Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Amanda de Souza Machado, Samantha Kowalski, Leonardo Marcel Paiz, Vladimir Pavan Margarido, Daniel Rodrigues Blanco, Paulo Cesar Venere, Sandra Mariotto, Liano Centofante e Orlando Moreira-Filho, Roberto Laridondo Lui (2021) Comparative cytogenetic analysis between species of *Auchenipterus* and *Entomocorus* (Siluriformes, Auchenipteridae). *Caryologia* 74(2): 89-101. doi: 10.36253/caryologia-1058

Received: August 21, 2020

Accepted: July 20, 2021

Published: October 08, 2021

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.

ORCID
ASM:0000-0002-1253-1357
SK: 0000-0002-4507-5714
LMP: 0000-0002-4761-8321
VPM: 0000-0002-0823-6646
DRB: 0000-0003-1619-2417
PCV: 0000-0001-7236-8857
SM: 0000-0003-4007-3100
LC: 0000-0003-0712-8149
OMF: 0000-0001-5137-0122
RLL: 0000-0003-4310-4865

Comparative cytogenetic analysis between species of *Auchenipterus* and *Entomocorus* (Siluriformes, Auchenipteridae)

Amanda de Souza Machado, Samantha Kowalski, Leonardo Marcel Paiz, Vladimir Pavan Margarido, Daniel Rodrigues Blanco, Paulo Cesar Venere, Sandra Mariotto, Liano Centofante, Orlando Moreira-Filho, Roberto Laridondo Lui*

Centro de Ciências Biológicas e da Saúde, Universidade Estadual do Oeste do Paraná, Cascavel, Paraná, Brazil; Departamento de Biologia Geral, Universidade Estadual de Londrina, Centro de Ciências Biológicas, Londrina, Paraná, Brazil; Universidade Tecnológica Federal do Paraná, Santa Helena, Paraná, Brazil; Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brazil; Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso, Cuiabá, Mato Grosso, Brazil; Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil
*Corresponding author. E-mail: roberto.lui@unioeste.br

Abstract. According to Auchenipteridae initial morphological data, *Auchenipterus* and *Entomocorus* have been considered phylogenetically close, and cytogenetic analyses are limited only to *Auchenipterus osteomystax*. Herein, we provide the first cytogenetic results about *Auchenipterus nuchalis* from Araguaia River and *Entomocorus radiosus* from Paraguay River. These data were generated in order to contribute to the investigation of the *Auchenipterus* chromosomal diversity and to attempt to better understand the phylogenetic relationship of these Auchenipterinae genera, mainly due to the existence of incongruous characters between *Entomocorus* and Centromochlinae. The two species presented 2n=58 chromosomes and had different karyotype formulas. The heterochromatin distribution was primarily shown in terminal regions, along with interstitial and/or pericentromeric blocks in submetacentric/subtelocentric pairs in *A. nuchalis* and *E. radiosus*. Single and terminal AgNORs were confirmed by 18S rDNA for the analyzed species, differing from *A. osteomystax* (cited as *A. nuchalis*) from Upper Paraná River. The variation in the number of 5S rDNA between species and its equilocality in *E. radiosus* suggest that the dispersion of the gene associated with the amplification of heterochromatic regions in the interphase, possibly promoted by the Rabl model system. The differences found between the species of *Auchenipterus* can work as species-specific characters and assist in studies of these taxa, which historically have been wrongly identified as a single species with wide distribution throughout the Neotropical region, when they are actually different species. Furthermore, there are cytogenetic similarities between *E. radiosus* and members of Centromochlinae like pointed out by recent morphological and molecular analyses in the family.

Keywords: Centromochlinae, equilocality, species-specific characters, Rabl, 5S rDNA.

INTRODUCTION

Vertebrates comprise more than 60.000 described species and about 32.000 of them are fish (Nelson 2016). In South America, a great ichthyofaunal diversity is reported, estimated to be over 9.100 species, which approximately 56% is from freshwater systems (Reis et al. 2016). The emergence and evolution of the freshwater ichthyofauna in the Neotropical region is large due to the humid tropical regions favorable for aquatic life (Albert et al. 2011). Furthermore, extensive geological events such as the formation of the Guiana Shield, the Brazilian Shield and the uplift of the Andes allowed the formation of important drainage axes that resulted in several speciation processes within and between the basins, thus reflecting the rich taxonomic composition of the freshwater ichthyofauna in the region (Reis et al. 2016).

Auchenipteridae, endemic to the Neotropical region, is subdivided into Centromochlinae and Auchenipterinae and consists of 25 genera and 127 species (Fricke et al. 2021). Moreover, it includes fishes known as inseminating and with external development (Calegari et al. 2019), just like in other Siluriformes families, such as Scoloplacidae and Astroblepidae (Spadella et al. 2006, 2012). This characteristic is directly associated with the sexual dimorphism related to modification of fins or barbels, which makes the internal insemination as a reproductive strategy in the group possible (Baumgartner et al. 2012; Calegari et al. 2019). Auchenipterinae comprises 18 genera, including *Auchenipterus* Valenciennes, 1840 and *Entomocorus* Eigenmann, 1917 (Fricke et al. 2021). According to morphological data, these taxa are considered sister-groups and constituting a clade with other groups. The phylogenetic relationships propositions between these genera of Auchenipteridae have undergone changes over time (e.g., Britski 1972; Ferraris 1988; Royero 1999; Akama 2004; Calegari et al. 2019).

Entomocorus is composed of 4 species, *Entomocorus benjamini* Eigenmann, 1917 distributed in the Upper Madeira River basin; *Entomocorus gameroi* Mago-Leccia, 1984 distributed in the drainages of the Orinoco River; *Entomocorus malaphareus* Akama and Ferraris, 2003 found in portions of the Lower and Middle Amazon River and *Entomocorus radiosus* Reis and Borges, 2006 endemic to the Paraguay River basin, the latter is described for the Pantanal region (Reis and Borges 2006; Fricke et al. 2021). Currently, the clade is reinforced by 41 molecular synapomorphies and 19 morphological synapomorphies (Calegari et al. 2019), a number that increased considerably after the previous review by Reis and Borges (2006), which presented 8 morphological synapomorphies for the genus.

Auchenipterus is reinforced by 9 morphological synapomorphies (Calegari et al. 2019) and is currently composed of 11 species widely distributed in the South American continent throughout the east of the Andean region (Fricke et al. 2021). Unlike most species of the genus, *Auchenipterus nuchalis* Spix and Agassiz, 1829 has a more restricted distribution and occurs only in a few portions of the Amazon River basin and low portions of the Tocantins River (Ferraris and Vari 1999); although it differs from more recent records in some locations (e.g., Fricke et al. 2021). On the other hand, *Auchenipterus osteomystax* Miranda Ribeiro, 1918 has a greater distribution from the Lower Amazon River basin, Tocantins River and the Prata River basin (Fricke et al. 2021). According to Ferraris and Vari (1999), these two species have already been wrongly identified in different hydrographic systems, as is the case of records of specimens of *A. osteomystax* identified as *A. nuchalis* in portions of the Paraná River, in the region of Itaipu reservoir, and in Porto Rico (PR, Brazil) (e.g., Agostinho et al. 1993; Cecilio et al. 1997; Ravedutti and Júlio Jr. 2001). Regarding the type species *A. nuchalis* (type locality: Amazon River), synonymization problems of new species in different locations overestimated its distribution (Ferraris and Vari 1999).

Auchenipterus nuchalis was the first species described for *Auchenipterus* Valenciennes, 1840, however, it was initially classified as *Hypophthalmus nuchalis* Spix and Agassiz, 1829 (Birindelli 2014). After the genus description, *A. nuchalis* was included and kept in Auchenipteridae since then, mainly due to the presence of sexual dimorphism (Miranda Ribeiro 1968), a character that proves to be very informative for the family (Calegari et al. 2019). On the other hand, *Entomocorus* was a target for some phylogenetic inconsistencies until a consensus was reached on its relationship with other close groups. According to Britski (1972), *Auchenipterus* was initially considered sister-group of the clade composed of *Epapterus* and *Pseudepapterus* (*Auchenipterus* (*Epapterus*, *Pseudepapterus*)), whereas *Entomocorus* was allocated close to *Trachelyichthys* and *Pseudauchenipterus* in a clade that is also made up of genera that currently belong to Centromochlinae (*Trachelyichthys* (*Entomocorus* (*Pseudauchenipterus* (*Centromochlus*, *Glanidium*)))). Subsequently, *Auchenipterus* and *Entomocorus* were relocated to the same clade (*Entomocorus* (*Auchenipterus*, *Epapterus*)), this closeness was reinforced by 14 morphological synapomorphies (Ferraris, 1988). Subsequent studies by Royero (1999) and Akama (2004) also kept *Entomocorus* and *Auchenipterus* close although, for these authors, the group (*Entomocorus*, *Auchenipterus*) has divergences in comparison with the *Epapterus* and *Pseudepapterus* taxa.

This clade has remained allocated in Auchenipterini tribe Bleeker, 1862, initially created to contain *Auchenipterus* Valenciennes, 1840 and, currently with the addition of *Pseudauchenipterus*, it is supported by 6 molecular synapomorphies and 9 morphological synapomorphies (*Pseudauchenipterus* (*Entomocorus* (*Pseudepapterus* (*Epapterus*, *Auchenipterus*))) (Calegari et al. 2019). Nonetheless, *Entomocorus* shares characters with Centromochlinae and other siluriforms and diverges by some diagnostic characteristics of Auchenipteridae (Reis and Borges 2006; Calegari et al. 2019). This set of characteristics shared among members of the clade and other groups of catfish, according to Birindelli (2014), is what could explain this group (*Entomocorus* (*Auchenipterus* (*Epapterus*)) as basal in the family, as proposed by Royero (1999). Regarding the relationship between *Entomocorus* and Centromochlinae, Bayesian Inference analyses (BI) based on molecular characters reinforced its inclusion in the subfamily, besides *Entomocorus* shares the genital tube anteriorly to the anal fin base and separated from its first rays like seen in members of Centromochlinae (Calegari et al. 2019). However, Calegari et al. (2019) still suggest that this relationship may be the result of events of genetic homoplasy (independent evolution) and not a common ancestry between the groups.

Regarding cytogenetic analyses in species of this clade, only *A. osteomystax* (cited as *A. nuchalis*) from the Upper Paraná River basin (e.g., Ravedutti and Júlio Jr. 2001) was studied and, together with data from some other species of the family (e.g., Fenocchio and Bertollo 1992; Fenocchio et al. 2008; Lui et al. 2009, 2010, 2013a, 2013b, 2015; Kowalski et al. 2020) (Table 1) have contributed to the understanding of evolutionary relationships and diversification mechanisms in Auchenipteridae. Due to the absence of chromosomal data about *A. nuchalis* and *E. radiosus*, this study aimed (1) to investigate the chromosomal characteristics of *A. nuchalis* from the Araguaia River basin, in search of species-specific characters that help to understand the diversity in *Auchenipterus*, considering the history of incongruences related to its taxa using morphological data, and (2) searching for chromosomal characters in *Entomocorus* and *Auchenipterus* that can add information to the evolutionary understanding between Auchenipteridae genera, specifically to the clade involving *Auchenipterus* and *Entomocorus*, since there are characters of morphological nature that approach *Entomocorus* to some Centromochlinae species.

MATERIAL AND METHODS

Chromosomal analyses were performed on four specimens of *Auchenipterus nuchalis* (Figure 1a), two males and two females, from the Araguaia River basin, between Aragarças (GO) and Barra do Garças (MT) (GPS: 15°53'03,9"S; 52°06'17,9"W); and eleven specimens of *Entomocorus radiosus* (Figure 1b), six males and five females, from the Paraguay River basin, Poconé (MT) (GPS: 16°25'40,9"S; 56°25'07,4"W) (Permanent license SISBIO 10538-1). The specimens of *A. nuchalis* e *E. radiosus* were deposited in the Zoology Museum of the University of São Paulo, under the respective vouchers: MZUSP 110805 and MZUSP 109791.

The specimens were euthanized with a clove oil overdose (Griffthis 2000) to remove the anterior kidney and prepare the mitotic chromosome suspensions as described by Bertollo et al. (1978) and Foresti et al. (1993), according to Committee of Ethics in Animal Experimentation and Practical Classes from Unioeste – (Protocol 13/09 - CEEAAP/Unioeste). The mitotic chromosomes were stained with Giemsa 5% diluted in phosphate buffer (Na_2HPO_4 x $12H_2O$ + KH_2PO_4 x $12H_2O$), pH = 6.8, for 7 minutes and classified according to Levan et al. (1964) in metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a). The C-banding technique followed the protocol according to Sumner (1972) with modifications suggested by Lui et al. (2012) and the detection of AgNORs through silver nitrate impregnation, according to Howell and Black (1980). The analysis of metaphases was done sequentially. Fluorescent *in situ* hybridization (FISH) was performed according to the methodology of Pinkel et al. (1986) with modifications suggested by Margarido and Moreira-Filho (2008), using the probes rDNA 18S (Hatanaka and Galetti Jr. 2004) and rDNA 5S (Martins et al. 2000). The rDNA 18S probe was labeled with biotin-16-dUTP by nick translation (Biotin Nick Translation Mix - Roche), with detection and amplification with avidin-FITC and anti-avidin biotin (Sigma) for both species. The 5S rDNA probe was labeled with digoxigenin-11-dUTP by nick translation (Dig 11 Nick Translation Mix - Roche) and detected with anti-digoxigenin-rhodamine for *A. nuchalis* and labeled with fluorescein-12-dUTP (FITC) by PCR for *E. radiosus*, using primers A (5'-TAC GCC CGA TCT CGT CCG ATC-3 ') and B (5'-CAG GCT GGT ATG GCC GTA AGC-3') (Pendás et al. 1994). Hybridizations were performed with 77% stringency (200 ng of each probe, 50% formamide, 10% dextran sulfate, 2xSSC; pH 7.0 - 7.2). FISH slides were analyzed using an epifluorescence photomicroscope Olympus BX60 under an appropriate filter.

Table 1. Cytogenetic data in Auchenipteridae.

Subfamily/Species	Locality	FN	2n	Karyotypic formula	AgNORs/ 18S rDNA	5S rDNA	Ref.
Centromochlinae							
Glanidium ribeiroi	Iguaçu River, Res. Salto Caxias, PR	112	58	28m+16sm+10st+4a	pair 17, p, i, sm	-	1
	Iguaçu River, Res. Segredo, PR	106	58	22m+16sm+10st+10a	pair 13, p, i sm	-	2
	Iguaçu River, Res. Salto Osório, PR	106	58	22m+16sm+10st+10a	pair 13, p, i sm	-	2
	Iguaçu River, Capanema, PR	110	58	22m+20sm+10st+6a	pair 14, p, i, sm	pair 16, q, i, sm	3
Tatia neivai	Machado River, Denise, MT	116	58	26m+26sm+6st	pair 28, p, t, st	pair 4, p, i, sm / pair 21, p, t, sm / pair 22, q, i, sm	4
Tatia jaracatia	Iguaçu River, Capanema, PR	116	58	20m+26sm+12st	pair 28, p, t, st	pair 4, p, i, m / pair 18, p, t, sm / pair 19, q, i, sm / pair 29, p, t, sm	4
Centromochlus heckelii	Solimões River, Manaus, AM	72	46	15m+6sm+5st+20a (ZW) 14m+6sm+6st+20a (ZZ)	pair ZW, p, t, m-st pair 20, p, t, a	-	9
Auchenipterinae							
Tympanopleura atronasus (cited as *Ageneiosus atronasus*)	Solimões River, Manaus, AM	100	56	16m+16sm+12st+12a	q, i, sm	-	5
Ageneiosus inermis (cited as *Ageneiosus brevifilis*)	Solimões River, Manaus, AM	102	56	20m+16sm+10st+10a	p, t, sm	-	5
Ageneiosus inermis	Araguaia River, Aragarças, GO	108	56	32m+16sm+4st+4a	pair 20, p, t, sm	pair 4, p, i, m	6
Auchenipterus osteomystax (cited as *Auchenipterus nuchalis*)	Paraná River, Porto Rico, PR	106	58	24m+14sm+10st+10a	pair 15, p, i, sm	-	1
Auchenipterus nuchalis	Araguaia River, Aragarças, GO	110	58	22m+16sm+14st+6a	pair 14, p, t, sm	pair 22, p, t, st	10
Entomocorus radiosus	Paraguai River, Poconé, MT	106	58	22m+12sm+14st+10a	pair 21, p, t, st	pair 12, p, t, sm / pair 13, p, t, sm / pair 14, p, t, sm / pair 15, p, t, sm / pair 16, p, t, sm / pair 18, p, t, st / pair 19, p, t, st	10
Trachelyopterus galeatus (cited as *Parauchenipterus galeatus*)	Paraná River, Porto Rico, PR	98	58	22m+12sm+6st+18a	pair 23, p, t, a	-	1
	Paraná River, Três Lagoas, MS	108	58	24m+18sm+8st+8a	pair 25, p, t, st	pair 16, p, i, sm / pair 17, q, i, sm	7
	Piumhi River, Capitólio, MG	108	58	20m+16sm+14st+8a	pair 24, p, t, st	pair 15, p, i, sm / pair 16, q, i, sm	7
	São Francisco River, Lagoa da Prata, MG	108	58	22m+16sm+12st+8a	pair 23, p, t, st	pair 16, p, i, sm / pair 17, q, i, sm	7, 8

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- Ravedutti and Júlio Jr. (2001); 2- Fenocchio et al. (2008); 3- Lui et al. (2015); 4- Lui et al. (2013a); 5- Fenocchio and Bertollo (1992); 6- Lui et al. (2013b); 7- Lui et al. (2010); 8- Lui et al. (2009); 9- Kowalski et al. (2020); 10- present study.

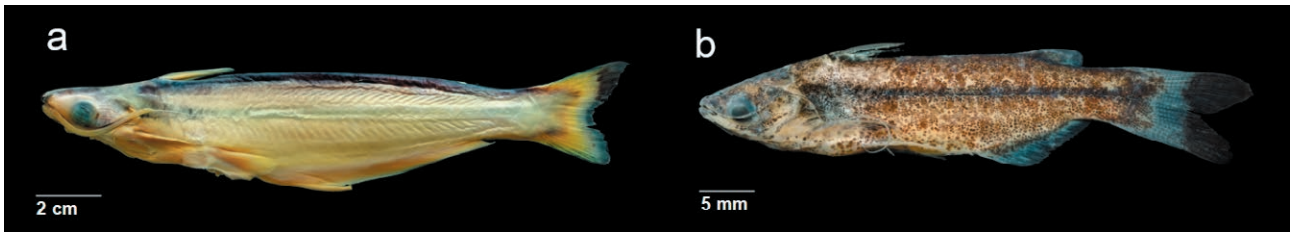

Figure 1. (**a**) Specimen of *Auchenipterus nuchalis* (Total length = 18.5 cm); (**b**) Specimen of *Entomocorus radiosus* (Total length = 4.96 cm).

RESULTS

Auchenipterus nuchalis - Araguaia River basin

The diploid number (2n) found for *A. nuchalis* was 58 chromosomes, 22 metacentric chromosomes, 16 submetacentric chromosomes, 14 subtelocentric chromosomes and 6 acrocentric chromosomes and fundamental number (FN) of 110 (Figure 2a). The heterochromatin distribution pattern showed blocks mainly in the terminal regions, as well as a pericentromeric block on the short arm of submetacentric pair 14 and an interstitial block on the long arm of submetacentric pair 16 and subtelocentric pair 20 (Figure 2b). Single AgNORs were detected in terminal position on the short arm of submetacentric pair 14 (Figure 2a, in box), and confirmed by fluorescent *in situ* hybridization (FISH/18S rDNA) (Figure 3a). The 5S rDNA sites were found in the terminal position on the short arm of the subtelocentric pair 22 (Figure 3a).

Entomocorus radiosus - Paraguay River basin

The diploid number (2n) found for *E. radiosus* was 58 chromosomes, 22 metacentric chromosomes, 12 submetacentric chromosomes, 14 subtelocentric chromosomes and 10 acrocentric chromosomes and fundamental number (FN) of 106 (Figure 2c). The heterochromatin distribution pattern showed blocks mainly in terminal regions, as well as strongly marked blocks in the pericentromeric position of submetacentric pair 13, subtelocentric pairs 18, 19 and 23 and acrocentric pairs (Figure 2d). Single AgNORs were detected in terminal position

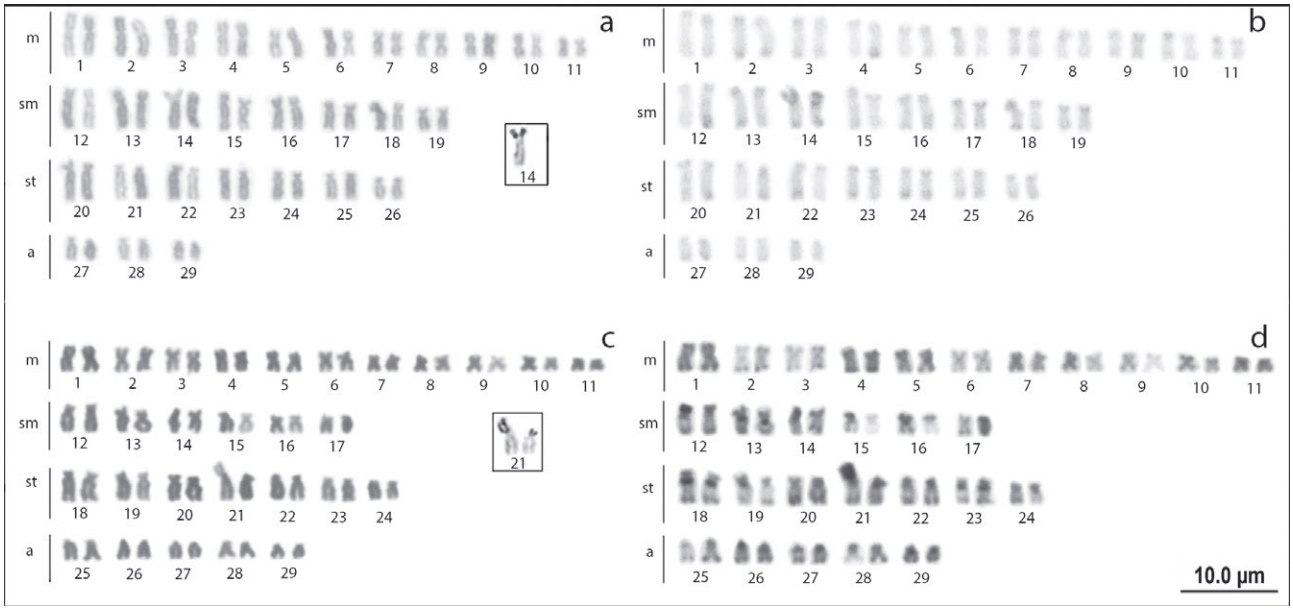

Figure 2. Karyotypes of *Auchenipterus nuchalis* (**a**, **b**) and *Entomocorus radiosus* (**c**, **d**) stained with Giemsa (**a**, **c**) and submitted to C-banding (**b**, **d**). AgNORs presented in boxes. The presence of only one marked chromosome (Fig 2a, in box) during the silver nitrate impregnation technique ($AgNOR_3$) in *A. nuchalis* suggests that the Nucleolus Organizer Region (NOR) on its corresponding chromosome was inactive during the previous interphase or even in due the region is small.

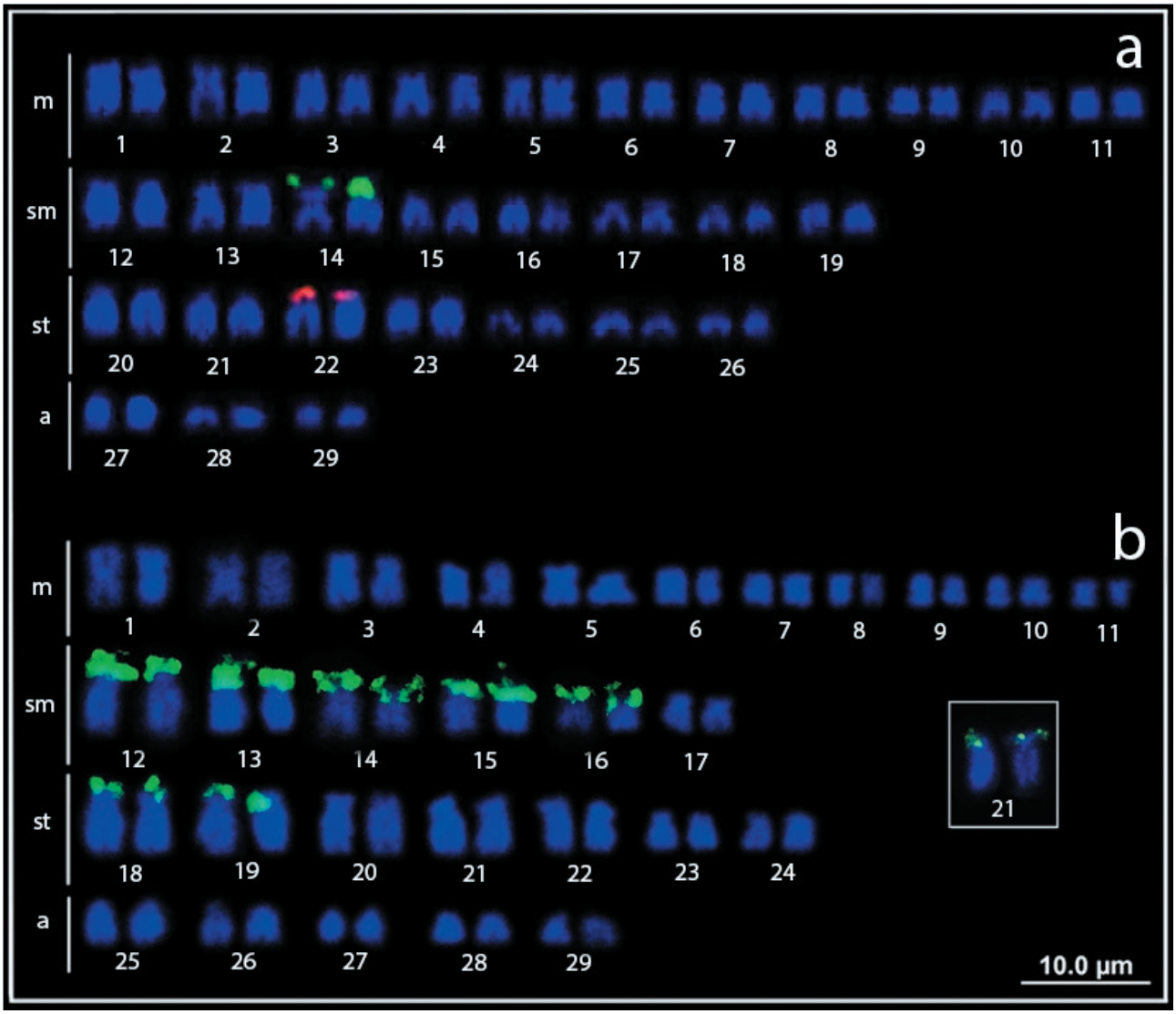

Figure 3. Karyotypes of *Auchenipterus nuchalis* (**a**) and *Entomocorus radiosus* (**b**) hybridized with rDNA 18S probes (pair 14 of *A. nuchalis* and pair 21 in box of *E. radiosus*, green signal) and rDNA 5S probes (red signal in the pair 22 of *A. nuchalis* and green signal in the pairs 12, 13, 14, 15, 16, 18 and 19 of *E. radiosus*), counterstained with DAPI. rDNA = ribosomal DNA and DAPI = 4',6-diamidino-2-phenylindole.

in the short arm of subtelocentric pair 21, confirmed by fluorescent *in situ* hybridization (FISH/18S rDNA) (Figure 3b, in box). Multiple sites of 5S rDNA were found in terminal position on the short arm of the submetacentric pairs 12, 13, 14, 15 and 16 and subtelocentric pairs 18 and 19 (Figure 3b).

DISCUSSION

In Auchenipteridae, cytogenetic analyses are restricted to few species and most of them present diploid number of 58 chromosomes (e.g., Ravedutti and Júlio Jr. 2001; Fenocchio et al. 2008; Lui et al. 2009, 2010, 2013a), except *Ageneiosus* and *Tympanopleura* with 56 chromosomes (Fenocchio and Bertollo 1992; Lui et al. 2013b) and *Centromochlus* with 46 chromosomes (Kowalski et al. 2020) (Table 1), caused by fusion events confirmed by the presence of ITS (Interstitial Telomere Sequence) (Lui et al. 2013b). In Doradidae, sistergroup of Auchenipteridae (e.g., Pinna 1998; Sullivan et al. 2006, 2008; Birindelli 2014; Calegari et al. 2019), the most frequent diploid number is also 58 chromosomes (Milhomen et al. 2008; Takagui et al. 2017, 2019), which reinforces it as a basal condition for both families and it is also corroborated by the data obtained in the species

of this study. In Neotropical fish, the variation of karyotypic formula among different populations of a given species or among species of the same family with maintenance of 2n is a common process resulted of chromosomal rearrangements, such as inversions or translocations (Ravedutti and Júlio Jr. 2001; Fenocchio et al. 2008; Lui et al. 2009, 2013a), as seen in *T. galeatus* (cited as *P. galeatus*) and *G. ribeiroi* (Lui et al. 2010, 2015).

The terminal heterochromatin distribution found in *A. nuchalis* and *E. radiosus* follows the pattern observed in Auchenipteridae (Lui et al. 2015), as well as for *A. osteomystax* (cited as *A. nuchalis*) (e.g., Ravedutti and Júlio Jr. 2001). However, interstitial and/or pericentromeric heterochromatins in some pairs in two species in this study (Figure 2b, 2d) diverge from what is more common to the family (e.g., Lui et al. 2009, 2010, 2015). *Auchenipterus osteomystax* (cited as *A. nuchalis*) from the Upper Paraná River (Ravedutti and Júlio Jr. 2001), the only species of this genus previously studied, presented only pale blocks in terminal and centromeric regions, in contrast to *A. nuchalis*, with some interstitial heterocromatins. On the other hand, similar markings have also been observed in *E. radiosus*, these heterochromatin data show greater similarity among species of different genera than between the two species of *Auchenipterus*. These small inconsistencies in the detection of heterochromatins are common among works performed by different authors and may be the result of artifacts of techniques, as observed between *A. nuchalis* from the Araguaia River and *A. osteomystax* (cited as *A. nuchalis*) from the Upper Paraná River, which used propidium iodide and Giemsa for the staining of the C-banding, respectively.

According to Lui et al. (2012), the use of some non-specific fluorescent dyes such as propidium iodide promote a greater contrast between heterochromatic and euchromatic regions, due to its greater interaction/absorbance in more compacted regions of the DNA (heterochromatin) and less interaction/absorbance in the DNA degraded during the C-banding process (euchromatin). This possibly explains that such inconsistencies between the populations of *Auchenipterus* may be due to the use of different dyes, since studies that use iodide has shown that the interstitial and/or pericentromeric markings found in *A. nuchalis* and *E. radiosus* can occur in other species of Auchenipteridae, from both subfamilies, such as *Ageneiosus*, *Tatia* and *Centromochlus* (e.g., Lui et al. 2013a, 2013b; Kowalski et al. 2020).

The NORs in the two species (Figure 2) resemble the heterochromatic pattern found in the family, such as *A. inermis*, *G. ribeiroi*, *T. galeatus*, *T. neivai* (e.g., Lui et al. 2009, 2013a, 2013b, 2015) and closer taxa like Doradidae (e.g., Eler et al. 2007; Takagui et al. 2017, 2019; Baumgärtner et al. 2018) and Aspredinidae (e.g., Ferreira et al. 2016). Single and terminal AgNORs/18S rDNA in submetacentric (*A. nuchalis*) and subtelocentric (*E. radiosus*) pairs (Figure 2, in boxes) coincided with those found in some species of the family, as in *T. galeatus* (subtelocentric pairs) (Lui et al. 2009), *A. inermis* (submacentric pair) (Fenocchio e Bertollo 1992; Lui et al. 2013b), *T. jaracatia* and *T. neivai* (subtelocentric pairs) (Lui et al. 2013a) (Table 1), as well as for most Doradidae species (e.g., Fenocchio et al. 1993; Eler et al. 2007; Milhomen et al. 2008; Takagui et al. 2017, 2019; Baumgärtner et al. 2018). Recently, data about *C. hechelli* demonstrated the first case of multiple and terminal NORs (acrocentric and ZW pairs) in Auchenipteridae (Table 1), an event that the authors propose to be the result of translocation between pairs during the interphase (e.g., Kowalski et al. 2020). Nevertheless, these results reinforce the presence of single and terminal NORs as the basal characteristic of the group, refuting data about *A. osteomystax* (cited as *A. nuchalis*) from the Upper Paraná River, which presented single and interstitial NORs (Table 1), initially suggested as standard in Auchenipteridae (Ravedutti and Júlio Jr. 2001).

Despite the differences related to the morphology of the pair carrying the 18S rDNA and the position of these cistrons on the chromosome among the Auchenipteridae species, we can suggest correspondence of this pair in the family, considering the similar size and the absence of multiple NORs for most Auchenipteridae species (Table 1), as well as for the pairs *A. nuchalis* and *E. radiosus* from this paper. Variations in the morphology and chromosome pair number in the karyotype must be related to chromosomal rearrangements, such as pericentric inversions or translocations (Lui et al. 2009, 2010, 2013a), as also observed in other families of Neotropical fishes, such as Doradidae (e.g., Eler et al. 2007; Milhomem et al. 2008), Loricariidae (e.g., Mariotto et al. 2019) and Rhamphichthyidae (e.g., Cardoso et al. 2011; Fernandes et al. 2019). Comparing the two species of *Auchenipterus*, it is possible to notice that both have NORs in submetacentric pairs and on the short arm, however in a terminal position in *A. nuchalis* and interstitial position in *A. osteomystax* (cited as *A. nuchalis*) (Table 1), representing a specific chromosomal marker between them. Thus, this difference may be useful in future studies of other populations these species, since there are some inconsistencies regarding the real geographic distribution of these species, especially as for *A. nuchalis*, which may be due to synonymizations and identification errors within the genus (Ferraris and Vari 1999).

Regarding repetitive sequence mapping data in Auchenipteridae, rDNAs are the most common, although limited to few species (Lui et al. 2009, 2010, 2013a, 2013b, 2015). Variations in the number of 5S rDNA sites in the family, from single to multiple, were observed in Centromochlinae and Auchenipterinae. Centromochlinae, *T. jaracatia* and *T. neivai* had multiple sites (Lui et al. 2013a), while *G. riberoi* had a single site (Lui et al. 2015) (Table 1). In Auchenipterinae, *T. galeatus* presented multiple sites (Lui et al. 2009) and *A. inermis* had only one pair containing the 5S rDNA (Lui et al. 2013b) (Table 1). Compared to close groups, the same scenario is observed for Doradidae (e.g., Baumgärtner et al. 2016, 2018; Takagui et al. 2017, 2019); while Aspredinidae, sister-group of Doradoidea (Auchenipteridae + Doradidae) (Sullivan et al. 2006, 2008; Calegari et al. 2019), presents 5S rDNA mapping data only for a species of the family with multiple sites (Ferreira et al. 2016, 2017).

There is still difficulty in determining the plesiomorphic condition related the 5S rDNA in Auchenipteridae, mainly due to (1) these variations (simple sites: multiple sites) in Doradoidea are distributed in an approximate ratio of 1:1, both in Auchenipteridae (Table 1) and in Doradidae (e.g., Baumgärtner et al. 2016, 2018; Takagui et al. 2017, 2019); and (2) analyzing the outgroup of Doradoidea (Aspredinidae), there is not enough data to understand the evolution of this gene in the groups, since there is only one species studied, which has polymorphic multiple condition related to the number of sites (Ferreira et al. 2016, 2017). However, despite these complicating factors, it would be coherent and parsimonious to hypothesize that single 5S rDNA sites are plesiomorphic in Doradoidea, or at least in Auchenipteridae. According to Martins and Galetti Jr. (1999), this is probably the ancestral condition for fish, as observed in Cichlidae (e.g., Nakajima et al. 2012; Paiz et al. 2017) and Pimelodidae (e.g., Girardi et al. 2018). On the other hand, the occurrence of multiple sites in different subfamilies of Auchenipteridae would be a result from independent dispersion events during the diversification of these species, just as the presence of transposition/translocation in species of *Pimelodus* is suggested (Girardi et al. 2018).

Considering the distribution of 5S rDNA in the terminal position of the short arm of the chromosome pairs in both species of this study (Table 1, Figure 3), it is possible to raise discussions about the dispersing mechanism of these sites in the genome of *E. radiosus*, which showed a significant higher number of chromosomes carrying this gene compared to the rest of the family. As a result, it would be possible to hypothesize that the dispersion these genes could (1) be associated with the distribution of heterochromatin or (2) be associated with transposing elements present in the genome (e.g., Gouveia et al. 2017; Glugoski et. al 2018; Primo et al. 2018). However, based on the arrangement of these sites, the hypothesis of dispersion related to the heterochromatic regions seems to be more likely because these genes have shown to correspond to terminal heterochromatins and are distributed evenly (equilocal) in the species genome, as already reported for Cyprinidae species (e.g., Saenjundaeng et al. 2020). According to Schweizer and Loidl (1987), this arrangement could explain the dispersion of sequences through transfer and amplification to other regions by proximity or physical contact between these stretches during the interphase nucleus. Furthermore, such movements could be favored because they are associated with heterochromatic regions (Schweizer and Loidl 1987) like already identified as recombination hotspots (Gornung 2013; Saenjundaeng et al. 2020). This characteristic corresponds to observed for *E. radiosus* from this study.

During the interphase, these mitotic chromosomes are organized into chromosomal territories (Cremer et al. 2018; Szalaj and Plewczynski 2018; Stam et al. 2019), thus they maintain their individuality during this phase and establish different and stable patterns with territories adjacent to each metaphasic cycle (Cremer et al. 1982; Fritz et al. 2015, 2019). These territories are designed from primary chromatin beams that depart from specific centromeric regions of the nucleus and extend, together with secondary and tertiary filaments, to the nuclear envelope until the telomeres, also called "Rabl Model" (Cremer and Cremer 2010). This arrangement would allow the spatial organization of equilocal telomeric regions proposed by Schweizer and Loidl (1987), facilitating the proximity and/or contact between homologous and non-homologous chromosomes and consequently the transfer and amplification of these regions in the genome (e.g., Prestes et al. 2019; Suaréz et al. 2019; Saenjundaeng et al. 2020; Takagui et al. 2020). This organization would explain the high number of terminal sites of 5S rDNA in *Entomocorus* which seems to be an apomorphy of the genus, or at least in *E. radiosus*. Although, these hypotheses need to be further investigated due to the lack of ribosomal analysis in *Auchenipterus*, as in *A. osteomystax* (e.g., Ravedutti and Júlio Jr. 2001) or other species of *Entomocorus*.

So far, *T. jaracatia* and *T. neivai* have a greater number of 5S rDNA sites after *E. radiosus* in Auchenipteridae (Table 1). These data can be interpreted in a similar way to what is proposed by Calegari et al. (2019) about the presence of possible homoplasies, it would explain

the proximity of *Entomocorus* to members of Centromochlinae, supported mainly by Bayesian Inference (BI) analyses. However, the monophyly of Auchenipterinae and Centromochlinae is well supported by Maximum Parsimony (MP) analyses of combined data (264 morphological characters and 1082 molecular sites), and they keep *Entomocorus* and the members of Centromochlinae phylogenetically distant (Calegari et al. 2019). Therefore, these similarities related to the number of 5S rDNA sites should not be considered as a common ancestry among these groups. However, it is interesting to mention that such phylogenetic inconsistencies generated by BI analyses, both of morphological and molecular data, can also be recognized through chromosomal markers.

In summary, differences in the karyotypic formula, fundamental number (FN), position of the NORs (Table 1) and distribution of heterochromatins can be pointed out as species-specific characters for the populations/species of *Auchenipterus* from the Araguaia and Upper Paraná River basins. At the moment, there is no data about 5S rDNA for *A. osteomystax* (cited as *A. nuchalis*) (Ravedutti and Júlio Jr. 2001), which would be useful and interesting to add to the data from the classic analyses, since this marker proves to be very informative for the group. Its variation in the group, mainly related to the number of sites, shows potential as a cytotaxonomic marker and raises discussions about its dynamics in the genomes of the group, like pointed out in this study for the equilocality in *E. radiosus*, suggesting to be related to scattering events associated with amplification of heterochromatic regions in the interphase. Furthermore, for this level of cytogenetic analysis, no apomorphies were found that reinforce the phylogenetic proximity between *A. nuchalis* and *E. radiosus*, resulting from two aspects: (1) the high similarity of the karyotype macrostructure observed by classical chromosomal markers, compared to others Auchenipteridae groups; and (2) absence of molecular chromosomal markers for the group, which considering the potential of 5S rDNA, should be better explored, since in the family some taxonomic/phylogenetic conflicts remain throughout history due to the lack of research beyond morphological diagnosis.

GEOLOCATION INFORMATION

Auchenipterus nuchalis from the Araguaia River basin, between Aragarças (Goiás State) and Barra do Garças (Mato Grosso State) (GPS: 15°53'03,9"S; 52°06'17,9"W), and *Entomocorus radiosus* from the Paraguay River basin, Poconé (Mato Grosso State) (GPS: 16°25'40,9"S; 56°25'07,4"W).

ACKNOWLEDGMENTS

The authours are grateful to the Universidade Estadual do Oeste do Paraná (UNIOESTE)/*Campus* Cascavel for the infrastructure for research development. We are grateful Dr. Heraldo Antonio Britski for the identification of the specimens and the laboratory technician Pedro Luis Gallo for his assistance in sampling. This study was funded by Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Paraná (FA), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

REFERENCES

Agostinho AA, Mendes VP, Suzuki HI, Canzi C. 1993. Avaliação da atividade reprodutiva da comunidade de peixes dos primeiros quilômetros a jusante do reservatório de Itaipu. Ver. UNIMAR. 15:175–189.

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

Albert JS, Petry P, Reis RE. 2011. Major Biogeographic and Phylogenetic Patterns. In: Albert JS, Reis RE, editors. Historical Biogeography of Neotropical Freshwaters Fishes. Califórnia (CA): University of California Press, p. 21–56.

Baumgartner G, Pavanelli CS, Baumgartner D, Bifi AG, Debona T, Frana VA. 2012. Peixes do baixo rio Iguaçu. Maringá (PR): Eduem; [accessed 2019 April 25]. http://books.scielo.org/id/sn23w.

Baumgärtner L, Paiz LM, Takagui FH, Lui RL, Moreira-Filho O, Giuliano-Caetano L, Portela-Castro ALB, Margarido VP. 2018. Comparative cytogenetics analysis on five genera of thorny catfish (Siluriformes, Doradidae): Chromosome review in the family and inferences about chromosomal evolution integrated with phylogenetic proposals. Zebrafish. 15(3):1–9. https://doi.org/10.1089/zeb.2017.1554

Bertollo LAC, Takahashi CS, Moreira-Filho O. 1978. Cytotaxonomic consideration on *Hoplias lacerdae* (Pisces, Erythrinidae). Braz. J. Genet. 1:103-120.

Birindelli JLO. 2014. Phylogenetic relationships of the South American Doradoidea (Ostariophysi: Siluriformes). Neotrop. Ichthyol. 12(3):451–564. https://doi.org/10.1590/1982-0224-20120027

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

Calegari BB, Vari RP, Reis RE. 2019. Phylogenetic systematics of the driftwood catfishes (Siluriformes: Auchenipteridae): a combined morphological and molecular analysis. Zool. J. Linn. Soc. 187(3):661–773. https://doi.org/10.1093/zoolinnean/zlz036

Cardoso AL, Pieczarka JC, Feldberg E, Milhomem SSR, Moreira-Almeida T, Silva DS, Silva PC, Nagamachi CY. 2011. Chromosomal characterization of two species of genus *Steatogenys* (Gymnotiformes: Rhamphichthyoidea: Steatogenini) from the Amazon basin: sex chromosomes and correlations with Gymnotiformes phylogeny. Rev. Fish Biol. Fish. 21(3):613–621. https://doi.org/10.1007/s11160-010-9196-0

Cecilio EB, Agostinho AA, Júlio Jr H, Pavanelli CS. 1997. Colonização ictiofaunística do reservatório de Itaipu e áreas adjacentes. Rev. Bras. Zool. 14(1):1–14. https://doi.org/10.1590/S0101-81751997000100001

Cremer T, Cremer C, Baumann H, Luedtke EK, Sperling K, Teuber V, Zorn C. 1982. Rabl's Model of the Interphase Chromosome Arrangement Tested in Chinese Hamster Cells by Premature Chromosome Condensation and Laser-UV-Microbeam Experiments. Hum. Genet. 60:46–56. https://doi.org/10.1007/BF00281263

Cremer T, Cremer C. 2010. Cromosome Territories. Cold Spring Harb. Perspect. Biol. 2(3):1–22. https://doi.org/10.1101/cshperspect.a003889

Cremer T, Cremer M, Cremer C. 2018. The 4D nucleome: Genome compartmentalization in an evolutionary context. Biochemistry. 83(4):313–325. https://doi.org/10.1134/S000629791804003X

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

Fenocchio AS, 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.

Fernandes CA, Aguiar AKM, Paiz LM, Baumgärtner L, Piscor D, Margarido VP. 2019. First chromosomal analysis of *Gymnorhamphichthys britskii*: the remarkable lowest diploid value within the family Rhamphichthyidae (Gymnotiformes). Neotrop. Ichthyol. 17(3):1–6. https://doi.org/10.1590/1982-0224-20190069

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

Ferraris Jr CJ, Vari RP. 1999. The South American catfish genus *Auchenipterus* Valenciennes, 1840 (Ostariophysi: Siluriformes: Auchenipteridae): monophyly and relationships, with a revisionary study. Zool. J. Linn. Soc. 126(4):387–450. https://doi.org/10.1006/zjls.1998.0177

Ferreira M, Garcia C, Matoso DA, Jesus IS, Feldberg E. 2016. A new multiple sex chromosome system X1X1X2X2/X1Y1X2Y2 in Siluriformes: cytogenetic characterization of *Bunocephalus coracoideus* (Aspredinidae). Genetica. 144(5):591–599. https://doi.org/10.1007/s10709-016-9927-9

Ferreira M, Garcia C, Matoso DA, Jesus IS, Cioffi MB, Bertollo LAC, Zuanon J, Feldberg E. 2017. The *Bunocephalus coracoideus* species complex (Siluriformes, Aspredinidae). Signs of a speciation process through chromossomal, genetic nad ecological diveristy. Front. Genet. 8(120):1–12. https://doi.org/10.3389/fgene.2017.00120

Foresti F, Oliveira O, Almeida-Toledo LF. 1993. A method for chromosome preparations from large fish specimens using in vitro short-term treatment with colchicine. Experientia. 49(9):810–813. https://doi.org/10.1007/BF01923555

Fricke R, Eschmeyer WN, Fong JD. 2021. Catalog of Fishes: Species by Family/subfamily. San Francisco (CA): California Academy of Sciences; [Accessed 2021 March 01].
http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp.

Fritz AJ, Barutcu AR, Martin-Buley L, Van-Wijnen AJ, Zaidi SK, Imbalzano AN, Lian JB, Stein JL, Stein GS. 2015. Chromosome at work: Organization of chromosome territories in the interphase nucleus. J. Cell. Biochem. 117(1):9–19. https://doi.org/10.1002/jcb.25280

Fritz AJ, Sehgal N, Pliss A, Xu J, Berezney R. 2019. Chromosome territories and the global regulation of the genome. Genes, Chromosomes and Cancer. 58(7):407–426. https://doi.org/10.1002/gcc.22732

Girard SC, Pavanelli CS, Margarido VP. 2018. Contributions to the systematic of Pimelodidae (Osteichthyes, Siluriformes): basic and molecular cytogenetics on seven species of *Pimelodus* from three Brazilian hydrographic systems. Neotrop. Ichthyol. 16(2):1–16. http://orcid.org/0000-0002-0823-6646

Gornung E. 2013. Twenty years of physical mapping of major ribosomal RNA genes across the Teleosts: A

review of research. Cytogenet. Genome Res. 141(2-3):90–102. https://doi.org/10.1159/000354832

Gouveia JG, Wolf IR, Vilas Boas L, Heslop-Harrison P, Schwarzacher T, Dias AL. 2017. Repetitive DNA in the catfish genome: rDNA, microsatellites and *Tc1-mariner* transposon sequences in *Imparfinis* species (Siluriformes, Heptapteridae). J. Hered. 108(6):650–657. https://doi.org/10.1093/jhered/esx065

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

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):239–244. https://doi.org/10.1007/s10709-004-2039-y

Howell WM, Black DA. 1980. Controlled silver staining of Nucleolus Organizer Regions with protective colloidal developer: a one-step method. Experientia. 36(8):1014–1015. https://doi.org/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(3):1–12. https://doi.org/10.1590/1982-0224-2020-0009

Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. 52(2):201–220. https://doi.org/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(5–6):552–559. https://doi.org/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(3):648–656. https://doi.org/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. https://doi.org/10.3109/10520295.2012.696700

Lui RL, Blanco DR, Margarido VP, Troy WP, Moreira-Filho O. 2013a. Comparative chromosomal analysis concerning two species of genus *Tatia* (Siluriformes, Auchenipteridae). Comp. Cytogenet. 7(1):63–71. https://doi.org/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(2):327–334. https://doi.org/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. https://doi.org/10.1590/1519-6984.10714

Marajó L, Viana PF, Ferreira M, Py-Daniel LHR, Feldberg E. 2018. Cytogenetics of two Farlowella species (Locariidae: Loricariinae): implications on the taxonomic status of the species. Neotrop. Ichthyol. 16(4):1–8. http://dx.doi.org/10.1590/1982-0224-20180029

Margarido VP, Moreira-Filho O. 2008. Karyotypic differentiation through chromosome fusion and number reduction in *Imparfinis hollandi* (Ostariophysi, Heptapteridae). Genet. Mol. Biol. 31(1 suppl):235–238. https://doi.org/10.1590/S1415-47572008000200012

Mariotto S, Centofante L, Venere PC, Ferreira DC, Artoni RF. 2019. New Comparative Cytogenetic Data on Three Genera of Armored Catfishes of Ancistrini (Loricariidae: Hypostominae). Cytogenet. Genome Res. 159:1–7. https://doi.org/10.1159/000504723

Martins C, Galetti Jr PM. 1999. Chromosomal localization of 5S rDNA genes in *Leporinus* fish (Anostomidae, Characiformes). Chromossome Res. 7(5):363–367. https://doi.org/10.1023/A:1009216030316

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*. Chromosome Res. 133(1):39–46. https://doi.org/10.1111/j.1601-5223.2000.00039.x

Milhomem SSR, Souza ACP, do 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(1):256–260. https://doi.org/10.1590/S1415-47572008000200017

Miranda Ribeiro P. 1968. Sobre o dimorfismo sexual no gênero *Auchenipterus* Valanciennes, 1840 (Pisces-Auchenipteridae). Bol. Mus. Nac. Rio de Janeiro. 261:1-11.

Nakajima RT, Cabral-de-Mello DC, Valente GT, Venere PC, Martins C. 2012. Evolutionary dynamics of

rRNA gene clusters in cichlid fish. BMC Evol. Biol. 12(1):2–11. https://doi.org/10.1186/1471-2148-12-198

Nelson JS, Grande TC, Wilson MVH. 2016. Fishes of the world. New Jersey: John Wiley & Sons.

Paiz LM, Baumgärtner L, Graça WJ, Margarido VP, Pavanelli CS. 2017. Cytogenetics of *Gymnogeophagus setequedas* (Cichlidae: Geophaginae), with comments on its geographical distribution. Neotrop. Ichthyol. 15(2):1–8. https://doi.org/10.1590/1982-0224-20160035

Pendás AM, Morán P, Freije JP, García-Vazquez E. 1994. Chromosomal mapping and nucleotide sequence of two tandem repeats of Atlantic salmon 5S rDNA. Cytogenet. Cell Genet. 67(1):31–36. https://doi.org/10.1159/000133792

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

Pinna MCC. 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; p. 279–330.

Prestes AB, Nardelli A, Paiz LM, Gavazzoni M, Margarido VP. 2019. Cytogenetic markers as tools in delimiting species of the highly diverse Neotropical fish *Bryconamericus* (Characiformes: Characidae). Neotrop. Ichthyol. 17(3):1–11. https://doi.org/10.1590/1982-0224-20190057

Primo CC, Glugoski L, Vicari MR, Nogaroto V. 2018. Chromosome mapping and molecular characterization of the *Tc1/Mariner* element in *Rinoloricaria* (Siluriformes: Loricariidae). 61:1–7. https://doi.org/10.1590/1678-4324-2018170623

Ravedutti CG, Júlio Jr HF. 2001. Cytogenetic Analysis of Three Species of the Neotropical Family Auchenipteridae (Pisces, Siluriformes) from the Paraná River Basin, Brazil. Cytologia. 66(1):65–70. https://doi.org/10.1508/cytologia.66.65

Reis RE, Borges TAK. 2006. The South American Catfish Genus *Entomocorus* (Ostariophysi: Siluriformes: Auchenipteridae), with the Description of a New Species from the Paraguay River Basin. Copeia. 2006(3):412–422. http://dx.doi.org/10.1643/0045-8511(2006)2006[412:TSACGE]2.0.CO;2

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(1):12–47. https://doi.org/10.1111/jfb.13016

Royero R. 1999. Studies on the systematics and phylogeny of the catfish Family Auchenipteridae (Teleostei: Siluriformes) [thesis]. Bristol: University of Bristol.

Saenjundaeng P, Supiwong W, Sassi FMC, Bertollo LAC, Rab P, Kretschmer R, Tanomtong A, Suwannapoom C, Reungsing M, Cioffi, MB. 2020. Chromosome of Asian cyprinid fishes: Variable karyotype patterns and evolutionary trends in the genus Osteochilus (Cyprinidae, Labeoninae, "Osteochilini"). Neotrop. Ichthyol. 43(4):1–8. https://doi.org/10.1590/1678-4685-gmb-2020-0195

Schweizer D e Loidl J. 1987. A model for heterochromatin dispersion and the evolution of C-band patterns. Chromosomes today. 9:61–74. https://doi.org/10.1007/978-94-010-9166-4_7

Spadella MA, Oliveira C, Quagio-Grassiotto I. 2006. Spermiogenesis and introsperm ultrastructure of *Scoloplax distolothrix* (Ostariophysi: Siluriformes: Scoloplacidae). Acta Zool. 87(4):341–348. https://doi.org/10.1111/j.1463-6395.2006.00250.x

Spadella MA, Oliveira C, Quagio-Grassiotto I. 2012. Spermiogenesis and sperm ultrastructure in ten species of Loricariidae (Siluriformes, Teleostei). Zoomorphology. 131(3):249–263. https://doi.org/10.1007/s00435-012-0153-4

Stam M, Tark-Dame M, Fransz P. 2019. 3D genome organization: a role for phase separationand loop extrusion? Curr. Opin. Plant Biol. 48:36–46. https://doi.org/10.1016/j.pbi.2019.03.008

Suaréz P, Barroso ICGP, Silva DS, Milhomem SSR, Cabral-de-Mello D, Martins C, Pieczarka JC, Nagamachi CY. 2017. Highest diploid number among Gymnotiformes: First cytogenetic insights into *Rhabdolichops* (Sternopygidae). Zebrafish. 14(3):1–8. https://doi.org/10.1089/zeb.2016.1405

Sullivan JP, Lundberg JG, Hardman M. 2006. A phylogenetic analysis of the major groups of catfishes (Teleostei: Siluriformes) using rag1 and rag2 nuclear gene sequences. Mol. Phylogenet. Evol. 41(3):636–662. https://doi.org/10.1016/j.ympev.2006.05.044

Sullivan JP, Peng Z, Lundberg JG, Peng J, He S. 2008. Molecular evidence for diphyly od the Asian catfish family Amblycipitidae (Teleostei: Siluriformes) and exclusion of the South American Aspredinidae from Sisoroidea. Proc. Acad. Nat. Sci. Philadelphia. 157(1):51–65. https://doi.org/10.1635/0097-3157(2008)157[51:MEFDOT]2.0.CO;2

Sumner AT. 1972. A simple technique for demonstrating centromeric heterocromatin. Exp. Cell Res. 75(1):304–306. https://doi.org/10.1016/0014-4827(72)90558-7

Szalaj P, Plewczynski D. 2018. Three-dimensional organization and dynamics of the genome. Cell Biol. Toxi-

col. 34:381–404. https://doi.org/10.1007/s10565-018-9428-y

Takagui FH, Moura LF, Ferreira DC, Centofante L, Vitorino CA, Bueno V, Margarido VP, Venere PC. 2017. Karyotype Diversity in Doradidae (Siluriformes, Doradoidea) and Presence of the Heteromorphic ZZ/ZW Sex Chromosome System in the Family. Zebrafish. 14(3):236–243. https://doi.org/10.1089/zeb.2016.1368

Takagui FH, Baumgärtner L, Baldissera JN, Lui RL, Margarido VP, Fonteles SBA, Garcia C, 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(5):1–9. https://doi.org/10.1089/zeb.2019.1769

Takagui FH, Baumgärtner L, Venturelli NB, Paiz LM, Viana P, Dionísio JF, Pompeo LRS, Margarido VP, Fenocchio AS, Rosa R, Giuliano-Caetano L. 2020. Unrevealing the karyotypic evolution and cytotaxonomy of armored catfishes (Loricariinae) with emphasis in Sturisoma, Loricariichthys, Loricaria, Proloricaria, Pyxiloricaria, and Rineloricaria. Zebrafish. 17(5):1–14. https://doi.org/10.1089/zeb.2020.1893