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Cytogenetic analysis in *Tetragonopterus franciscoensis* (Characiformes): another piece to the karyoevolutionary puzzle of tetra fishes

Mauricio Barros Fernandes, Jamille de Araújo Bitencourt, Joandson Calixto dos Santos, José Henrique Galdino*, Paulo Roberto Antunes de Mello Affonso

Department of Biological Sciences, Universidade Estadual do Sudoeste da Bahia (UESB), Jequié - BA, Brazil
*Corresponding author. E-mail: galdino.jhsgb@gmail.com

Abstract. *Tetragonopterus* is a taxonomically complex genus in Characidae, being currently represented by nine species according to integrative approaches. One of them, *T. franciscoensis* was recently validated in rivers from northeastern Brazil. Even though molecular and morphological data have been collected in *Tetragonopterus*, the cytogenetic analyses in this group are scarce despite of the role of chromosomal variation in speciation. Herein, we present the first detailed karyotypic study in *T. franciscoensis* along with a comparative analysis with published cytogenetic data in characin fish. All specimens shared 2n=52 distributed in 12 metacentric (m), 12 submetacentric (sm), and 28 subtelocentric/acrocentric (st/a) chromosomes for both sexes as well as single nucleolus organizer regions on short arms of pair 8 and several GC-rich sites. The mapping of telomeric sequences (TTAGGG)n revealed no telomeric interstitial signals. While subtle cytogenetic differences were observed between samples from northeastern basins in Brazil, corroborating a recent genetic divergence, distinct karyotypes were detected in relation to congeneric taxa from other Brazilian regions. Therefore, the origin of large biarmed pairs in species with low 2n values should be related to occurrence of centric fusions.

Keywords: Characidae, Characins, cytotaxonomy, neotropical fish, Tetragonopterinae.

INTRODUCTION

The genus *Tetragonopterus* (Characidae) was proposed by Cuvier (1816) to describe the species *T. argenteus* based on a unique specimen from South America. In the second half of the 19th century, Günther (1864) added 32 new species to this taxon and proposed the subfamily Tetragonopterinae which would include most of small characins or tetras (e.g., *Astyanax, Hemigrammus, Moenkhausia, Psalidodon*).

Over the following decades, the group was extensively revised and it turned to be one of the most intriguing taxa among Characidae. In a series of studies carried out by Carl H. Eigenmann, several species previously allocated in *Tetragonopterus* were reassigned to different genera, like *Bryconamericus, Ctenobrycon,* and *Deuterodon* (Eigenmann, 1917; Eigenmann, 1918; Eigenmann, 1921; Eigenmann and Myers, 1929). Later, the number of species in *Tetragonopterus* was reduced to four evolutionary units, comprising *T. argenteus, T. chalceus. T. gibossus*, and *T. huberi*. On that occasion, the reassignment of *T. georgiae* and *T. rarus* to *Moenkhausia*, for example, was justified by the lack of a complete lateral line greatly bent downwards at the anterior portion, a common feature of *Tetragonopterus*. Follow-up taxonomic reviews reallocated *T. argenteus* and *T. chalceus* as the only representatives of this genus (Reis *et al.,* 2003). However, this scenario has changed considerably, as DNA-based studies provided important insights about the taxonomic relationships of *Tetragonopterus* and other tetras (Araújo and Lucinda, 2014; Mirande, 2019).

Accordingly, molecular analysis recognized eight previously described species in *Tetragonopterus* (*T. anostomus, T. araguaiensis, T. argenteus, T. carvalhoi, T. chalceus, T. denticulatos. T. georgiae*, and *T. rarus)* and cases of cryptic diversity (Silva *et al.,* 2016). These authors revealed that the populations of *T. chalceus* from São Francisco, Paraguaçu, and Itapicuru river basins actually encompassed a distinct species, referred to as *T. fransciscoensis* (Silva *et al*., 2016). In addition, three new species were also described (*T. jurema, T. kulene*, and *T. ommotus*) and new evidence reallocated *Moenkhausia georgiae* back to *Tetragonopterus* (*T. georgiae*), as also supported by other authors (Silva *et al.,* 2016; Melo *et al.,* 2016; Terán *et al*., 2020).

Even though the abovementioned studies were particularly informative to resolve the taxonomic uncertainties in *Tetragonopterus*, cytotaxonomic analyses that could add new pieces of evidence to this subject remain limited to a few reports based on conventional analyses in *T. argenteus* Cuvier, 1816 and *T. chalceus* Spix & Agassiz, 1829. Both species shared a modal diploid number of 2n = 52, a single NOR system and few heterochromatin regions, but they diverge in their karyotype formulae (Portela *et al.,* 1988; Alberdi and Fenocchio, 1997). Interestingly, populations of *T. argenteus* from Cuiabá River were differentiated by the presence of two cytotypes (1 and 2). While the cytotype 1 is represented by specimens with 2n=50 and a karyotype of 14m+4sm+4st+28a, the cytotype 2 presents 2n=52 distributed into 14m+4sm+4st+30a chromosomes (Miyazawa, 2015).

A striking cytogenetic feature commonly reported in small characins is the presence of a large first metacentric pair when compared to other chromosomes in the karyotype (Scheel, 1973). In fact, this metacentric pair and a modal number of 2n=50 have been regarded as plesiomorphies for this fish group (Morelli *et al*., 1983; Portela-Castro *et al*.,1998; Tenório *et al*., 2013), being also observed in Bryconidae (Almeida-Toledo *et al.,* 1996; Mariguela *et al.,* 2010; Yano *et al.,* 2021).

In turn, the highly conserved morphology of small characins, including *Tetragonopterus* (Eigenmann, 1917), indicates that species complexes or cryptic species might be present, thus hindering reliable estimates of richness and endemicity rates in these Neotropical fishes. In this context, cytogenetic methods can help reveal such overlooked diversity, as exemplified by studies in the genus *Psalidodon* (e.g. Bertaco *et al.,* 2006; Ferreira-Neto *et al.,* 2012). Therefore, the goal of the present study was to report the first detailed cytogenetic characterization of *T. franscicoensis* from an isolated drainage from Northeastern Brazil to shed some light on the taxonomy and species delimitation in *Tetragonopterus*. In addition, we carried out a comprehensive comparative cytogenetic analysis in characin species to provide insights about the karyoevolutionary trends in the subfamily Tetragonopterinae.

MATERIAL AND METHODS

Thirteen individuals of *T. franciscoensis* Silva, Melo, de Oliveira & Benine, 2016 (8 males and 5 females) were collected at the Itapicuru-Mirim River (Itapicuru River Basin) in the municipality of Tucano, state of Bahia, northeastern Brazil (11°12'15.3"S/40°29'15.1"W) (Fig. 1). The collection license was granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBIO n. 26752-2). The procedures and experiments were approved by the Committee of Ethics in Experimentation with Animals from the State University of Southwestern Bahia (CEUA/UESB 32/2013).

To stimulate cell division, the fish specimens were inoculated with fungal antigens and kept in tanks for 48 to 72 hours (Lee and Elder, 1980). Afterwards, the specimens were euthanized in cold water (Blessing *et al*., 2010), and the anterior kidney was removed to obtain metaphase cells, according to Netto *et al*., (2007). The cell suspension containing the mitotic chromosomes were dropped on glass slides, air dried and stained with 10% Giemsa in phosphate buffer (pH 6.8).

The heterochromatin was visualized by C-banding technique (Sumner, 1972), and active nucleolar organizer regions (Ag-NOR) were detected by silver staining (Howell and Black, 1980). Sequential staining with the base-specific fluorochromes Chromomycin A_3 (CMA_3) and 4'-6-diamino-2-fenilindole (DAPI) to detect GC-

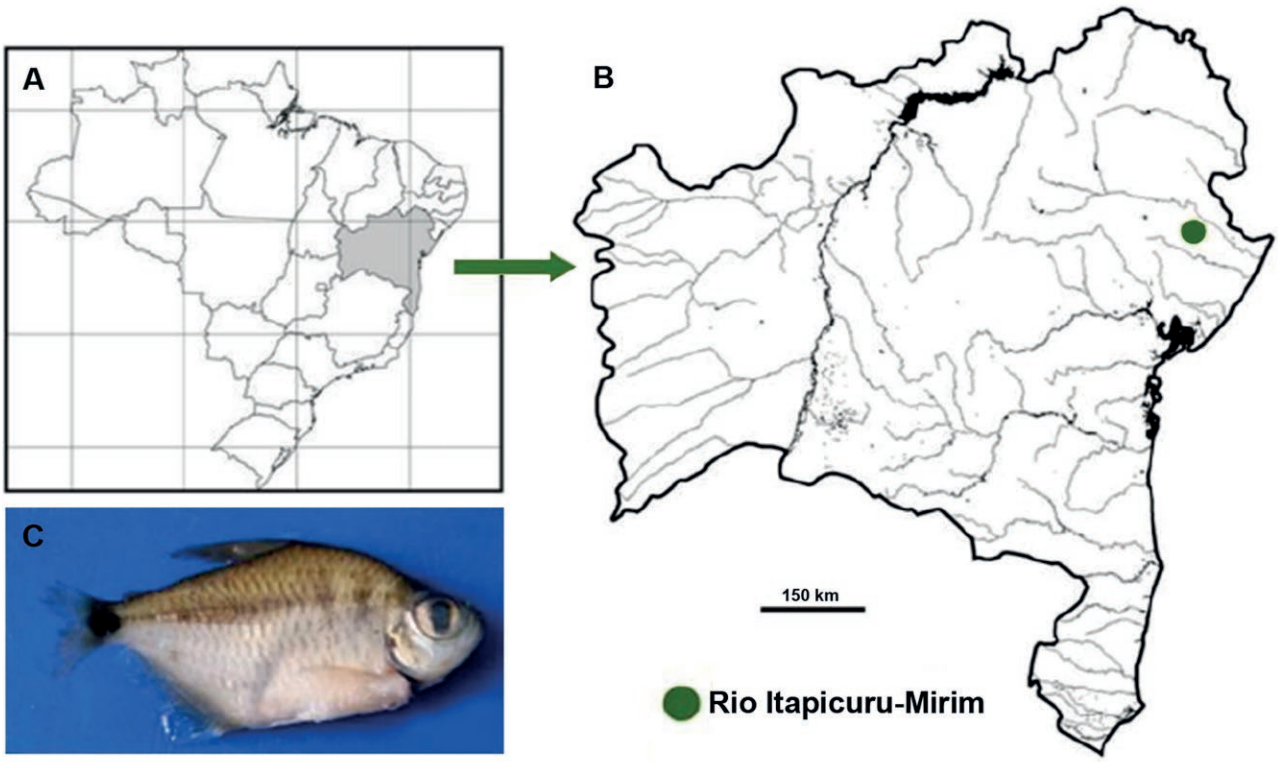

Figure 1. Map of Brazil highlighting the state of Bahia (a) and the collection site in Itapicuru-Mirim River (b) of *T. franciscoensis* (c).

and AT-rich regions, respectively, were carried out according to Schmid (1980).

The physical mapping of telomers was performed based on fluorescence *in situ* hybridization (FISH) according to Pinkel *et al.* (1986) under high stringency (77%) conditions to evaluate the putative presence of internal telomere sequences (ITS) that could reveal structural rearrangements. The telomere (TTAGGG) n probes were obtained via PCR without template DNA (Ijdo et al., 1991). The probes were labeled with digoxigenin-11-dUTP and detected with anti-digoxigenin-Rhodamine conjugate, according to the manufacturer's instructions (Roche). The chromosomes were counterstained with DAPI and the slides were mounted in a Vectashield medium.

A mean number of 10 metaphase spreads per specimen were analyzed using an epifluorescence microscope (Olympus BX-51) attached to a digital camera and equipped with the software Image-Pro Plus® v. 6.2 for photo documentation. The chromosomes were measured using the software Easy Idio 1.0 (Diniz and Melo, 2006). Then, they were classified according to their arm ratio (Levan *et al.* 1964), and the chromosomal pairs were systematically organized into karyotypes in decreasing size order within each morphological category.

RESULTS

A modal diploid number of 2n = 52 was observed in all specimens of *T. franciscoensis*, while the karyotype was invariably organized into 12 metacentric (m), 12 submetacentric (sm), and 28 subtelocentric/acrocentric (st/a) chromosomes (Figure 2a). No heteromorphic sex chromosomes were detected.

The silver staining revealed a single NOR-bearing pair (8) with heteromorphic ribosomal cistrons at interstitial regions on short arms. On the other hand, the C-banding revealed few heterochromatin blocks restricted to centromeres (Figure 2b). The GC-rich sites (CMA_3^+/$DAPI^-$) were coincident with Ag-NORs on pair 8 (Figure 3). Furthermore, additional CMA_3 signals were observed in, at least, three other chromosomal pairs (Figure 3). The mapping of (TTAGGG)n sequences by FISH revealed conspicuous signals on telomeres of all chromosomes and no internal telomere sequences (ITS) (Figure 4).

DISCUSSION

The karyotype macrostructure of *T. franciscoensis* (2n=52 and a karyotype formula of 12m+12sm+28st/a)

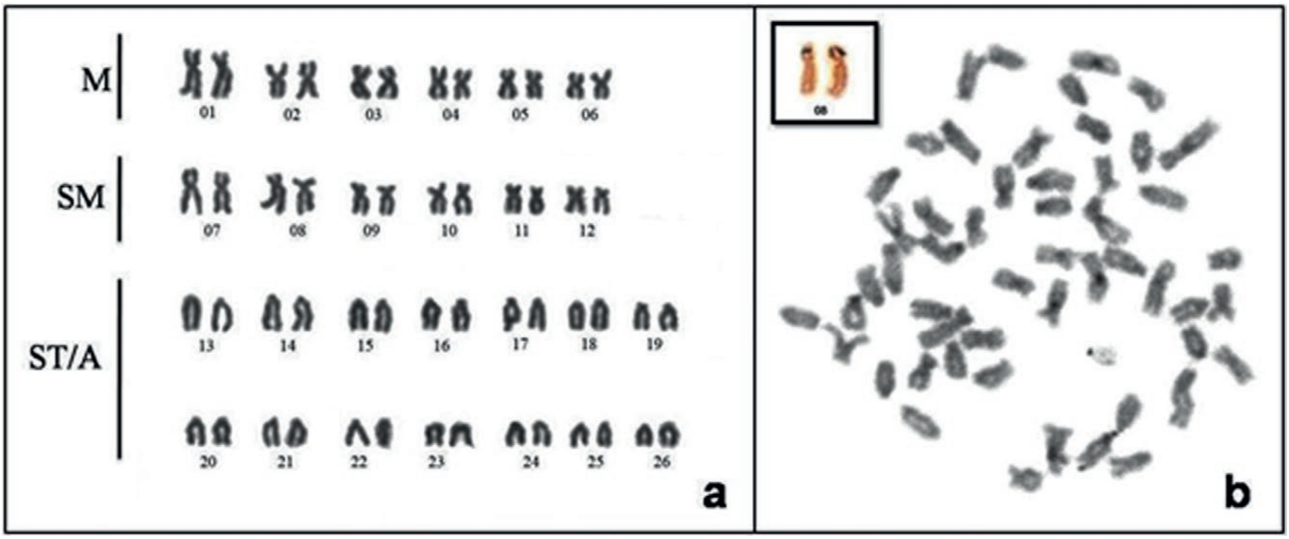

Figure 2. Giemsa-stained (a) and C-banded (b) karyotypes of *T. franciscoensis*. The NOR-bearing pair after silver nitrate in shown inbox.

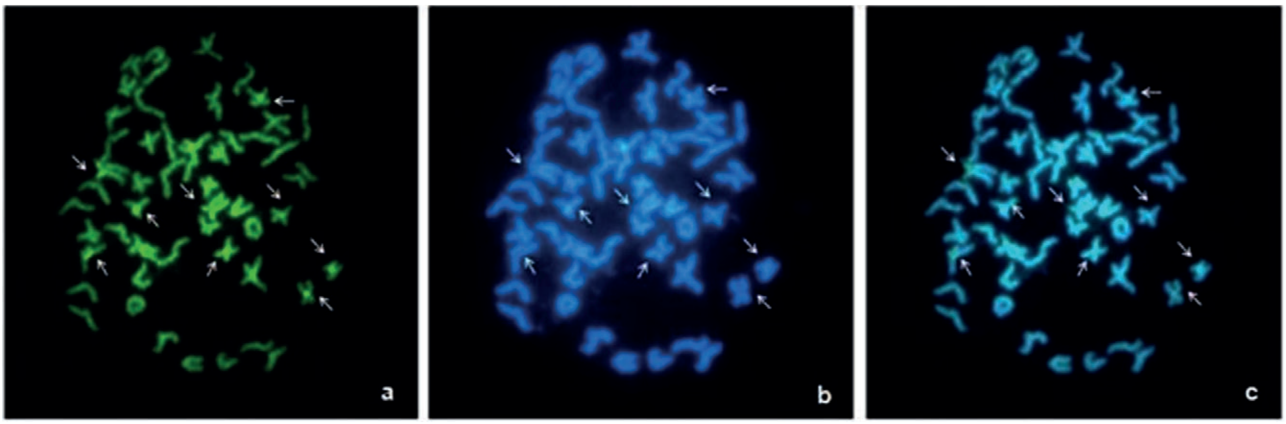

Figure 3. Somatic metaphases after CMA_3 (a); DAPI (b); and overlapped CMA_3/DAPI (c) staining, showing the GC-rich (CMA_3^+/$DAPI^-$ signals) indicated by arrows.

is similar to that reported in populations of *T. chalceus* (=*T. franscicoensis* sensu Silva *et al.,* 2016) from São Francisco River (26m/sm+26st/a) (Portela *et al*., 1988). The only difference refers to the presence of an additional subtelocentric/acrocentric pair in specimens from Itapicuru-Mirim (present study). This result suggests a genetic divergence among these lineages from each hydrographic system driven by pericentric inversions in a chromosome pair. Nevertheless, artifactual effects could also account for these such as distinct levels of chromosome condensation or the criteria for determining the chromosomal morphology between authors.

On the other hand, remarkable macrostructural differences are observed when the cytogenetic data in *T. franciscoensis* from the present study are compared to those reported in closely related species, such as *T. argenteus* from Paraná (16m/sm+2st+34a), Paraguay (14m+4sm+4st+28a), and (De La Plata) river basins (Alberdi and Fenocchio, 1997; Miyazawa, 2015). In the latter, the specimens presented interindividual variation in both number and morphology of chromosomes (2n-50, 14m+4sm+4st+28a and 2n=52, 16m+4sm+4st+28a) (Miyazawa, 2015; Supplementary Table 1). These findings indicate that inversions and fusions/fissions are involved in the karyoevolution of *Tetragonopterus* and that cryptic species are likely to be present in this group, as commonly observed in small characins (Medrado *et al*., 2018).

Furthermore, *T. franciscoensis* lacks the typical large metacentric pair described in other tetras, a condition putatively associated with the presence of 2n=52

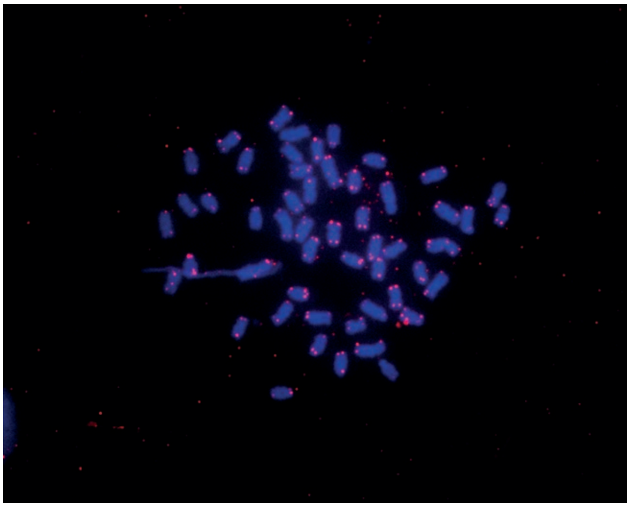

Figure 4. Metaphase of *T. franciscoensis* after FISH with (TTAGGG) n probes, revealing the positive signals (in magenta) on telomeres.

(Figure 2a), thus diverging from the pattern observed in several genera of Characidae like *Astyanax, Psalidodon*, *Moenkhausia*, and *Cheirodon* (Tenório *et al.*, 2013; Soto *et al.,* 2018; Nascimento *et al.,* 2020). Such difference reinforces the divergence of *T. franciscoensis* and congeners in relation to other small characin lineages, corroborating their allocation in a distinct subfamily (Tetragonopterinae) (Mirande, 2018; Terán *et al.*, 2020).

In fact, a distinctive first large metacentric pair is also found in representatives from other closely related and basal families of Characiformes (Supplementary Table 1), such as Bryconidae (Almeida-Toledo *et al.,* 1996, Mariguela *et al.,* 2010; Silva *et al.,* 2012), indicating that this is a plesiomorphic condition. Moroever, this condition (presence or absence of large metacentric pairs) varies remarkably among distinct taxonomic units in Characidae. Such variation has been reported even within some genera such as *Astyanax, Psalidodon*, and *Hyphessobrycon*, and within species, like *Bryconamericus* aff. *exodon* and *Bryconamericus* aff. *iheringii*, indicating putative species complexes or cryptic diversity (Supplementary Table 1).

On the other hand, the absence of a long metacentric pair appears to be ubiquitous in *Odontostilbe, Piabina, Serrapinnus,* and *Knodus* (Supplementary Table 1). Moreover, according to the present revision, the lack of this large metacentric pair is correlated with species characterized by 2n=52 (Supplementary Table 1). Therefore, it is reasonable to hypothesize that independent chromosomal fusion events could account for the very large size of the first pair of biarmed chromosomes and the reduction of diploid numbers (2n < 50) in characins. However, these findings are insufficient to fully understand the karyoevolutionary trends in Characidae because several genera and species in this family remain poorly studied in relation to their cytogenetic traits. Therefore, further basic chromosomal studies should be carried out to test the role of centric fusions in the karyoevolution of small characins and the utility of the largest metacentric pair as a cytotaxonomic marker in tetras.

Similarly, the number and distribution of NORs in *T. franciscoensis* (Figure 2b) resembles that of *T. chalceus* (Portela *et al.*, 1988) and *T. argenteus* (Miyazawa, 2015), following a common trend among characins (Medrado *et al.*, 2008). In addition, the presence of GC-rich (CMA_3^+) sites co-located with NORs are considered a basal trait for fish and amphibianli (Schmid, 1980; Tenório *et al.*, 2013; Monteiro *et al.*, 2022). On the other hand, the presence of additional GC-rich sites at centromeric regions (Figure 3) represent a unique and putatively apomorphic condition since AT-rich sites near centromeres are more frequently reported in small characins (Sánchez *et al.*, 2021), thus indicating a heterogenous composition of satellite DNAs. These results show the importance of detailed chromosomal analyses to infer the dynamics of genome organization and the role of microarrangements in speciation of tetra fishes.

The mapping of telomeric sequences on chromosomes of *T. franciscoensis* (Figure 4) followed the expected pattern in vertebrates, revealing positive signals at terminal portions of chromosomal pairs (Meyne *et al.*, 1989; Ferro *et al.,* 2003; Schmid *et al.*, 2006) and no evidence of ITS. Nonetheless, this pattern should not reject the occurrence of chromosomal rearrangements in the analyzed species. Actually, ITS are often lost or degenerated in rearranged chromosomes, particularly when the chromosomal changes have occurred in early stages of differentiation among clades (Meyne *et al.,* 1990; Bolzan, 2017).

In general, the present study revealed subtle cytogenetic differences in *Tetragonopterus* from São Francisco and Itapicuru River basins in northeastern Brazil, contrasting with the distinct karyotypes of congeneric species from other Brazilian regions (e.g., *T. argenteus*). These findings provide additional support to the validation of these populations as *T. franciscoensis* as proposed by morphological data (Silva *et al.,* 2016). At last, the lack of the typical large metacentric pair and the predominance of 2n=52 in *Tetragonopterus* when compared to other small characins reinforced the status of Tetragonopterinae as a monophyletic subfamily. In addition, cytotaxonomic markers were reported for *T. franciscoensis* that can be properly used to resolve taxonomic uncertainties in Neotropical tetras.

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Table 1. Cytogenetic data of small characins and closely related groups, according to Mirande (2018) and Terán *et al.* (2020) (the asterisks indicate the taxa whose scientific names were updated).

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Family Characidae					
Subfamily Aphyocharacinae					
Prionobrama filigera	52	12m/sm+ 40st/a	Not Informed	Present	Arefjev (1990)
Subfamily Characinae					
Phenacogaster cf. pectinatus	46	12m+2st+32a	São Francisco stream - AC	Present	Carvalho *et al.* (2002)
Subfamily Cheirondotinae					
Cheirodon australe	52	8m+6sm+4st+33a	La Poza Lake - Chile	Present	Soto *et al.* (2018)
Cheirodon galusdae	52	6m+6sm+4st+34a	Andalién River - Chile	Present	Soto *et al.* (2018)
Cheirodon interruptus	52	6m+6sm+4st+34a	Marga-Marga River - Chile	Present	Soto *et al.* (2018)
Cheirodon kiliani	52	8m+6sm+4st+33a	Calle-Calle River - Chile	Present	Soto *et al.* (2018)
Cheirodon pisciculus	52	6m+6sm+4st+34a	Angostura River - Chile	Present	Soto *et al.* (2018)
Odontostilbe pequira	52	14m+20sm+14st+4a	Onça stream - MS	**Absent**	Nishiyama *et al.* (2015)
Odontostilbe pequira	52	24m+12sm+12st+4a	Cuiabá River - MT	**Absent**	Troy *et al.* (2010)
Serrapinnus calliurus	52	36m+12sm+6st	Bento Gomes River - MT	**Absent**	Troy *et al.* (2010)
Serrapinnus heterodon	52	16m+20sm+14st+2a	São Francisco River - MG	**Absent**	Peres *et al.* (2007)
Serrapinnus kriegi	52	24m+18sm+10st	Cuiabá River - MT	**Absent**	Troy *et al.* (2010)
Serrapinnus microdon	52	30m+12sm+8st+4a	Bento Gomes River - MT	**Absent**	Troy *et al.* (2010)
Serrapinnus piaba	52	16m+20sm +14st+2a	São Francisco River- MG	**Absent**	Peres *et al.* (2007)
Subfamily Stethaprioninae					
Astyanax abramis	50	4m+30sm+8st+8a	Iguaçu River - PR	Present	Gavazzoni *et al.* (2018)
Astyanax altiparanae	50	6m+28sm+4st+12a	Pântano Stream and Jordão River - PR, Feijão Stream - MG	Present	Ferreira-Neto *et al.* (2009)
Astyanax altiparanae	50	6m+28sm+8st+8a	Tibagi River - PR	Present	Domingues *et al.* (2007)
Astyanax altiparanae	50	6m+30sm+8st+6s	Iguaçu River - PR	Present	Domingues *et al.* (2007
Astyanax altiparanae	50	16m+24sm+4st+6a	Queixada River - PR	Present	Da Silva *et al.* (2016)
Astyanax altiparanae	50	16m+20sm+4st+10a	Esperança stream - PR	Present	Da Silva *et al.* (2016)
Astyanax altiparanae	50	16m+20sm+4st+10a	Jacutinga River - PR	Present	Da Silva *et al.* (2016)
Astyanax altiparanae	50	6m+28sm+4st+12a	Paraná River - PR	Present	Gavazzoni *et al.* (2018)
Astyanax asuncionensi	50	18m+22sm+6st+4a	Miranda River - MS	Present	Da Silva *et al.* (2016)
Astyanax asuncionensis	50	8m+24sm+6st	Iguaçu River - PR	Present	Gavazzoni *et al.* (2018)
Astyanax aff. *bimaculatus*	50	4m+14sm+24st+8a	Dois de Agosto stream - PA	Present	Sousa *et al.* (2023)
Astyanax bimaculatus	50	8m+34sm+2st+6a	São Francisco River - PR	Present	Peres *et al.* (2012)
Astyanax bimaculatus	50	8m+32sm+2st+8a	Grande River - PR	Present	Peres *et al.* (2012)
Astyanax bimaculatus	50	8m+33sm+2st+7a	Piumhi River - PR	Present	Peres *et al.* (2012)
Astyanax bimaculatus	50	8m+31sm+2st+9a	Piumhi River - PR	Present	Peres *et al.* (2012)
Astyanax bimaculatus	50	8m+30sm+2st+10a	Piumhi River - PR	Present	Peres *et al.* (2012)
Astyanax bimaculatus	50	10m+18sm+12 st+10a	Aguapeí River - SP	Present	Alberdi and Fenocchio (1997)
Astyanax bimaculatus	50	6m+28sm+8st+8a	Caeté River - PA	Present	Sousa *et al.* (2023)
Astyanax lacustris	50	10m+24sm+6st+10a	Itaipu Lake, Paraná River basin - PR	Present	Tonello *et al.* (2022)

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Astyanax lacustris	50	6m+12sm+14st+18a	Pirassununga River - SP	Present	Goes *et al.* (2020)
Astyanax jacuhiensis	50	10m+26sm+6st+8a	Tramandaí River basin - RS	Present	Da Silva *et al.* (2012)
Astyanax jacuhiensis	50	8m+30sm+4st+8a	Guaíba Lake - RS	Present	Pacheco *et al.* (2010)
Astyanax jacuhiensis	50	8m+28sm+6st+8a	Ijuá River - PR	Present	Gavazzoni *et al.* (2018)
Astyanax scabripinnis	50	8m+20s+8st+14a	Macacos River - PR	Present	Kavalco and Moreira-Filho (2003)
Astyanax scabripinnis	50	6m+22sm+10st+12a	Córrego das Pedras stream - SP	Present	Mestriner *et al.* (2000)
Astyanax scabripinnis	50	8m+22sm+12st+6a	Mogi-Guaçu River basin - SP	Present	Pazza *et al.* (2008)
Astyanax scabripinnis	50	12m+20sm+10st+4a	Paranapanema River basin - SP	Present	Pazza *et al.* (2008)
Astyanax scabripinnis	50	6m+22sm+10st+12a	Córrego das Pedras stream - SP	Present	Salvador and Moreira-Filho (1992)
Astyanax scabripinnis	50	10m+20sm+8st+12a	São Francisco River - PR	Present	Klassmann and Martins-Santos (2017)
Astyanax scabripinnis	48	11m+18sm+9st+10a	São Francisco River - PR	Present	Klassmann and Martins-Santos (2017)
Astyanax scabripinnis	48	10m+20sm+8st+10a	Ivaí River - PR	Present	Alves and Martins-Santos (2002)
Astyanax sp.	50	4m+22sm+8st+16a	Piraquara, Upper Paraná River basin - PR	Present	Kantek *et al.* (2008)
Astyanax sp.	50	4m+22sm+8st+16a	Bicudo River, Upper Paraná River basin - PR	Present	Kantek *et al.* (2008)
Astyanax sp.	50	4m+24sm+6st+16a	Bicudo River, Upper Paraná River basin - PR	Present	Kantek *et al.* (2008)
Astyanax sp.	52	22m+26sm+4a	Upper Paraná River basin - PR	**Absent**	Tenório *et al.* (2013)
Brachychalcinus retrospina	50	6m+24sm+6st+4a	Angelim River - MT	Present	Krinski and Miyazawa (2012)
Ctenobrycon hauxwellianus	50	10m+6sm+34st	São Francisco stream - AC	Present	Carvalho *et al.* (2002)
Deuterodon (*Astyanax*) *giton**	50	6m+8sm+8st+28a	Paraitinga River - SP	Present	Kavalco and Moreira-Filho (2003)
Deuterodon (*Astyanax*) *intermedius**	50	6m+18sm+12st+10a	Paraitinga River - SP	Present	Kavalco and Moreira-Filho (2003)
Deuterodon (*Astyanax*) *janeiroensis**	50	6m+14sm+14st+16a	Betari River - SP	Present	Carvalho *et al.* (2002)
Deuterodon pedri	50	12m+12sm+20st+6a	Santo Antônio - River	Present	Coutinho-Sanches and Dergam (2015)
Deuterodon pedri	50	14m/sm+36st/a	Pedri River - SP	Present	Portela *et al.* (1988)
Deuterodon stigmaturus	50	8m+6sm+2st+34a	Maquiné River - RS	Present	Mendes *et al.* (2011)
Gymnocorhimbus ternetzi	50	14m+12sm+6st	Paraná River - PR	Present	Alberdi and Fenocchio (1997)
Hasemania crenuchoides	50	6m+26sm+16st+2a	Alto-Tocantins River	Present	Duarte *et al.* (2018)
Hasemania marginata	50	12m+18sm +10st+10a.	Not Informed	Present	Arefjev (1990)
Hasemania nana	50	8m+42sm	São Francisco River basin - MG	Present	Moreira *et al.* (2007)
Hemigrammus hyanuary	52	22m/sm+30st/a.	Not Informed	Present	Arefjev (1990)
Hemigrammus marginatus	50	10m+34sm+6a	Upper Paraná River basin -PR	Present	Portela-Castro and Júlio-Jr (2002)
Hollandichthys multifasciatus	50	8m+10sm+32st	Iguapé River - SP	Present	Soares *et al.* (2021)
Hollandichthys multifasciatus	50	10m+12sm+28st	Grande River - SP	Present	Carvalho *et al.* (2002)
Hyphessobrycon anisitsi	50	6m+16sm+12st+16a	Upper Paraná River - PR	Present	Centofante *et al.* (2003)
Hyphessobrycon anisitsi	50	10m+2sm+20st+18a	Pirassununga River -SP	Present	Goes *et al.* (2020)
Hyphessobrycon anisitsi	50	18m+10sm+6st+16a	Tibagí River - PR	Present	Mendes *et al.* (2011)
Hyphessobrycon eques	52	10m+20sm+8st+14a	Piracicaba River - SP	**Absent**	Piscor *et al.* (2020)
Hyphessobrycon eques	52	14m+16sm+4st+18a	Capivara River - SP	**Absent**	Martinez *et al.* (2012)
Hyphessobrycon eques	52	12m+26sm+8st+14a	Ribeirão Claro River - SP	**Absent**	Piscor and Parise-Maltempi (2015)
Hyphessobrycon flammeus	52	18m/sm+32st+2a.	Not informed	Present	Arefjev (1990)
Hyphessobrycon herbertaxelrodi	52	10m/sm+42st/a.	não informado	**Absent**	Arefjev (1990)
Hyphessobrycon luetkenii	50	6m + 8sm + 36a	Maquiné River - RS	Present	Mendes *et al.* (2011)
Hyphessobrycon reticulatus	50	14m+20sm+16st	Jequiá River - SP	Present	Carvalho *et al.* (2002)

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Hyphessobrycon scholzei	50	8m+20sm+14a	Not Informed	Present	Arefjev (1990)
Hyphessobrycon vinaceus	50	8m+12sm+30a	Pardo River - BA	Present	Nishiyama *et al*. (2015)
Moenkhausia cosmops	50	14m+30sm+6st	Verde River - MT	Present	Nascimento *et al*. (2020)
Moenkhausia costae	50	50m/sm	São Francisco River – MG	Present	Portela *et al*. (1988)
Moenkhausia dichroura	50	22m+22sm+6st	Upper Paraná River - Argentina	Present	Sánchez *et al*. (2021)
Moenkhausia forestii	50	10m+32sm+8st	Sangue River - MT	Present	Nascimento *et al*. (2020)
Moenkhausia intermedia	50	16m+28sm+6st	Upper Paraná River - Argentina	Present	Sánchez *et al*. (2021)
Moenkhausia intermedia	50	50m/sm	Lagoa do Mato - SP	Present	Portela *et al*. (1988)
Moenkhausia intermedia	50	16m+34sm	Paraná River - PR	Present	Portela-Castro and Júlio-Jr (2002)
Moenkhausia nigromarginata	50	14m+32sm+4a	Verde River - MT	Present	Nascimento *et al*. (2020)
Moenkhausia oligolepis	50	12m+32sm+6st	Xapuri River- AC	Present	Nascimento *et al*. (2020)
Moenkhausia sanctaefilomenae	50	6m+23sm+12st	Batalha River, Tietê River basin - SP	Present	Voltolin *et al*. (2012)
Moenkhausia sanctaefilomenae	50	48m/sm+2a	Capivara and Tietê River - SP	Present	Forestii *et al*. (1989)
Moenkhausia sanctaefilomenae	50	12m+36sm+2a	Paraná River - PR	Present	Portela-Castro and Júlio-Jr (2002)
Moenkhausia sanctaefilomenae	50	12m+32sm+6st	Upper Paraná River - Argentina	Present	Sánchez *et al*. (2021)
Moenkhausia sanctaefilomenae	50	48m/sm+2st/a	Aguapeí River - Argentina	Present	Alberdi and Fenocchio (1997)
Nematobrycon palmeri	50	8m/sm+10st+32a	Not Informed	Present	Arefjev (1990)
Nematocharax venustus	50	8m+26sm+14st+2a	Contas River - BA/Jequitinhonha - MG	Present	Barreto *et al*. (2016)
Oligosarcus acutirostris	50	4m+14sm+18st	Paraibuna River - ES	Present	Cunha *et al*. (2021)
Oligosarcus hepsetus	50	6m+10sm+16+18a	Paraitinga and Paraíba do Sul River basin -SP	Present	Kavalco *et al*. (2005)
Oligosarcus hepsetus	50	2m+16sm+16st+16a	Paraíba do Sul River - SP	Present	Hattori *et al*. (2007)
Oligosarcus jenynsii	50	2m+24sm+10st+14a	Uruguay River - SC	Present	Hattori *et al*. (2007)
Oligosarcus longirostris	50	4m+10sm+16st+20a	Iguaçu River - PR	Present	Rupert and Margarido (2007)
Oligosarcus paranensis	50	8m+18sm+10st+14a	Tibagi River basin - PR	Present	Usso *et al*. (2018)
Oligosarcus paranensis	50	4m+10sm+16st+20a	Piquiri River basin - PR	Present	Rupert and Margarido (2007)
Oligosarcus pintoi	50	4m+10sm+16st+20a	Piquiri River basin - PR	Present	Rupert and Margarido (2007)
Oligigosarcus pintoi	50	4m+12sm +14st +20a	Ivaí River - PR	Present	Mari-Ribeiro *et al*. (2022)
Oligosarcus pintoi	50	2m+20sm+12st+16a	Mogi-Guaçu River - SP	Present	Hattori *et al*. (2007)
Oligosarcus sp.	50	6m+14sm+18st+12a	Velhas River basin - Ouro Preto - MG	Present	De-Barros *et al*. (2015)
Oligosarcus sp.	50	4m+14sm+20st+12a	Doce River basin - MG	Present	De-Barros *et al*. (2015)
Orthospinus franciscensis	50	22m+20sm+2st+6a	São Francisco River - MG	Present	Moreira *et al*. (2007)
Poptella paraguayensis	50	10m+26sm+8st+6a	Miranda River - MT	Present	Freitas and Galetti (1998)
Psalidodon (*Astyanax*) *bockmanni**	50	10m+12sm+12st+16a	Paranapanema River basin, São Miguel Arcanjo and Pilar do Sul - SP	Present	Kavalco *et al*. (2008)
Psalidodon (*Astyanax*) *bockmanni**	50	8m+14sm+12st+16a	Capivara River, Tietê River basin - SP	Present	Silva *et al*. (2013)
Psalidodon (*Astyanax*) *bockmanni**	50	8m+14sm+14st+14a	Água Madalena stream, Paranapanema River basin - SP	Present	Silva *et al*. (2013)
Psalidodon (*Astyanax*) *bockmanni**	50	6m+20sm+8st+16a	Iguatemi River basin - MS	Present	Fernandes *et al*. (2010)
Psalidodon (*Astyanax*) aff. *fasciatus**	50	16m+12sm+6st+16a	Tributary of Cabeça River - SP	Present	Piscor *et al*. (2017)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	10m+20sm+8st+10a	Tributary of Ribeirão Claro River - SP	Present	Piscor *et al*. (2017)
Psalidodon (*Astyanax*) *eigenmanniorum**	48	14m+24sm+4st+10a	Araguari River Basin - MG	Present	Torres-Mariano and Morelli (2008)
Psalidodon (*Astyanax*) *eigenmanniorum**	48	10m+16sm+10st+12a	Laguna dos patos - RS	Present	Mendes *et al*. (2011)

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Psalidodon (*Astyanax*) *fasciatus**	48	8m+20sm+16st+4a	São Francisco River - MG	Present	Peres *et al.* (2009)
Psalidodon (*Astyanax*) aff. *fasciatus**	50	8m+26sm+6st+10a	Afluente do rio Corumbataí - SP	Present	Piscor *et al.* (2017)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	10m+12sm+12st+14a	Pirassununga - SP	Present	Goes *et al.* (2020)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+20sm+12st+8a	Preto do Costa River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+18sm+14st+8a	Mutum River- BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+24sm+10st+6a	Oricó River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+28sm+8st+4a	Criciúma River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+18sm+16st+6a	Gongogi River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+16sm+16st+8a	Mineiro River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+16sm+18st+6a	Itapicuru River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+24sm+10st+6a	Braço River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+22sm+10st+8a	Cachoeira River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+20sm+16st+4a	Contas River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) *marionae**	48	4m+24sm+10st+6a	Rio claro stream, Paraguai River basin - MS	Present	Piscor *et al.* (2017)
Psalidodon (*Astyanax*) *parabybae**	48	8m+18sm+12st+10a	Paraitinga River - SP	Present	Kavalco and Moreira-Filho (2003)
Psalidodon (*Astyanax scabripinnis paranae*) *paranae**	50	4m+34sm+4st+6a	Araquá River - SP	Present	Maistro *et al.* (1992)
Psalidodon (*Astyanax*) *schubarti**	36	10m+10sm+10st+6a	Pirassununga -SP	Present	Goes *et al.* (2020)
Psalidodon (*Astyanax*) *schubarti**	36	14m+14sm/6st+2a	Paraná River - PR	Present	Alberdi and Fenocchio (1997)
Rhoadsia altipinna	50	10m+26+14a	Das Bocas River - Equador	Present	Sanchez-Romero *et al.* (2015)
Subfamily Stevardiinae					
Bryconamericus aff. *exodon*	52	16m+12sm+6st+18a	Tibagí River - PR	**Absent**	Paintner-Marques *et al.* (2002)
Bryconamericus aff. *exodon*	52	10m+24sm+6st+12a	Tibagí River - PR	**Absent**	Paintner-Marques *et al.* (2002)
Bryconamericus aff. *iheringii*	52	12m+10sm+16st+14a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	18m+14sm+10st+10a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	20m+18sm+4st+10a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	20m+14sm+12st+6a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	22m+18sm+8st+4a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	18m+24sm+6st+4a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	12m+18sm+8st+ 14a	Maringá stream, Paraná River basin - PR	**Absent**	Capistano *et al.* (2008)
Bryconamericus aff. *iheringii*	52	8m+28sm+6st+ 10a	Keller River, Paraná River basin - PR	**Absent**	Capistano *et al.* (2008)
Bryconamericus aff. *iheringii*	52	8m+20sm+8st+16a	Tatupeba stream, Paraná River basin - PR	**Absent**	Capistano *et al.* (2008)
Bryconamericus aff. *iheringii*	52	10m+16sm+14st+12a	Upper Uruguai River basin	Present	Prestes *et al.* (2009)

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Bryconamericus aff. *iheringii*	52	12m+18sm+8st+14a	Ocoí River - PR	**Absent**	Nishiyama *et al.* (2015)
Bryconamericus aff. *iheringii*	52	10m+14sm+18st+10a	Corumbataí River - SP	**Absent**	Piscor *et al.* (2013)
Bryconamericus coeruleus	52	14m+20sm+8st+10a	Upper Paraná River basin - PR	Present	Prestes *et al.* (2009)
Bryconamericus ecai	52	10m+16sm+8st +18a	Forquetinha River - RS	**Absent**	Santos *et al.* (2017)
Bryconamericus ecai	52	8m+16sm+14st+14a	Forquetinha River - RS	**Absent**	Santos *et al.* (2017)
Bryconamericus ecai	52	10m+16sm+8st+18a	Forquetinha River - RS	**Absent**	Santos *et al.* (2017)
Bryconamericus ecai	52	10m+10sm+8st+24a	Forquetinha River - RS	**Absent**	Dos Santos *et al.* (2012)
Bryconamericus ecai	52	10m+18sm+8st+16a	Forquetinha River - RS	**Absent**	Dos Santos *et al.* (2012)
Bryconamericus ecai	52	14m+14sm+6st+18a	Forquetinha River - RS	**Absent**	Dos Santos *et al.* (2012)
Bryconamericus ecai	52	10m+24sm+14st+4a	Forquetinha River - RS	**Absent**	Dos Santos *et al.* (2012)
Bryconamericus ecai	52	10m+16sm+14st+12a	Upper Uruguai River basin - PR	**Absent**	Prestes *et al.* (2009)
Bryconamericus eigenmanni	52	6m+16sm+16st+14a	Upper Uruguai River basin - PR	Present	Prestes *et al.* (2009)
Bryconamericus sp.	52	16m+14sm+10st+12a	Vermelho stream, Ivaí River basin - PR	**Absent**	Santos *et al.* (2017)
Bryconamericus sp.	52	2m+12sm+20st+20a	Cambuta River, Ivaí River basin - PR	**Absent**	Santos *et al.* (2017)
Bryconamericus sp. A	52	6m+30sm+6st+10a	Piracicaba river - SP	**Absent**	Wasko and Galetti-Jr (1998)
Bryconamericus sp. B	52	6m+10sm+20st+16a	Piracicaba river - SP	**Absent**	Wasko and Galetti-Jr (1998)
Bryconamericus sp. C	52	6m+18sm+14st+14a	Tibagi River - PR	**Absent**	Wasko and Galetti-Jr (1998)
Bryconamericus sp. D	52	8m+14sm+16st+14a	Garças River - MT	**Absent**	Wasko and Galetti-Jr (1998)
Bryconamericus sp. E	54	10m+16sm+22st+6a	Garças River - MT	**Absent**	Wasko and Galetti-Jr (1998)
Bryconamericus sp. A	52	6m+30sm+ 6st+10a	Piracicaba river - SP	**Absent**	Wasko *et al.* (1996)
Bryconamericus sp. B	52	10m+6sm+18st+18a	Piracicaba river - SP	**Absent**	Wasko *et al.* (1996)
Bryconamericus stramineus	52	26m/sm+26st/a	Mogi Guaçu River - SP	**Absent**	Portela *et al.* (1988)
Bryconamericus stramineus	52	6m+10sm+16st+20a	Iguatemi River basin - MS	**Absent**	Fernandes *et al.* (2010)
Bryconamericus stramineus	52	6m+10sm+16st+20a	Guaçu stream, Iguatemi River basin - MS	**Absent**	Piscor *et al.* (2013)
Bryconamericus turiuba	52	8m+10sm+14st+20a	Passo-Cinco River - SP	**Absent**	Piscor *et al.* (2013)
Glandulocauda melanogenys	52	4m+12sm+22st+14a	Paranapiacaba - SP	**Absent**	Guimarães *et al.* (1995)
Knodus cf. *chapadae*	52	14m+14sm+ 14st+10a	Tangará da Serra - MT	**Absent**	Krinski *et al.* (2008)
Markiana nigripinnis	52	8m+22sm+22st/a	Miranda River - MT	**Absent**	Monteiro *et al.* (2022)
Mimagoniates laterallis	52	6m+20sm+16st+10a	Itanhém - SP	**Absent**	Guimarães *et al.* (1995)
Mimagoniates microlepis	52	12m+18sm+14+8a	Iguaçu River basin and Piraquara River - PR	**Absent**	Torres *et al.* (2008)
Piabina anhembi	52	8m+10sm+16st+18a	Salesópolis - SP	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	26m/sm+26st/a	Mogi Guaçu - SP	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	8m+14sm+16st+14a	São Francisco - MG	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	4m+22sm+10s+16a	Itatinga - SP	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	8m+18sm+18st+10a	Botucatu - SP	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	4m+24sm+10st+14a	Bauru - SP	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	26m/sm+26st/a	Mogi Guaçu River - SP	**Absent**	Portela *et al.* (1988)
Piabina argentea	52	8m+14sm+16st+14a	São Francisco River - MG	**Absent**	Moreira *et al.* (2007)
Piabina argentea	52	6m+24sm+12st+10a	Iguatemi River - MS	**Absent**	Fernandes *et al.* (2010)
Subfamily Tetragonopterinae					
Tetragonopterus argenteus	52	16m+4sm+4st+28a	Cuiabá River - MT	**Absent**	Miyazawa (2015)
Tetragonopterus argenteus	52	24m+8sm+4st+16a	Bento Gomes River - MT	**Absent**	Miyazawa (2015)
Tetragonopterus argenteus	50	14m+4sm+4st+28a	Cuiabá River - MT	**Absent**	Miyazawa (2015)
Tetragonopterus argenteus	52	16m/sm+2st+34a	Paraná River - PR	**Absent**	Alberdi and Fenocchio (1997)
Tetragonopterus franciscoensis	52	12m+26sm+14a	Itapicuru River - BA	**Absent**	Present study
Tetragonopterus franciscoensis (*chalceus*)*	52	13m/sm+13st/a	São Francisco River - MG	**Absent**	Portela *et al.* (1988)

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Family Bryconidae					
Brycon amazonicus	50	22m+14sm+14st	Orinoco basin -Venezuela	Present	Mariguela *et al.* (2010)
Brycon cf. *cephalus*	50	26m+24sm/st	Amazon basin - AM	Present	Almeida-Toledo *et al.* (1996)
Brycon cf. *reinhardti*	50	22m+28sm/st	Paraíba do Sul River - SP	Present	Almeida-Toledo *et al.* (1996)
Brycon insignis	50	24m+21sm/st	Paraíba do Sul River - SP	Present	Almeida-Toledo *et al.* (1996)
Henochilus wheatlandii	50	26m+12sm+12st	Santo Antônio River - MG	Present	Silva *et al.* (2012)
Family Gasteropelecidae					
Carnegiella strigata	50	Not Informed	Manaus - MA	**Absent**	Yano *et al.* (2021)
Gasteropelecus levis	54	Not Informed	Manaus - MA	**Absent**	Yano *et al.* (2021)
Thoracocharax stellatus	54	Not Informed	Barra do Bugres - MT	**Absent**	Yano *et al.* (2021)
Family Triportheidae					
Agoniates halecinus	52	Not Informed	Manaus - AM	**Absent**	Yano *et al.* (2021)
Lignobrycon myersi	52	28m+18sm+6a	Almada River - BA	**Absent**	Dos-Santos et al. (2016)
Triportheus auritus	52	Not Informed	Ponta do Araguaia - MT	**Absent**	Yano *et al.* (2021)
Triportheus nematurus	52	13m+23sm+16st	Piracicaba River - SP	**Absent**	Diniz *et al.* (2008)
Triportheus pantanensis	52	Not Informed	Paraguay basin	**Absent**	Yano *et al.* (2016)
Triportheus aff. *rotundatus*	52	Not Informed	Paraguay basin	**Absent**	Yano *et al.* (2016)