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Contributions to the taxonomy of *Trachelyopterus* (Siluriformes): comparative cytogenetic analysis in three species of Auchenipteridae

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Auchenipteridae is divided into subfamilies Centromochlinae and Auchenipterinae. *Parauchenipterus* is included in the latter and is subject of taxonomic discussions concerning its validation or synonymization with *Trachelyopterus*. Herein, three species from two hydrographic basins were cytogenetically analyzed: *Parauchenipterus striatulus* from Doce River and two sympatric species, *P. galeatus* and *Trachelyopterus coriaceus*, from the Araguaia River. Diploid number of 58 chromosomes was verified for all species, but *P. striatulus* has different karyotype formula from the others. The three species have heterochromatin located in terminal regions of almost all chromosomes and in pericentromeric region on acrocentric chromosomes. Simple NORs was verified on a subtelocentric chromosome for all species. 5S rDNA sites were detected in three submetacentric chromosome pairs in *P. striatulus*; in a metacentric chromosome pair and submetacentric pair in *T. coriaceus*; and in one metacentric chromosome pair in *P. galeatus*. The similarities found in the karyotypes of the three species suggest the existence of only one genus, *Trachelyopterus*; therefore, our data refutes the validation of *Parauchenipterus*. Moreover, the differences in 5S rDNA distribution in *P. galeatus* in comparison with other populations already studied, indicate the existence of a new taxonomic unit, which suggests a species complex in *P. galeatus*.

Keywords: Cryptic species, FISH, *Parauchenipterus*, Problematic taxonomy, 5S rDNA.

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Auchenipteridae é dividida nas subfamílias Centromochlinae e Auchenipterinae. *Parauchenipterus* encontra-se incluído na última e tem sido alvo de discussões relacionadas com a problemática taxonômica de validação ou sinonimização com *Trachelyopterus*. Foram analisadas citogeneticamente três espécies de duas bacias hidrográficas: *Parauchenipterus striatulus* do rio Doce, *P. galeatus* e *Trachelyopterus coriaceus*, simpátricas do rio Araguaia. Todas as espécies analisadas apresentaram número diploide de 58 cromossomos, com diferença na fórmula cariotípica de *P. striatulus*. A heterocromatina foi localizada nas regiões terminais de quase todos os cromossomos e na região pericentromérica nos cromossomos acrocêntricos das três espécies. AgNORs e DNAr 18S detectaram RONs simples em um par de cromossomos subtelocêntricos nas três espécies. DNAr 5S foi detectado em três pares de cromossomos submetacêntricos em *P. striatulus*; em um par de cromossomos metacêntricos e um par submetacêntrico em *T. coriaceus*; e em apenas um par de cromossomos metacêntricos em *P. galeatus*. As semelhanças encontradas nos cariótipos das três espécies analisadas indicam a existência de somente *Trachelyopterus* (não validação de *Parauchenipterus*) e a diferença encontrada na distribuição de DNAr 5S de *P. galeatus* em relação às outras populações já estudadas sugere a existência de uma nova unidade taxonômica, portanto *P. galeatus* compreende um complexo de espécies.

Palavras-chave: Diversidade críptica, DNAr 5S, FISH, *Parauchenipterus*, Problemática taxonômica.

INTRODUCTION

Auchenipteridae belongs to the order Siluriformes and contains 25 genera and 126 species (Fricke *et al*., 2021) divided into two subfamilies: Centromochlinae (with nine genera) and Auchenipterinae, the latter including the species of *Trachelyopterus* Valenciennes, 1840 and *Parauchenipterus* Bleeker, 1862. Auchenipterids are small to medium-sized catfishes restricted to the Neotropical region. Several synapomorphies support the hypothesis of a monophyletic family, and its main characteristics are sexual dimorphism and internal insemination (Akama, 2004; Birindelli, 2014). Cytogenetic analyzes in this family are scarce and restricted to *Ageneiosus* Lacepède, 1803, *Auchenipterus* Valenciennes, 1840, *Glanidium* Lütken, 1874, *Tatia* Miranda Ribeiro, 1911 and *Parauchenipterus* (Tab. 1). Most species have 58 chromosomes (Ravedutti, Júlio, 2001; Fenocchio *et al*., 2008; Lui *et al*., 2010, 2013a), except for *Ageneiosus* and *Tympanopleura* Eigenmann, 1912 species that have 56 chromosomes (Fenocchio, Bertollo, 1992; Lui *et al*., 2013b), and *Centromochlus heckelii* (De Filippi, 1853) that has 46 chromosomes (Kowalski *et al*., 2020). Heterochromatin is distributed in the terminal regions of most chromosomes (Lui *et al*., 2010, 2015). Nucleolus Organizing Regions (NORs) are typically found on the short arm of one chromosome pair of most Auchenipteridae species studied (Lui *et al*., 2010, 2015). Exceptions to the most common in the group include *Ageneiosus*, *Tympanopleura*, *Tatia neivai* (Ihering, 1930) and *Centromochlus* Kner, 1858 (*e.g*., Fenocchio, Bertollo, 1992; Lui *et al*., 2013a,b;

Kowalski *et al*., 2020). In *Parauchenipterus*, only *P. galeatus* (Linnaeus, 1766) has chromosomal data available, and all populations have 58 chromosomes (Ravedutti, Júlio, 2001; Lui *et al*., 2010; Araújo, Molina, 2013). However, small differences in the karyotype formula and in the AgNORs bearing pair were found. This diversity is supported by the putative chromosomal rearrangements, like pericentric inversions (Ravedutti, Júlio, 2001; Lui *et al*., 2009, 2010; Araújo, Molina, 2013). Moreover, according to Araújo, Molina (2013), this species is likely a species complex.

The lack of morphological diversity causes confusion in taxonomic classification in some fish groups and makes systematic organization difficult (for review, see Keat-Chuang Ng *et al*., 2017). The validation of *Parauchenipterus* has been the subject of extensive discussion for over two centuries, which has been considered valid by some researchers (Curran, 1989; Royero, 1999; Akama, 2004; Buckup *et al*., 2007; Graça, Pavanelli, 2007). Akama (2004) attempted to sort it out and pondered this genus valid, dividing it into two groups: "*galeatus* group", composed of *Parauchenipterus* sp. n. and *P. galeatus*, which would have wide distribution occurring in the Amazon, Orinoco, Paraná-Paraguay, Guyana, São Francisco and Northeast Brazil drainages; and "*striatulus* group", composed by *Parauchenipterus ceratophysus* (Kner, 1858), *Parauchenipterus striatulus* (Steindachner, 1877) and *Parauchenipterus porosus* (Eigenmann & Eigenmann, 1888), distributed in the Amazon basin, Paraná-Paraguay basin, drainages from eastern Brazil and possibly in the northeastern of Brazil. This classification was based on differences of osteological characters and male gonadal apparatus. Even though some researchers disagree with the validity of *Parauchenipterus*, following studies to the review by Akama (2004), suggest that *Parauchenipterus* is synonym of *Trachelyopterus* (Ferraris, 2007; Birindelli, 2014; Calegari *et al*., 2019).

The taxonomic conflict between *Parauchenipterus* and *Trachelyopterus* is due to the great morphological similarity between these two nominal genera. Therefore, three species of these genera were cytogenetically analyzed (*P. striatulus* from Doce River basin, and *P. galeatus* and *Trachelyopterus coriaceus* Valenciennes, 1840 from Araguaia River basin) to highlight the historical taxonomic problematic linked to this group of Neotropical fishes. In addition, the present work attempt to contribute to the taxonomic problem of *Parauchenipterus*, analyzing species from the "*galeatus* group" and "*striatulus* group". These groups were defined in the last research, which focused in discussing *Parauchenipterus* validation (Akama, 2004), what did not happen until now.

MATERIAL AND METHODS

Individuals of *Parauchenipterus striatulus* (7 males 5 females) were collected in Verde Lake, Doce River basin in Mariléia city, Minas Gerais State, Brazil, 19°49'44.5"S 42°37'52.5"W. *Parauchenipterus galeatus* (9 males 10 females) and *Trachelyopterus coriaceus* (6 males 9 females) were collected from the Marginal Lake to the Córrego do Medo, tributary of the Araguaia River in São Miguel do Araguaia city, Ana Maria farm, Goiás State, Brazil, 13°08'52.7"S 50°25'02.8"W, with fishing nets (Permanent License SISBIO 10538-1). The specimens were kept in aquaria and subsequently euthanized by clove oil overdose (Griffiths, 2000; Pereira-Da-Silva *et al*., 2009) (according to the Animal Experimentation Ethics Committee and Unioeste practical classes: 13/09

TABLE 1 | Cytogenetic data in Auchenipteridae. FN: fundamental number; 2n: diploid number; Res.: Reservoir; m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric; p: short arm; q: long arm; W: sex chromosome. References (Ref.): 1. Ravedutti, Júlio (2001); 2. Fenocchio *et al.* (2008); 3. Lui *et al.* (2015); 4. Lui *et al.* (2013a); 5. Kowalski *et al.* (2020); 6. Fenocchio, Bertollo (1992); 7. Lui *et al.* (2013b); 8. Lui *et al.* (2010); 9. Araújo, Molina (2013); 10. Present study.

Species	Locality	FN	2n	Karyotype formula	AgNORs/18S rDNA	5S rDNA	Ref.
Centromochlinae							
Glanidium ribeiroi	Iguaçu River, Res. Salto Caxias, Paraná State	112	58	28m+16sm+10st+4a	pair 17, p, sm	-	1
	Iguaçu River, Res. Segredo, Paraná State	106	58	22m+16sm+10st+10a	pair 13, p sm	-	2
	Iguaçu River, Res. Salto Osório, Paraná State	106	58	22m+16sm+10st+10a	pair 13, p, sm	-	2
	Iguaçu River, Capanema, Paraná State	110	58	22m+20sm+10st+6a	pair 14, p, sm	pair 16, q, sm	3
Tatia neivai	Machado River, Denise, Mato Grosso State	116	58	26m+26sm+6st	pair 28, p, st	pair 4, p, sm / pair 21, p, sm pair 22, q, sm	4
Tatia jaracatia	Iguaçu River, Capanema, Paraná State	116	58	20m+26sm+12st	pair 28, p, st	pair 4, p, m / pair 18, p, sm pair 19, q, sm / pair 29, p, sm	4
Centromochlus heckelii	Solimões River, Manaus, Amazonas State	72	46	14m+6sm+6st+20a (♂) 15m+6sm+5st+20a (♀)	pair 20, p, a pair 13, p (W)	-	5
Auchenipterinae							
Tympanopleura atronasus (cit. *Ageneiosus atronasus)*	Solimões River, Manaus, Amazonas State	100	56	16m+16sm+12st+12st	q, sm	-	6
Ageneiosus inermis (cit. *Ageneiosus brevifilis*)	Solimões River, Manaus, Amazonas State	102	56	20m+16sm+10st+10s	p, sm	-	6
	Araguaia River, Aragarças, Goiás State	108	56	32m+16sm+4st+4a	pair 20, p, sm	pair 4, p, m	7
Auchenipterus osteomystax (cit. *Auchenipterus nuchalis*)	Paraná River, Porto Rico, Paraná State	106	58	24m+14sm+10st+10a	pair 15, p, sm	-	1
Parauchenipterus galeatus	Paraná River, Porto Rico, Paraná State	98	58	22m+12sm+6st+18a	pair 23, p, a	-	1
	Paraná River, Três Lagoas, Mato Grosso do Sul State	108	58	24m+18sm+8st+8a	pair 25, p, st	pair 16, p, sm / pair 17, q, sm	8
	Piumhi River, Capitólio, Minas Gerais State	108	58	20m+16sm+14st+8a	pair 24, p, st	pair 15, p, sm / pair 16, q, sm	8
	São Francisco River, Lagoa da Prata, Minas Gerais State	108	58	22m+16sm+12st+8a	pair 23, p, st	pair 16, p, sm / pair 17, q, sm	8
	Pium River, Rio Grande do Norte State	108	58	24m+16sm+10st+8a	p, sm	-	9
	Araguaia River, Goiás State	108	58	20m+18sm+12st+8a	pair 23, p, st	pair 3, p, m	10
Parauchenipterus striatulus	Doce River, Minas Gerais State	106	58	18m+20sm+10st+10a	pair 23, p, st	pair 10, p, sm / pair 13, p, sm pair 15, q, sm	10
Trachelyopterus coriaceus	Araguaia River, Goiás State	108	58	20m+18sm+12st+8a	pair 24, p, st	pair 3, p, m / pair 16, q, sm	10

– CEEAAP/Unioeste), to remove tissues for cytogenetic preparations. The collected specimens were deposited in the Museu de Zoologia da Universidade de São Paulo, São Paulo, under the voucher numbers: MZUSP 109798 for *P. striatulus*, MZUSP 109793 and 110803 for *P. galeatus*, and MZUSP 106766 for *T. coriaceus*.

Metaphasic mitotic chromosomal cell suspensions were prepared with anterior kidney cells (Bertollo *et al.*, 1978; Foresti *et al.*, 1993). The best metaphases were selected for karyotyping. Chromosomal morphology was determined according to Levan *et al.* (1964). The heterochromatin distribution pattern was determined according to Sumner (1972) with modifications in the staining process (Lui *et al.*, 2012). Nucleolus organizing regions (AgNORs) were identified using silver nitrate ($AgNO_3$) staining (Howell, Black, 1980). Both heterochromatin and NOR detection methods were carried out after Giemsa staining in order to follow a sequential analysis. Fluorescent *in situ* Hybridization (FISH) with 18S ribosomal (rDNA) and 5S rDNA probes was performed under stringency of 77% and followed the method by Pinkel *et al.* (1986),

with modifications suggested by Margarido, Moreira-Filho (2008). 5S rDNA and 18S rDNA probes were obtained by DNA from *Leporinus elongatus* Valenciennes, 1850 (Martins, Galetti, 1999) and *Prochilodus argenteus* Spix & Agassiz, 1829 (Hatanaka, Galetti, 2004), respectively. Probes were labeled by nick translation with digoxigenin-11-dUTP (18S rDNA for *P. striatulus*; 5S rDNA for *P. galeatus*, and *T. coriaceus*) and biotin-16-dUTP (5S rDNA for *P. striatulus*; 18S rDNA for *P. galeatus*, and *T. coriaceus*), in accordance with the manufacturer's instructions (Roche). Chromosomes were counterstained with DAPI (50 µg/mL). The slides were photographed through BX61 epifluorescence microscope (Olympus America Inc., Center Valley, PA, United States of America) with an attached Olympus DP71 digital camera and DP Controller 3.2.1.276 software.

RESULTS

***Parauchenipterus striatulus*: Doce River basin.** Diploid number (2n) was 58 chromosomes and the karyotype was composed of 18 metacentric, 20 submetacentric, 10 subtelocentric, and 10 acrocentric chromosomes (Fig. 1A). $AgNO_3$ staining demonstrated simple NORs allocated in terminal region on the short arm of the submetacentric pair 23 (Fig. 1A, in box). C-banding technique evidenced pale heterochromatin in terminal regions of almost all chromosome pairs (Fig. 1D). 18S rDNA-FISH confirmed previous findings by $AgNO_3$ staining and revealed these sequences only on pair 23. 5S rDNA-FISH detected these sequences in three submetacentric pairs, on the short arm of the pair 10 and 13, and on the long arm of the pair 15 (Fig. 1G).

***Parauchenipterus galeatus*: Araguaia River basin.** Diploid number was 58 chromosomes and karyotype was composed of 20 metacentric, 18 submetacentric, 12 subtelocentric, and 8 acrocentric (Fig. 1B). $AgNO_3$ staining revealed simple NORs allocated in the terminal region on the short arm of the submetacentric pair 24 (Fig. 1B, in box). C-banding evidenced pale heterochromatin in terminal regions of almost all chromosome pairs (Fig. 1E). 18S rDNA-FISH confirmed previous finding by $AgNO_3$ staining, simple NORs on pair 24. 5S rDNA-FISH detected these sites on short arm of the metacentric chromosome pair 3 (Fig. 1H).

***Trachelyopterus coriaceus*: Araguaia River basin.** Diploid number was 58 chromosomes and karyotype was composed of 20 metacentric, 18 submetacentric, 12 subtelocentric, and 8 acrocentric (Fig. 1C). $AgNO_3$ staining revealed simple NORs allocated in terminal region on the short arm of the submetacentric chromosome pair 23 (Fig. 1C, in box). C-banding evidenced pale heterochromatin in terminal regions on almost all karyotype pairs (Fig. 1F). 18S rDNA-FISH also revealed only the pair 23 with these sites, corresponding to what was evidenced by the $AgNO_3$. 5S rDNA-FISH probe detected these sites on the short arm of the metacentric pair 3 and on the long arm of the submetacentric pair 16 (Fig. 1I).

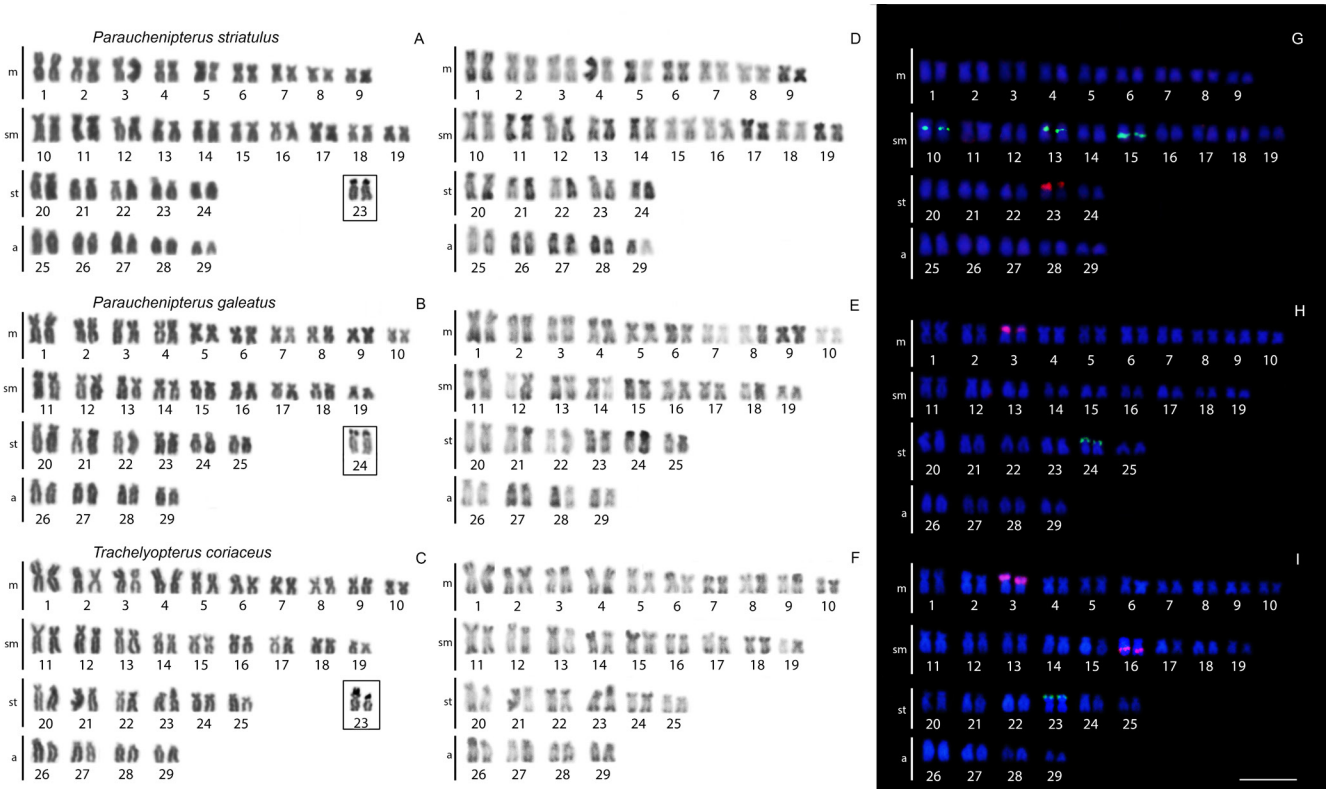

FIGURE 1 | Karyotypes of **A**. *Parauchenipterus striatulus*, **B**. *Parauchenipterus galeatus* and **C**. *Trachelyopterus coriaceus*. Giemsa stained karyotypes. The chromosome pairs marked with silver nitrate are in the boxes; **D**, **E**, **F**. C-banding sequentially karyotypes; **G**, **H**, **I**. Karyotypes hybridized with 5S rDNA and 18S rDNA probes. **G**. rDNA probe 18S (rhodamine, red signal), 5S rDNA probe (FITC, green signal). **H**, **I**. rDNA probe 18S (FITC, green signal), 5S rDNA probe (rhodamine, red signal).

DISCUSSION

The same diploid number of 58 chromosomes was found in all three species analyzed herein, which is considered basal for Auchenipteridae (Lui *et al*., 2013a, 2015), due to its prevalence within the species with available chromosome data (Tab. 1) and mainly because cytogenetic data in Doradidae (*e.g*., Eler *et al*., 2007; Milhomem *et al*., 2008; Baumgärtner *et al*., 2016; Takagui *et al*., 2017, 2019), sister-group of Auchenipteridae (Sullivan *et al*., 2006; Nelson *et al*., 2016). According Takagui *et al*. (2019), the most probable ancestor karyotype in Doradidae had 58 chromosomes. *Ageneiosus inermis* (Linnaeus, 1766) (Fenocchio, Bertollo, 1992; Lui *et al*., 2013b), *Tympanopleura atronasus* (Eigenmann & Eigenmann, 1888) (cited as *A. atronasus*) (Fenocchio, Bertollo, 1992) and *C. heckelii* (Kowalski *et al*., 2020) are exceptions, because they have lower diploid numbers. These reduced diploid numbers are probably result of fusion events (Lui *et al*., 2013b; Kowalski *et al*., 2020), which were confirmed by the presence of ITS (Interstitial Telomeric Sequence) in a metacentric chromosome pair detected in *A. inermis* (Lui *et al*., 2013b).

Small karyotype formula differences are found among the species analyzed in the present study. *Parauchenipterus striatulus* has higher amount of acrocentric (10 chromosomes) than *P. galeatus* and *T. coriaceus* (8 chromosomes). The presence of eight

acrocentric chromosomes has already been reported in other three populations of *P. galeatus* (Lui *et al*., 2010), and their karyotype formulas are also very similar to the population from this study. Therefore, it shows irrelevant karyotype variation between these taxa, and these karyotype differences might be related to non-Robertsonian events, which do not imply diploid number changes. Nonetheless, the population of *P. galeatus* from Porto Rico in the Paraná River had 18 acrocentric chromosomes, and this difference is probably related to methodological problems, such as the chromosomal condensation pattern, which difficult the classification of chromosomes by different researchers, as already proposed for other fish groups (*e.g*., Carvalho, Dias 2005; Moraes-Neto *et al*., 2011). The two sympatric species (*P. galeatus* and *T. coriaceus*) had the same karyotype formula that indicates large chromosomal similarity between these two species. Moreover, researchers suggest that these species belong to different genera, supported by morphological data, as initially considered in this paper by Akama (2004); however, these chromosomal data reinforce the phylogenetic closeness between these two nominal genera.

Heterochromatin distribution was similar among the three analyzed species. Pale heterochromatin was found in terminal region of almost all chromosomes, as observed in other *P. galeatus* populations (Ravedutti, Júlio, 2001; Lui *et al*., 2009, 2010). This pattern is typically found in other Auchenipteridae species, such as *Ageneiosus inermis* (cited as *A. brevifilis*), *Tympanopleura atronasus* (cited as *A. atronasus*), *Auchenipterus osteomystax* (Miranda-Ribeiro, 1918) (cited as *A. nuchalis*), and *Glanidium ribeiroi* Haseman, 1911 (Fenocchio, Bertollo, 1992; Ravedutti, Júlio, 2001; Fenocchio *et al*., 2008; Lui *et al*., 2013b, 2015), suggesting to be a common family trait. On the other hand, small differences are found in some of these species: heterochromatin in terminal regions is strongly reported for *A. inermis* (Lui *et al*., 2013b); heterochromatin in centromeric region of chromosomes is observed for *Tatia jaracatia* Pavanelli & Bifi, 2009, and a conspicuous block in the interstitial region of a submetacentric pair is reported for *T. neivai* (Lui *et al*., 2013a).

$AgNO_3$ impregnation and FISH with 18S rDNA probe evidenced simple NORs in the terminal position on a subtelocentric pair 23 in *P. galeatus* and *P. striatulus*, and on a subtelocentric pair 24 in *T. coriaceus*. According to Ravedutti, Júlio (2001), simple NORs may be a common characteristic of Auchenipteridae, which has been confirmed by recent studies on this family (Tab. 1). Even though only one chromosomes pair bearing NORs is found in almost all auchenipterids, the location varies (terminal and interstitial), due to pericentric inversions, as aforementioned to explain the differences between karyotypic formulas. The three species in this study also had 18S rDNA present in subtelocentric chromosomes, as in most *P. galeatus* populations studied (Lui *et al*., 2009, 2010), however, there are populations with this marker located either in a pair of acrocentric (Ravedutti, Júlio, 2001) or submetacentric chromosomes (Araújo, Molina, 2013). As mentioned above, these differences might be associated with different chromatin condensation levels, which hinder their classification by researchers. Moreover, by analyzing the karyotypes of these last two papers, the pair bearing the NORs could have been classified as subtelocentric.

Despite the conservatism in the number of 18S rDNA sites for this family and small variation in the bearing pair, 5S rDNA has been found to be the chromosomal marker with the highest variability among Auchenipteridae species (Tab. 1). 5S rDNA has

shown to be more dynamic, also evident in the data presented in this study. These cistrons were found in only one pair of chromosomes in *G. ribeiroi* and *A. inermis* (Lui *et al*., 2013b, 2015), but multiple 5S rDNA is more prevalent in the species of the genus *Tatia*: four pairs in *T. jaracatia* (metacentric pair 4, submetacentric pairs 18 and 19, and subtelocentric pair 29) and three pairs in *T. neivai* (metacentric pair 4 and submetacentric pairs 21 and 22) (Lui *et al*., 2013a). 5S rDNA submetacentric pairs reported for *Tatia* and *Parauchenipterus* species can be considered corresponding to the submetacentric pairs found in the species of this study, as they have similar measures, morphology, and position. Physical mapping of rDNA is considered a promising implement for evolutionary and taxonomic analyzes in fishes (Moraes-Neto *et al*., 2011), and the three species analyzed in this study indicate variation in the number of 5S rDNA bearing chromosome pairs. Therefore, this marker seems to be remarkable to understand chromosomal evolution of Auchenipteridae.

Regarding 5S rDNA data in *Parauchenipterus* and *Trachelyopterus*, FISH made it possible to identify differences among the three species of this paper. There are similar results found in other populations of *P. galeatus*, from different watersheds and all of these populations have two 5S rDNA submetacentric pairs (Lui *et al*., 2010). *Trachelyopterus coriaceus* also has two chromosome pairs carrying 5S rDNA sites, one metacentric and one submetacentric. Even though the morphology of these chromosomes is different from the two pairs of *Parauchenipterus*, these chromosomes can be considered corresponding. Similarly, *P. striatulus* also has two chromosomes (one with these clusters on short arm and the other one on the long arm) that might be corresponding to the same pairs. Moreover, the extra third 5S rDNA carrier submetacentric pair of *P. striatulus*, can be considered an autapomorphy of this species. It confirms that 5S rDNA is a good marker for the group, because these data differentiate *P. striatulus* from other phylogenetically close species. It is important to highlight that this species has distribution in coastal basins of the South American continent, different from *P. galeatus* and *T. coriaceus*, which are present in basins in the interior of the continent, thus there is no overlap in distribution and it suggests ancient diversification of *P. striatulus* from the other *Trachelyopterus* and explains the exclusive third 5S rDNA chromosome pair of this species.

Comparison of 5S rDNA distribution data among all populations of *P. galeatus* previously studied, *T. coriaceus* and *P. striatulus* shows greater similarity between *P. galeatus* from Araguaia River and *T. coriaceus* than with other *P. galeatus* populations. At a minimum, these data reinforce the phylogenetic proximity between these two nominal genera, and may even give rise to an interpretation contrary to *Parauchenipterus* validation. Thus, the differences in *P. galeatus* 5S rDNA data from Araguaia River basin, regarding the variation in number of sites from all other populations in this group, and the greater similarity with the sympatric population of *T. coriaceus* make it possible to suggest the existence of a new taxonomic unity. According to Birindelli *et al*. (2012), *Trachelyopterus* can be diagnosed by its gas bladder morphology among other characters. *Trachelyopterus galeatus* specimens analyzed showed differences in the gas bladder morphology (Birindelli *et al*., 2012), which could be interpreted as an indicative of cryptic diversity. Our data also suggest a population as a potentially new taxon in this fish group.

Considering the proposal of Akama (2004), the species belonging to *Trachelyopterus* analyzed in this study are the first report of cytogenetic data for this genus, and also the first record for a *P. striatulus* population. Comparing the data of *Parauchenipterus* and

Trachelyopterus described here with those already found in the literature, it is possible to note the great similarity concerning chromosomal markers (diploid number, heterochromatin distribution, number and location of nucleolus organizing regions) and differences in 5S rDNA number and location. This way, our data reaffirm that *Parauchenipterus* should be considered synonymous of *Trachelyopterus*, contrary to what was suggested by last diagnosis reviewed of *Parauchenipterus* and closely related species (Akama, 2004), but in agreement with some other more recent studies in Auchenipteridae that were not focused on this issue (Ferraris, 2007; Birindelli, 2014; Calegari *et al*., 2019). It is noteworthy that maintaining *Parauchenipterus* as a junior synonym of *Trachelyopterus* is based on the greatest similarity between the sympatric species *P. galeatus* and *T. coriaceus*, compared to all others Auchenipteridae previously studied, mainly *Trachelyopterus* species, through the following chromosomal data: equal karyotype formula and metacentric pair 3 with marking on the short arm bearing rDNA 5S (for more detail, see Tab. 1). The 5S rDNA is an excellent marker to understand chromosomal evolution of Auchenipteridae and helped to suggest the existence of a new taxonomic unit distributed in the Araguaia River basin, which reaffirms the status of species complex in *P. galeatus*.

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ETHICAL STATEMENT

Permanent License SISBIO 10538-1. The specimens were kept in aquaria and subsequently euthanized by clove oil overdose (Griffiths, 2000; Pereira-Da-Silva *et al*., 2009) (according to the Animal Experimentation Ethics Committee and Unioeste practical classes: 13/09 – CEEAAP/Unioeste).

COMPETING INTERESTS

The authors declare no competing interests.

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