitive genes. J Genet. 96(4):665–671. doi: 10.1007/s12041-017-0813-8.

Ravedutti CG, Júlio Jr H.F. 2001. Cytogenetic analysis of three species of the neotropical family Auchenipteridae (Pisces, Siluriformes) from the Paraná River Basin, Brazil. Cytologia. 66:65–70. doi: 10.1508/cytologia.66.65.

Regan CT. 1911. The classification of the teleostean fishes of the order Ostaripophysi. 2. Siluroidea. Ann Mag Nat. 8(8):552–577.

Reis RE, Albert JS, Di Dario F, Mincarone MM, Petry P, Rocha LA. 2016. Fish biodiversity and conservation in South America. J Fish Biol. 89:12–47. doi: 10.1111/jfb.13016.

Royero RL. 1999. Studies on the systematics and phylogeny of the catfish family Auchenipteridae (Teleostei: Siluriformes) [unpublished dissertation]. Bristol (BS): University of Bristol.

Santos DP, Felicetti D, Baumgärtner L, Margarido VP, Blanco DR, Moreira-Filho O, Lui RL. 2021. Contributions to the taxonomy of *Trachelyopterus* (Siluriformes): comparative cytogenetic analysis in three species of Auchenipteridae. Neotrop Ichthyol. 19:e200115. doi: 10.1590/1982-0224-2020-0115.

Stivari MK, Martins-Santos IC. 2004. Karyotype diversity in two populations of *Rhamdia quelen* (Pisces, Heptapteridae). Cytologia. 69(1):25–34. doi: 10.1016/0014-4827(72)90558-710.1508/cytologia.69.25.

Sumner AMT. 1972. A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res. 75:304–306. doi: 10.1016/0014-4827(72)90558-7.

Takagui FH, Dias AL, Birindelli JLO, Swarca AC, Rosa R, Lui RL, Fenocchio AS, Giuliano-Caetano L. 2017. First report of B chromosomes in three Neotropical thorny catfishes (Siluriformes, Doradidae). Comp Cytogenet. 11:55–64. doi: 10.3897/CompCytogen.v11i1.10496.

Takagui FH, Baumgärtner L, Baldissera JN, Lui RL, Margarido VP, Fonteles SBA, Garcia G, Birindelli JO, Moreira-Filho O, Almeida FS, Giuliano-Caetano L. 2019. Chromosomal diversity of thorny catfishes (Siluriformes-Doradidae): a case of allopatric speciation among Wertheimerinae species of São Francisco and Brazilian Eastern coastal drainages. Zebrafish. 16:477–485. doi: 10.1089/zeb.2019.1769.

Takagui FG, Viana P, Baumgartner L, Bitencourt JA, Margarido VP, Lui RL, Feldberg E, Birindelli JLO, Almeida FS, Giuliano-Caetano L. 2021. Reconstruction of the Doradinae (Siluriformes-Doradidae) ancestral diploid number and NOR pattern, reveals new insights about the karyotypic diversification of the Neotropical thorny catfishes. Genet Mol Biol. 44:e20200068. doi: 10.1590/1678-4685-GMB-2020-0068.

Tenório RCCO, Vitorino CA, Souza IL, Oliveira C, Venere PC. 2013. Comparative cytogenetics in *Astyanax* (Characiformes, Characidae) with focus on the cytotaxonomy of the group. Neotrop Ichthyol. 11(3):553–564. doi: 10.1590/S1679-62252013000300008.

Toussaint A, Charpin N, Brosse S, Villéger S. 2016. Global functional diversity of freshwater fish is concentrated in the Neotropics while functional vulnerability is widespread. Sci Rep. 6:22125. doi: 10.1038/srep22125.