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Reconstruction of the Doradinae (Siluriformes-Doradidae) ancestral diploid number and NOR pattern reveals new insights about the karyotypic diversification of the Neotropical thorny catfishes

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Abstract

Doradinae (Siluriformes: Doradidae) is the most species-rich subfamily among thorny catfishes, encompassing over 77 valid species, found mainly in Amazon and Platina hydrographic basins. Here, we analyzed seven Doradinae species using combined methods (e.g., cytogenetic tools and Mesquite ancestral reconstruction software) in order to scrutinize the processes that mediated the karyotype diversification in this subfamily. Our ancestral reconstruction recovered that 2n=58 chromosomes and simple nucleolar organizer regions (NOR) are ancestral features only for Wertheimerinae and the most clades of Doradinae. Some exceptions were found in Trachydoras paraguayensis (2n=56), Trachydoras steindachneri (2n=60), Ossancora punctata (2n=66) and Platydoras hancockii whose karyotypes showed a multiple NOR system. The large thorny catfishes, such as Pterodoras granulosus, Oxydoras niger and Centrodoras brachiatus share several karyotype features, with subtle variations only regarding their heterochromatin distribution. On the other hand, a remarkable karyotypic variability has been reported in the fimbriate barbells thorny catfishes. These two contrasting karyoevolution trajectories emerged from a complex interaction between chromosome rearrangements (e.g., inversions and Robertsonian translocations) and mechanisms of heterochromatin dispersion. Moreover, we believe that biological features, such as microhabitats preferences, populational size, low vagility and migratory behavior played a key role during the origin and maintenance of chromosome diversity in Doradinae subfamily.

Keywords: Karyotypic diversification, Cytotaxonomy, 5S rDNA, 18S rDNA, Heterochromatin.

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Introduction

Cytogenetic studies have provided valuable information about the evolutionary trends and relationships in a range of vertebrate species, such as amphibians (Bruschi et al., 2019), reptiles (Viana et al., 2019, 2020), birds (Damas et al., 2019, Sigeman et al., 2019), mammals (Graphodastsky et al., 2011) and fish (Sember et al., 2018; Takagui et al., 2019). Different softwares for reconstruction of ancestral characters (e.g., Chromoevol, Mesquite) have been incorporated into cytogenetic analyses in recent years and provided a better understanding regarding the karyotype evolution in several organisms, as seen in plants (Burchardt et al., 2018), insects (Castillo et al., 2018; Micolino et al., 2019), birds (Damas et al., 2018) and mammals (Kim et al., 2017).

Despite the paucity of studies involving this kind of evolutionary approach in fish, analysis combining cytogenetic data and reconstruction of ancestral features have emerged in recent years (Cardoso et al., 2018, Terra et al., 2019). Therefore, these studies demonstrate the efficiency of combined analysis between robust phylogenetic relationships and pre-establishes chromosomal patterns in generating accurate estimates of ancestral chromosomal states in fish, especially in groups that possess a huge karyotype diversity, as for instance the Doradidae family.

Within Neotropical Siluriformes, Doradidae stands out as one of the most diverse and representative families, with over 96 species (Fricke et al., 2020), commonly known as thorny or...
spiny catfishes. They are a remarkable group, easily recognized by the presence of a single rows of scutes with thorns along the lateral line. Thorny catfishes are widely distributed across the largest hydrographic basins in South America, although the highest diversity is found in the Amazon and La Plata basins (Ferraris, 2007; Birindelli, 2014). The relationships among Doradidae species were already investigated through morphological and molecular data and the monophyly of this family as well as its subfamilies are usually corroborated by both approaches (Arce et al., 2013; Birindelli, 2014).

Doradidae is classified into three subfamilies: Wertheimerinae (3 species), Astrodoradinae (15 species), and Doradinae (78 species) (Fricke et al., 2020). The latter, represents the most diverse of all subfamilies and includes large species that are found mainly in the main channel of large rivers and exhibit migratory behavior during reproduction, represented by species as Pterodoras granulosus Valenciennes, 1821, Oxydoras nigro Valenciennes, 1821, Centrodoras brachiatus Cope, 1872, Megalodoras uranoscopus Eigenmann & Eigenmann, 1888, Lithodoras dorsalis Bleeker, 1862 (Goulding, 1980; Agostinho et al., 2003; Birindelli and Sousa 2017). On the other hand, Doradinae also includes tiny species, characterized by the presence of fimbriate barbels, such as Hemidoras, Trachydoras, Ossancora and Tenellus (Sabaj, 2005; Arce et al., 2013; Birindelli, 2014; Birindelli and Sousa 2017). The latter group, which has a wide morphological variability and behavioral lability, not only includes sedentary species but also others with high vagility (Sabaj, 2005; Birindelli and Sousa, 2017).

Karyotype data is available solely for 19 out of the 96 Doradidae species, most of them having 58 chromosomes, except for Anadoras sp. “araguaia” and Trachydoras paraguayensis Eigenmann & Ward 1907 (2n=56 chromosomes), and Ossancora punctata Kner, 1853 (2n=66 chromosomes), the highest diploid number in the family to date. Additionally, a considerable cytogenetic variability is also observed in the structural level (i.e., karyotype formulas, heterochromatin patterns and rDNA sites distribution), supernumerary chromosomes, as seen in Ossancora punctata, Pterodoras granulosus and Platydyros armatus Valenciennes, 1840 and a unique ZZ/ZW sex chromosome system in Tenellus trimaculatus Boulenger, 1898 (Table 1). Thus, it is believed that the origin of the current karyotype diversity in Doradidae has been assigned to numerical (Robertsonian translocations), structural (pericentric inversions) and different mechanisms of repetitive DNA dispersion (Baumgärtner et al., 2018; Takagui et al., 2019).

To unravel the evolutionary processes that drove the karyotype diversification of the Neotropical Doradidae and to better characterize its likely ancestral karyotype state, we applied an extensive suite of cytogenetic tools in a range of Doradinae subspecies, which allowed us to identify patterns of homologies and independent diversification in some particular clades of this subfamily. In addition, we also recovered ancestral features regarding the macro and micro karyotype structure based on a robust phylogeny, providing a better understanding about the karyotype evolution of the Neotropical thorny catfishes.

Material and Methods

Species and collection sites

Our representative sampling encompassed a total of 35 individuals of seven different thorny catfish species from different Brazilian hydrographic basins. All specimens here analyzed were collected under permission granted by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) number 11399-1. All procedures and experiments used in this study were approved, performed in accordance with all relevant guidelines and fulfill the rules of the Ethics Committee for Animal Use of the Londrina State University (Protocol: 60/2017). The individuals were properly identified by morphological criteria and subsequently deposited in the Museum of Zoology of the State University of Londrina (MZUEL), available online via SpeciesLink (Table 2).

Mitotic chromosomes preparations, chromosomal banding and Fluorescence in situ hybridization (FISH)

All individuals were treated with an intraperitoneal injection of 2 mL (1 mL/50 g) body weight) of bacterial lyase Broncho-vaxom (7 mg/mL), to trigger an inflammatory response and hence increase the number of renal cells in mitotic division (Molina et al., 2010). The mitotic chromosomes were obtained from kidney cells according to Bertollo et al. (1978). Heterochromatin was detected according to Sumner (1972) with modification in the staining step (Giemsa was replaced by propidium iodide) according to Lui et al. (2012).

Fluorescence in situ hybridization (FISH) was performed according to Pinkel et al. (1986). The rDNA probes were obtained by Mini-Prep (i.e., extraction of plasmidial DNA), 18S rDNA probe from Prochilodus argenteus Spix & Agassiz, 1829 (Hatanaka and Galetti, 2004) and 5S rDNA from Megaleporinus elongatus Valenciennes, 1850 (Martins and Galetti, 1999). The rDNA probes were labelled by nick translation (Roche) (according to the manufacturer’s instructions) using biotin-16-dUTP or digoxigenina-11-dUTP. Hybridizations were conducted under a high stringency (77%). The detection of the signals was performed using anti-digoxigenin-rhodamine (Roche) and avidin-FITC (Sigma-Aldrich). The karyotype morphology analysis followed Levan et al. (1964), but modified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a).

Reconstruction of ancestral characters using the Mesquite software

We performed a reconstruction of the ancestral chromosome number (2n) and NOR pattern using Mesquite software (Maddison and Maddison, 2011). For that, we incorporated the molecular species-level phylogeny of Doradidae and two outgroups from Auchenipteridae (its sister group), Trachelyopterus galeatus Linnaeus, 1766 and Ageneiosus inermis Linnaeus, 1766 (Arce et al., 2013). This study encompassed three datasets that included two mitochondrial DNA fragments (COI, n= 39 and 16S, n=41) and one nuclear DNA fragment (Rag 1, n=37) from previous studies available in online databases Genbank (Table 3). We reconstructed the phylogenetic relationships using Maximum
## Table 1 – Cytogenetic data available for the Neotropical freshwater fishes of Doradidae family.

| GENERA/ SPECIES | 2n | KARYOTYPE | Ag-NORs | 18S rDNA | 5S rDNA | REFERENCES |
|-----------------|----|-----------|---------|----------|---------|------------|
| **Wertheimerinae Subfamily** | | | | | | |
| Wertheimeria maculata | 58 | 24m+14sm+8st+12a | Pair 20 (p arm) | – | – | Eler et al. (2007) |
| Wertheimeria maculata | 58 | 24m + 12sm + 8st + 14st–a | – | Pair 22 (p arm) | Pair 22 (p arm) | Takagui et al. (2019) |
| Kalyptodoras bahiensis | 58 | 24m + 12sm + 8st + 14st–a | – | Pair 22 (p arm) | Pair 22 (p arm)/ Pair 19 (p arm) | Takagui et al. (2019) |
| Franciscodoras marmoratus | 58 | 24m + 12sm + 8st + 14st–a | – | Pair 22 (p arm) | Pair 22 (p arm)/ Pair 19 (p arm) | Takagui et al. (2019) |
| **Astrodoradinae Subfamily** | | | | | | |
| Anadoras sp. “araguaia” | 56 | 24m+10sm+8st+14a | Pair 28 (q arm) | Pair 28 (q arm) | Pair 15 (p arm) | Baumgärtner et al. (2018) |
| Platydoras cf. costatus | 58 | 26m+16sm+4st+2a | Pair 20 (p arm) | – | – | Milhomem et al. (2008) |
| Platydoras armatulus | 58 | 22m+14sm+18st+4a | – | – | – | Takagui et al. (2017a) |
| Platydoras armatulus | 58 | 24m+14sm+20st | Pair 25 (p arm) | Pair 25 (p arm) | Pairs 18, 25 | Baumgärtner et al. (2018) |
| Pterodoras granulosus | 58 | 16m +16sm+14st+12a | – | – | – | Takagui et al. (2017a) |
| Oxydoras niger | 58 | 20m+16sm+8st+14a | Pair 15 (p arm) | – | – | Fenocchio et al. (1993) |
| Rhinodoras dorbignyi | 58 | 20m+20sm+4st+14a | Pair 16 (p arm) | – | – | Fenocchio et al. (1993) |
| Rhinodoras dorbignyi | 58 | 18m+16sm+12st+12a | Pair sm (p arm) | – | – | Fenocchio et al. (1993) |
| Rhinodoras dorbignyi | 58 | 24m+12sm+12st+10a | Pair 24 (p arm) | Pair 24 (p arm) | Pairs 18,24,26 | Baumgärtner et al. (2018) |
| Ossancora punctata | 66 | 12m+8sm+6st+40a | – | – | – | Takagui et al. (2017a) |
| Ossancora eigenmanni | 58 | 30m+14sm+14st | Pair 17 (p arm) | Pair 17 | Pairs 10, 17,23 | Baumgärtner et al. (2018) |
| Trachydoras paraguayensis | 56 | 32m+20sm+4st | sm Pair | – | – | Fenocchio et al. (1993) |
| Trachydoras paraguayensis | 56 | 36m+16sm+4st | Pair 11 (Interstitial) | Pair 11 (Interstitial) | Pair 22 | Baumgärtner et al. (2016) |
| Tenellus leporinus | 58 | 36m+18sm+4st | Pair 23 (q arm) | Pair 23 | Pair 10 | Takagui et al. (2017b) |
| Tenellus trimaculatus | 58 | 21 m+18sm+12st+7a | Pair 22 (p arm) | Pair 22 (p arm) | Four sites | Takagui et al. (2017b) |
| Tenellus ternetzi | 58 | 44m+12sm+2a | Pair 24 (q arm) | – | – | Milhomem et al. (2008) |
| Hassar orestis | 58 | 32m+20sm+6a | Pair 22 (p arm) | – | – | Milhomem et al. (2008) |
| Hassar cf. orestis | 58 | 32m+18sm+8a | Pair 20 (p arm) | – | – | Milhomem et al. (2008) |
| Hassar wilderi | 58 | 32m+16sm+10a | Pair 25 (p arm) | – | – | Eler et al. (2007) |
| Hassar wilderi | 58 | 26m+20sm+12st | Pair 28 (p arm) | Pair 28 (p arm) | Four sites | Takagui et al. (2017b) |
| Hassar sp. | 58 | 42m+14sm+2a | Pair 7 (p arm) | – | – | Milhomem et al. (2008) |
| Leptodoras cataniae | 58 | 24m+16sm+14st+4a | Pair 23 (p arm) | Pair 23 (p arm) | Four sites | Takagui et al. (2017b) |

Legend: 2n=diploid number; m=metacentric; sm=submetacentric; st=subtelocentric; a=acrocentric; Ag-NORs=Nitrate impregnation for detect the NORs sites; rDNA=ribossomal desoxiribonucleic acid; p arm=short arm; q arm=long arm. The information’s produced by dissertations, PhD thesis or abstracts in national/international congresses were not included in the table.
Table 2 – Information about the species under study, their sex, collection sites and Vouchers in Ichthyological Collections.

| Species                  | Number of individuals | Localities          | Coordinates                  | Vouchers       |
|--------------------------|-----------------------|----------------------|------------------------------|----------------|
| Platydoras hancockii     | 3 males               | Negro River – Central Amazon basin | 0°58’31.68”S 62°55’40.79”W | MZUEL17318   |
| Centrodoras brachiatus   | 2 females             | Solimões River – Central Amazon basin | 3°14’28.32”S 59°56’29.19”W | MZUEL17831   |
| Pterodoras granulosus    | 4 males / 2 females    | Solimões River – Central Amazon basin | 3°09’34.11”S 59°54’04.34”W | MZUEL20294   |
| Ossancora punctata       | 5 males / 3 females   | Miranda River – Middle Paraguay basin | 19°31’25”S 57°02’26”W | MZUEL12170   |
| Oxydoras nigra           | 6 females             | Catalão Lake – Central Amazon basin | 3°09’49.8”S 59°54’47.5”W | MZUEL17317   |
| Trachydoras steindachneri| 4 females / 1 male    | Solimões River – Central Amazon basin | 3°09’34.11”S 59°54’04.34”W | MZUEL17802   |
| Hemidoras stenopelitis   | 3 females / 2 male    | Negro River – Central Amazon basin | 0°58’31.68”S 62°55’40.79”W | MZUEL17807   |

Legend: [S]= South; [W]= West; [MZUEL]= Museum of Zoology of Londrina State University; [MZUSP]= Museum of Zoology of Sao Paulo University.

Table 3 – Molecular (GenBank access numbers of genes used in the phylogenetic reconstruction) and cytogenetic data (diploid number and NOR pattern) used by the Mesquite software to estimate the ancestral diploid number and NORs pattern for Doradidae. Legend: Rag1= recombination activating gene 1; Co1= cytochrome c oxidase subunit 1; 16s= ribosomal RNA 16s; 2n= diploid number; NOR= nucleolar organizator region.

| Species                  | Molecular data identifier | Cytogenetic data |
|--------------------------|---------------------------|------------------|
|                          | Rag1 | Co1 | 16s | Source | 2n | NORs Pattern | Source |
| Trachelyopterus galeatus | –    | EU490848.1 | JX899742.1 | Genbank | 58 | Simple NORs (Two sites) | Lui et al. (2010) |
| Ageneiosus inermis       | KC555823.1 | –    | KC555843.1 | Genbank | 56 | Simple NORs (Two sites) | Lui et al. (2013) |
| Anadoras sp. “araguaia” | KC555726.1 | –    | KC555850.1 | Genbank | 56 | Simple NORs (Two sites) | Baumgärtner et al. (2018) |
| Physopyxis ananas        | KC555793.1 | KC555674.1 | KC555928.1 | Genbank | – | – | – |
| Scorpiodoras heckelli    | KC555813.1 | KC555695.1 | KC555948.1 | Genbank | – | – | – |
| Hypodoras forficulatus   | KC555747.1 | KC555619.1 | KC555877.1 | Genbank | – | – | – |
| Astrodoras asterions     | KC555729.1 | KC555597.1 | KC555855.1 | Genbank | – | – | – |
| Amblyodoras nheco        | KC555724.1 | KC555642.1 | KC555897.1 | Genbank | – | – | – |
| Acanthodoras sp. 2       | KC555714.1 | KC555580.1 | KC555837.1 | Genbank | – | – | – |
| Wertheimeria maculata    | –    | KC555709.1 | KC555963.1 | Genbank | 58 | Simple NORs (Two sites) | Takagui et al. (2019) |
| Kalypodoras bahiensis    | KC555748.1 | KC555622.1 | KC555878.1 | Genbank | 58 | Simple NORs (Two sites) | Takagui et al. (2019) |
| Franciscodoras marmoratus| KC555741.1 | KC555610.1 | KC555868.1 | Genbank | 58 | Simple NORs (Two sites) | Takagui et al. (2019) |
| Agamyxis pectinifrons    | KC555718.1 | KC555841.1 | KC555841.1 | Genbank | – | – | – |
| Rhynocodoras woodsi      | KC555810.1 | KC555693.1 | KC555946.1 | Genbank | – | – | – |
| Orinocodoras eigenmanni  | –    | KC555664.1 | KC555918.1 | Genbank | – | – | – |
| Rhinodoras dorbignyi     | KC555807.1 | KC555690.1 | KC555943.1 | Genbank | 58 | Simple NORs (Two sites) | Baumgärtner et al. (2018) |
| Pterodoras granulosus    | KC555802.1 | KC555686.1 | KC555939.1 | Genbank | 58 | Simple NORs (Two sites) | This study |
| Dorasps zuloagai         | KC555736.1 | KC555604.1 | KC555862.1 | Genbank | – | – | – |
| Oxydoras nigra           | KC555791.1 | KC555672.1 | KC555926.1 | Genbank | 58 | Simple NORs (Two sites) | This study |
| Centrochir crocodilli    | KC555731.1 | KC555999.1 | KC555861.1 | Genbank | – | – | – |
| Platydoras hancockii     | KC555798.1 | KC555679.1 | KC555933.1 | Genbank | 58 | Multiple NORs (Four sites) | This study |
| Platydoras costatus      | KC555797.1 | KC555678.1 | KC555932.1 | Genbank | 58 | Simple NORs (Two sites) | Milhomem et al. (2008) |
Table 3 – Cont.

| Species                  | Molecular data identifier | Cytogenetic data |
|--------------------------|---------------------------|------------------|
|                           | Rag1          | Col1         | 16s         | Source | 2n     | NORs Pattern | Source             |
| Platydoras armatulus     | KC555795.1   | KC555676.1  | KC555930.1  | Genbank | 58     | Simple NORs (Two sites) | Baumgärtner et al. (2018) |
| Centrodoras brachiatus   | KC555733.1   | KC555601.1  | KC555858.1  | Genbank | 58     | Simple NORs (Two sites) | This study            |
| Lithodoras dorsalis      | KC555763.1   | KC555639.1  | KC555895.1  | Genbank | –      | –             | –                  |
| Megalodoras goyanensis   | KC555764.1   | KC555640.1  | KC555896.1  | Genbank | –      | –             | –                  |
| Ossancora punctata       | KC555788.1   | KC555670.1  | KC555924.1  | Genbank | 66     | Simple NORs (Two sites) | This study            |
| Doras higuchii           | –             | KC555606.1  | –           | –       | –      | –             | –                  |
| Trachydoras paraguayensis| KC555818.1   | KC555704.1  | KC555958.1  | Genbank | 56     | Simple NORs (Two sites) | Baumgärtner et al. (2016) |
| Trachydoras steindachneri| –             | KC555708.1  | KC555962.1  | Genbank | 60     | Simple NORs (Two sites) | This study            |
| Anduzeadoras oxyrhynchus | KC555728.1   | KC555594.1  | KC555852.1  | Genbank | –      | –             | –                  |
| Leptodoras cataniae      | KC555750.1   | KC555624.1  | KC555882.1  | Genbank | 58     | Simple NORs (Two sites) | Takagui et al. (2017b) |
| Tenellus ternetzi        | KC555783.1   | KC555661.1  | KC555915.1  | Genbank | 58     | Simple NORs (Two sites) | Milhomem et al. (2008)  |
| Tenellus leporinus       | KC555773.1   | KC555653.1  | KC555907.1  | Genbank | 58     | Simple NORs (Two sites) | Takagui et al. (2017b) |
| Nemadoras elongatus      | KC555765.1   | KC555643.1  | KC555898.1  | Genbank | –      | –             | –                  |
| Tenellus trimaculatus    | KC555778.1   | KC555656.1  | KC555910.1  | Genbank | 58     | Simple NORs (Two sites) | Takagui et al. (2017b) |
| Hassar wilderi           | KC555744.1   | KC555616.1  | KC555874.1  | Genbank | 58     | Simple NORs (Two sites) | Takagui et al. (2017b) |
| Hassar orestis           | KC555743.1   | KC555615.1  | KC555873.1  | Genbank | 58     | Simple NORs (Two sites) | Milhomem et al. (2008)  |
| Ossancora fimbriata      | –             | KC555667.1  | KC555921.1  | Genbank | –      | –             | –                  |
| Opsodoras morei          | KC555781.1   | KC555659.1  | KC555913.1  | Genbank | –      | –             | –                  |
| Hemidoras stenopeltis    | KC555746.1   | KC555618.1  | KC555876.1  | Genbank | 58     | Simple NORs (Two sites) | This study            |

Likelihood (ML) in the software packages RAxML-HPC v. 8.2.10 (Stamatakis, 2014) performed in the CIPRES Science Gateway 3.3 (http://www.phylo.org/index.php/portal/).

The ancestral state was inferred using Maximum Likelihood analysis and Markov model 1 state (MK1), which considers that all changes are equally possible. The cytogenetic data used in the reconstruction were obtained from the literature (Table 3), including the data of the present study. The characters were treated as non-ordered and multi-state, with five states being considered for the diploid number (data absent; 2n=56; 2n=58; 2n=60; 2n=66) and three states for the NORs pattern (data absent; Simple NORs, Multiple NORs). The likely ancestor character was determined for each node, and the probabilistic values were organized in Table 4.

**Results**

**Platydoras hancockii:** Valenciennes 1840: had 2n=58 chromosomes (26m + 14sm + 18st-a) (Figure 1A). Heterochromatin was detected on short arm of the pairs 13, 15, 16, 20, 26, 28 and on long arm of the pair 3, 6 and 21; on both arms of the pairs 4 and 8; and in interstitial position (near to the centromere) on short arms of the pair 2 (Figure 1B).

The FISH using the 18S rDNA probes, evidenced multiple sites in terminal position on short arms of the pairs 26 and 28. The FISH with 5S rDNA probes, revealed hybridized sites on the short arm of the pair 26, the same chromosome pair were the 18S rDNAs sites were detected (Figure 1 box).

**Centrodoras brachiatus:** had 2n=58 chromosomes (22m + 16sm + 20st-a) (Figure 1C). C-banding evidenced heterochromatin blocks on short arms of the pairs 9, 18, 22, 24 and 27; on long arms of the pair 6; interstitial blocks on long arms of the pairs 20 and 26; in both arms of the pair 5; in pericentromeric and terminal regions on short arm of the pair 3 (Figure 1D). The FISH with rDNA probes, evidenced the presence of 18S rDNA sites and 5S rDNA sites on short arm of the pair 24, being that the 18S rDNA sites are located on terminal position, whereas 5S rDNA sites occurs in interstitial position, near to the centromere (Figure 1 box).

**Pterodoras granulosus:** had 2n=58 chromosomes (22m + 16sm + 20st-a) (Figure 1E). Heterochromatic blocks were detected on short arms of the pairs 9, 18, 24, 27; on long arms of the pair 6; interstitial blocks on long arms of the pairs 20 and 26; in both arms of the pair 5; in pericentromeric and terminal regions on short arm of the pair 3 (Figure 1D). The FISH with rDNA probes revealed the presence of DNA 18S rDNA in terminal position on short arm of the pair 24, adjacent to the 5S rDNA sites (Figure 1 box).
Table 4 – Probabilistic values calculated after, maximum likelihood ancestral state reconstructions of diploid number and NORs pattern, based on Mk1 model using the Mesquite software in Doradidae species. The values highlighted in red, are the most probably ancestral character for each node.

| Nodes | Diploid Numbers | NORs Pattern | Clades |
|-------|----------------|--------------|--------|
|       | Undefined | 2n=56 | 2n=58 | 2n=60 | 2n=66 | Undefined | Simple | Multiple |        |
| 1     | 35.4      | 32.7 | 22.3 | 4.7 | 4.7 | 23.3 | 69.5 | 7.0 | Doradoidea |
| 2     | 11.7      | 45.4 | 36.8 | 2.9 | 2.9 | 3.4 | 94.9 | 1.6 | Auchenipteridae |
| 3     | 64.7      | 22.2 | 8.9  | 2.1 | 2.1 | 43.5 | 51.7 | 4.6 | Doradidae |
| 4     | 63.1      | 29.8 | 3.6  | 1.6 | 1.6 | 43.0 | 53.2 | 3.7 | Astro doradinae |
| 5     | 95.8      | 2.6  | 0.6  | 0.4 | 0.4 | 89.5 | 8.4  | 2.0 | |
| 6     | 99.2      | 0.3  | 0.1  | 0.1 | 0.1 | 97.3 | 1.7  | 0.8 | |
| 7     | 99.2      | 0.3  | 0.1  | 0.1 | 0.1 | 98.7 | 0.6  | 0.5 | |
| 8     | 99.2      | 0.3  | 0.1  | 0.1 | 0.1 | 99.0 | 0.6  | 0.4 | |
| 9     | 85.1      | 4.7  | 7.8  | 1.0 | 1.0 | 65.9 | 30.6 | 3.4 | |
| 10    | 75.6      | 2.3  | 19.5 | 1.2 | 1.2 | 55.6 | 40.7 | 3.6 | |
| 11    | 6.4       | 0.7  | 91.3 | 0.6 | 0.6 | 0.8  | 89.4 | 1.9 | Wertheimerinae |
| 12    | 0.5       | 0.1  | 98.9 | 0.1 | 0.1 | 0.1  | 97.7 | 0.07 | |
| 13    | 90.1      | 0.6  | 8.1  | 0.5 | 0.5 | 78.4 | 18.9 | 2.6 | |
| 14    | 86.2      | 0.6  | 11.9 | 0.6 | 0.6 | 73.0 | 23.9 | 0.2 | Doradinae |
| 15    | 92.2      | 0.4  | 6.4  | 0.4 | 0.4 | 84.2 | 13.5 | 2.1 | |
| 16    | 84.1      | 0.8  | 13.3 | 0.8 | 0.8 | 74.0 | 23.2 | 2.6 | |
| 17    | 59.4      | 0.9  | 37.7 | 0.9 | 0.9 | 46.4 | 50.1 | 3.4 | |
| 18    | 57.4      | 0.9  | 39.5 | 0.9 | 0.9 | 47.1 | 49.7 | 3.1 | |
| 19    | 33.4      | 0.8  | 63.8 | 0.8 | 0.9 | 2.0  | 77.0 | 2.7 | |
| 20    | 38.7      | 0.9  | 58.2 | 0.9 | 1.1 | 23.3 | 73.3 | 3.2 | |
| 21    | 41.1      | 1.0  | 55.5 | 1.0 | 1.1 | 34.9 | 60.2 | 4.8 | |
| 22    | 3.5       | 0.4  | 95.0 | 0.4 | 0.4 | 9.5  | 83.2 | 0.7 | |
| 23    | 0.3       | 0.1  | 99.2 | 0.1 | 0.1 | 0.1  | 74.8 | 20.1 | |
| 24    | 41.7      | 1.6  | 52.4 | 1.6 | 2.5 | 20.0 | 77.8 | 2.8 | |
| 25    | 42.6      | 1.3  | 53.1 | 1.3 | 1.5 | 26.2 | 70.6 | 3.0 | |
| 26    | 94.2      | 0.5  | 4.1  | 0.5 | 0.5 | 88.6 | 9.4  | 1.9 | |
| 27    | 41.9      | 4.4  | 40.7 | 4.4 | 8.5 | 15.2 | 82.3 | 2.4 | |
| 28    | 54.1      | 2.9  | 13.4 | 2.9 | 26.5 | 24.4 | 72.7 | 2.7 | |
| 29    | 26.9      | 10.6 | 46.7 | 10.6 | 4.9 | 0.6 | 91.4 | 1.7 | |
| 30    | 10.0      | 35.2 | 15.7 | 35.2 | 3.7 | 1.2 | 98.0 | 0.06 | |
| 31    | 22.4      | 2.9  | 69.8 | 2.9 | 1.8 | 9.9  | 87.9 | 2.0 | |
| 32    | 28.1      | 1.6  | 67.1 | 1.6 | 1.3 | 20.6 | 76.6 | 2.6 | |
| 33    | 13.5      | 0.9  | 83.6 | 0.9 | 0.7 | 8.9  | 89.0 | 2.0 | |
| 34    | 15.7      | 0.8  | 81.6 | 0.8 | 0.8 | 14.6 | 82.7 | 2.6 | |
| 35    | 0.1       | 0.2  | 98.0 | 0.2 | 0.2 | 2.2  | 96.7 | 0.9 | |
| 36    | 57.0      | 1.0  | 39.8 | 1.0 | 1.0 | 56.0 | 40.7 | 3.2 | |
| 37    | 55.5      | 1.0  | 41.3 | 1.0 | 1.0 | 53.9 | 43.0 | 3.0 | |
| 38    | 7.7       | 0.5  | 90.6 | 0.5 | 0.4 | 7.2  | 90.9 | 1.7 | |
| 39    | 14.5      | 0.8  | 82.9 | 0.8 | 0.8 | 18.6 | 78.7 | 2.5 | |
| 40    | 0.6       | 0.1  | 98.8 | 0.1 | 0.1 | 1.3  | 97.9 | 0.7 | |
Figure 1 – Karyotypes of the Doradinae subfamily: *Platydoras hancockii* (a) Giemsa, (b) C-band; *Centrodoras brachiatus* (c) Giemsa, (d) C-band; *Pterodoras granulosus* (e) Giemsa, (f) C-band; *Oxydoras niger* (g) Giemsa, (h) C-band. The boxes contain the chromosome pairs bearing the 18S and 5S rDNA sites. The scale bar corresponds at 10 µm.
**Oxydoras niger**: had 2n=58 chromosomes (22m + 16m + 20st-a) (Figure 1G). C-banding evidenced heterochromatic blocks on short arms of the pairs 5, 6, 24 and on long arm of the pairs 14, 17, 21, 28 on both arms of the pairs 3, 9 and in interstitial position on long arms of the pair 23 (Figure 1H). FISH also revealed 18S and 5S rDNA sites on short arms of the pair 24, being the NORs sites in terminal position, while 5S rDNA sites was detected interstitially, near to the centromere (Figure 1 box).

**Hamiodora stenoptera** Steindachner 1881: had 2n=58 chromosomes (34m + 16sm + 8a) (Figure 2A). The heterochromatin was detected in terminal position on short arms of the pairs 3, 5, 7, 8, 21, 22; on long arms of the pair 28; in pericentric region of the pairs 2, 18, 19, 24, 27 and on both arms of the pair 4 (Figure 2B). The 18S rDNAs showed hybridized signals in terminal position on long arms of the pair 28, whereas 5S rDNA sites were detected on short arms of the pairs 7 and 8 (Figure 2 boxed).

**Trachydoras steindachneri** Perugia 1897: had 2n=60 chromosomes (30m + 14sm + 16a) (Figure 2C). C-banding evidenced terminal heterochromatic blocks on short arms of the pairs 10, 11, 18 and 28; on long arms of the pairs 6, 15, 23, 24, 27, 29; in pericentric regions in the pairs 5, 10, 11 and 16; on both arms of the pairs 3, 7, 26; in pericentric and terminal position on short arm of the pair 1 (Figure 2D). FISH revealed 18S rDNA sites on short arms of the pair 28 and 5S rDNA sites on short arms of the pair 18 (Figure 2 boxed).

**Ossancora punctata**: had the karyotype and heterochromatin pattern previously described by Takagui et al. (2017a) and shows 2n=66 chromosomes, the largest diploid number in the family. Here, we present unpublished data about the distribution of rDNA sites in the karyotype of this species. The rDNA sites were detected in distinct chromosomal pairs, but both located in terminal position and on short arms, being that the 18S rDNA sites in the pair 33 and 5S rDNA sites in the pair 11 (Figure 2E).

Reconstruction of ancestral chromosome characters in Doradidae clades

(a) Diploid number

When we integrated the diploid number data available for thorny catfishes with the molecular phylogenetic analysis carried out by Arce et al. (2013), we observed that the probabilistic values obtained for the basal nodes are low and very close to each other. Thus, it is not yet possible to determine which would be the ancestral state for diploid number for the Doradidae family. Our data indicate that both 2n=56 and 58 chromosomes might be considered equally parsimonious ancestral conditions for Doradoidea (node 1), Auchenipteridae (node 2) and Doradidae (node 3). Moreover, establishing the ancestral 2n in Astrodoradinae was hampered by the low number of species cytogenetically analyzed so far. On the contrary, the 2n=58 chromosomes in Wertheimerinae is the ancestral condition with 99.9% of support. The lack of chromosomal data in basal clades of Doradinae might also make it impossible to define which 2n would be the ancestral condition for the subfamily (node 14), as well as for other terminal clades (nodes 15, 16, 17, 18, 26, 28, 36, 37). Doras + Ossancora and Trachydoras clades have a greater 2n variability reported in its analyzed species; hence, increasing the studies in other species of these genera is required prior to reconstructing their likely ancestral 2n with accuracy (Figure 3, Table 4).

(b) NORs pattern

Our analyses show that simple NORs pattern is likely to be the ancestral condition for Doradidae, however, the value that supports such condition (51.7%) is not significantly high and sufficient to confirm this hypothesis. Only one species from Astrodoradinae has cytogenetic data available; therefore, insufficient samples to define the pattern of NORs for this subfamily (nodes 4,5,6,7,8). On the other hand, simple NORs was confirmed as an ancestral condition with high support values (89.4% and 97.7%) in Wertheimerinae. Simple NORs was defined as an ancestral trait in most clades of Doradinae, except for the basal clades (nodes 14, 15 e 16) and a part of the apical ones (26, 36 e 37) (Figure 3, Table 4).

Discussion

The origin of the current karyotype diversity in Doradidae has been assigned to numerical (Robertsonian translocations), structural (pericentric inversions) and different mechanisms of repetitive DNA dispersion (Baumgärtner et al., 2018; Takagui et al., 2019). The hypothesis that the contemporary thorny catfishes diversified from an ancestor with a karyotype composed by 58 chromosomes and simple NORs has been inferred by several studies (Eler et al., 2007; Milhomem et al., 2008; Baumgärtner et al., 2016; Takagui et al., 2017a; 2017b; Baumgärtner et al., 2018; Takagui et al., 2019). In fact, these characteristics occur in most Doradinae species, as well as in related groups, such as Auchenipteridae (Lui et al., 2010, 2013a, 2013b, 2015; Felicetti et al., 2021). In this scenario, a very intriguing question emerge: would the prevalence of 2n=58 chromosomes and simple NORs in Doradinae and Auchenipteridae (sister group) be enough arguments to support such characteristics as plesiomorphies in the family?

The reconstruction analysis of ancestral characters based on the likelihood method and Markov MK1 model imply that none of the evaluated characteristics (diploid number and NORs) had sufficient support values to be confirmed as plesiomorphic conditions for Doradidae. In fact, the hypothesis of 2n=58 chromosomes and simple NORs as ancestral states is applicable solely to Wertheimerinae and part of Doradinae clades, groups in which most of the cytogenetic studies are concentrated. Therefore, this would be the reason that led some authors to attempt to define ancestral conditions for the whole family. The uncertainty of the ancestral patterns for Doradinae is a reflect of the paucity of karyotype data in the basal-most clades. Cytogenetic studies in Astrodoradinae, as well as in Acanthodoras and Agamysix will be required to confirm or refute the ancestral karyotype hypothesis previously claimed for the group.

The presence or absence of fimbriate barbells, divides Doradinae into two large clades (Birindelli, 2014), also supported by molecular data (Arce et al., 2013). From a cytogenetic perspective, the ancestral karyotype remained highly conserved among the non-fimbriate barbells thorny catfishes, such as Platydoras, Rhinodoras, Pierodoras,
**Figure 2** – Karyotypes of the Doradinae subfamily “fimbriate barbells thorny catfishes”: *Hemidoras stenopeltis* (a) Giemsa, (b) C-band; *Trachydoras steindachneri* (c) Giemsa, (d); The boxes contain the chromosome pairs bearing the 18S and 5S rDNA rDNA sites. (e) karyotype of *Ossancora punctata* after FISH with 18S and 5S rDNA probes. The scale bar corresponds at 10 µm.

**Oxydoras** and **Centrodoras**, where all the species have 2n=58, however, most of them has variable chromosomal morphology (Table 1). These differences have been mainly attributed to pericentric inversions, which are considered, in a general context, the most important rearrangement for karyotypic diversification in Doradidae (Eler *et al*., 2007, Milhomem *et al*., 2008; Baumgärtner *et al*., 2018; Takagui *et al*., 2019). From an evolutionary point of view, the pericentric inversions promote genetic variability and could be involved with reproductive isolation, and therefore, contribute to the speciation process (King, 1993; Noor *et al*., 2001), as already reported in several fish groups such as *Loricarichthys* (Takagui *et al*., 2014), *Apterontus* (Takagui *et al*., 2017c; Fernandes *et al*., 2017), *Chronicichla* (Frade *et al*., 2019), *Boalengerella* (de Souza *et al*., 2017), *Brachyhypopomus* (Cardoso *et al*., 2018), *Exallodontus* and *Propimelodus* (Terra *et al*., 2019).

The large thorny catfishes *Centrodoras brachiatus*, *Pterodoras granulosus* and *Oxydoras niger*, shared the same diploid number, karyotypic formulae and rDNAs sites array. These similarities in their karyotypes reinforce the close relationship among these species, which are cytogenetically distinguished only by the distribution of the heterochromatin.
Figure 3 – Mirror trees showing maximum likelihood ancestral state reconstructions of diploid number and NORs pattern, based on Mk1 model using
the Mesquite software. This evolutionary analysis integrated cytogenetic data available for Doradidae species (including the present study) and two
Auchenipteridae species (sister group) with sequences of two mitochondrial DNA fragments (COI and 16S) and one nuclear DNA fragment (Rag 1)
obtained from the molecular phylogeny of Arce et al. (2013).

According to Motta-Neto et al. (2019), the karyotype stasis
(in different levels), is a multifactorial process resultant
by phylogenetic (recent or ancient radiation), biological
(dispersion capacity, populational size, habitat preferences),
and biogeographic contexts (presence of geographic barriers,
stable environments). The three thorny catfishes species
aforementioned, constitute demes with a high number of
individuals that seasonally perform migration movements
during the reproductive period (Goulding, 1980; Agostinho
et al., 2003; Birindelli and Sousa 2017). Thus, we can infer
that the population size, high vagility, phylogenetic proximity
and stabilizing natural selection mechanisms, may be decisive
factors that act synergistically, underscoring the chromosome
conservatism in this group. This correlation, also occurs in
other Neotropical fish species, such as Anostomidae (Martins
and Galetti, 1998), Prochilodontidae (Völtin et al., 2013),
Tetraodontidae (Viana et al., 2017) and in large catfishes of
the subfamily Sorubiminae (Swarça et al., 2013).

A greater cytogenetic variability was observed among
the fimbriate-barbells clade when compared to the other clades
placed into Doradinae subfamily (Table 1). This group shows
different diploid numbers ranging from 2n=56 to 2n=66,
supernumerary chromosomes (Takagui et al., 2017a) and a
unique ZZ/ZW differentiated sex chromosome system (Takagui
et al., 2017b). Derived diploid numbers was observed in
Trachydoras paraguayensis, which has 2n=56 chromosomes,
originated from a chromosomal fusion (Baumgärtner et al.,
2016), Trachydoras steindachneri with 2n=60 product of
one centric fission (present study) and Ossancora punctata
with 2n=66 chromosomes, which possibly arose due to four
centric fissions and multiple pericentric inversions from an
ancestral karyotype composed by 58 chromosomes (Takagui
et al., 2017b). Such diversity may be interpreted as a reflect
of the non-migratory behavior. These species occur mainly
in sandbanks, at the deep of the main channels of large rivers
or in marginal lagoons, associated with floating or riparian
vegetation (Birindelli and Sousa, 2017). The sedentarism
and microhabitat preference associated with small population sizes,
are characteristics that may be enhancing the chromosomal
rearrangements fixation along the same hydrographic basin.
This hypothesis has been corroborated by different groups
of fish widely distributed in the Amazon basin, as seen in
Ancistrus (de Oliveira et al., 2009), Farlowella (Marajo et al.,
2018) and in the species complex Bunocephalus coracoideous
(Ferreira et al., 2017).
Simple NORs in terminal position, appears as a plesiomorphic condition with high support values in Doradinae, although it remains an issue to be further investigated in most clades of the subfamily. In the Platydoras clade, a multiple NORs system was observed only in Platydoras hancockii, such configuration apparently represents a derived condition in Doradidae and hitherto particular to this species. The spreading of NORs sites between different chromosomes has often been related to the presence of transposable/mobile elements, which may insert itself in regions of DNaR 18S and spread them to other chromosomal sites (Raskina et al., 2004; Eickbush and Eickbush, 2007; Porto et al., 2014, among others). Another plausible and widely discussed possibility is the occurrence of non-reciprocal translocations involving terminal or sub-terminal segments (Hirai, 2020; Takagui et al., 2020.). In this case, the proximity of these regions during the meiotic interphase (Rabl’s Model), would facilitate the exchange of 18S DNaR segments in the terminal regions between non-homologous chromosomes (Cremer et al., 1982; Schweizer and Loidl 1987).

The localization of 18S and 5S rDNA sites in the same chromosome pair is unusual in closely related groups to Doradidae family: few Aspredinidae species possess such condition (Ferreira et al., 2017, 2020), also, the sister family Auchenipteridae has no evidence of syntenic rDNA sites (Lui et al., 2010, 2013a, 2013b, 2015; Felicetti et al., 2021). According to Baumgärtner et al. (2018), the presence of 18S and 5S rDNA sequences adjacently organized on short arms of one subtelocentric pair could indeed represent an ancestral condition for Doradidae species. Recently, Takagui et al. (2019) also revealed a sole subtelocentric pair bearing 18S and 5S rDNA for all Wertheimerinae species, reinforcing this trait as a plesiomorphic condition, once Wertheimerinae is considered one of the most ancient lineages among thorny catfishes, sister group to Doradinae. Our data also highlights that this association is maintained for at least the large thorny catfishes species in Doradinae, as seen in P. granulosus, P. hancockii, O. niger and C. brachiatus. However, syntenic breakage events might have occurred at the very beginning of fimbriate-barbell thorny catfishes differentiation. Notably, excepting Ossancora eigenmanni, all species of this clade do not have 18S and 5S rDNA sharing the same location on a chromosome pair.

The 5S rDNA distribution, when compared to 18S rDNA, is so much more variable and unstable, holding numerical and structural variability and also representing an excellent cytotoxiconomic marker for Doradidae species (Table 1). For instance, Platydoras hancockii and Platydoras armatus (Baumgärtner et al., 2018) can be easily differentiated from each other by the presence of differential 5S rDNA sites, and the same occurs among Tenellus species (Takagui et al., 2017b) and in Wertheimerinae (Takagui et al., 2019). In Auchenipteridae, 5S rDNA sites distribution pattern has also been useful to characterize species of Tatia (Lui et al., 2013a), as well as populations of Trachelyopterus galeatus (Lui et al., 2009; Lui et al., 2010). In general, most variability in the 5S rDNA distribution is attributed to the presence of different repetitive DNA classes in non-transcribed regions (NTS) of 5S rDNA, which is common in fish groups, including transposable elements such as LINES, SINES and non-LTR retrotransposons (Rebordinos et al., 2013; Gouveia et al., 2017), histones DNA (Hashimoto et al., 2011; Piscor et al., 2018), small nuclear RNA (Silva et al., 2015) as well as different microsatellites motifs (Gouveia et al., 2017).

Our results combined, shed light on the karyotype diversification of Doradinae, the most representative subfamily among thorny catfishes. Our cytogenetic analyses and reconstruction of ancestral states brought important new insights into evolutionary pathways traced by doradids, providing thus, two striking evolutionary trajectories: low variation and conservatism of several chromosomal features in large thorny catfishes (non-fimbriate barbells) and remarkable diversity in tiny species from fimbriate barbells group, often mediated by dynamic behaviors and complex evolutionary processes, still far from being fully solved. However, the available data suggest that the main mechanisms responsible for the current karyotype variability are: pericentric inversions (Baumgärtner et al., 2018), chromosomal fusions (Baumgärtner et al., 2016), centric fissions (Takagui et al., 2017a), paracentric inversion (Takagui et al., 2017b) and differential dispersion of heterochromatin regions driven by transposable elements activity (Takagui et al., 2019).

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Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

FHT conduced the experiments, analyzed the data, conceived and designed the study and wrote; FHT, PV, LGC collected the specimens; JLOB helped to identify the specimens; LB, PV assisted in obtaining chromosomal preparations, in fluorescence hybridizations in situ (FISH) reactions and wrote the manuscript; JAB helped in the Ancestral state reconstruction by Mesquite software; VPM, RLL, EF, FSA, LGC provides laboratorial structure for some cytogenetics analysis and helped in designed the study and wrote the manuscript. All authors read and approved the final version.

References

Agostinho AA, Gomes LC, Suzuki HI and Júlio HF (2003) Migratory fishes of the Upper Paraná river basin, Brazil. In: Carolsfeld J, Harvey B, Ross C, Baer A (eds) Migratory fishes of South America: biology, fisheries and conservation status. IDRC and World Bank, Washington, 372p.
Eickbush TH and Eickbush DG (2007) Finely orchestrated movements: Evolution of the ribosomal RNA genes. Genetics 175:477–485
Eler ES, Dermaj GA, Venere PC, Paiva LC, Miranda GA, Oliveira AA (2007) The karyotypes of the thorny catfishes Wertheimera maculata Steindachner, 1877 and Hassar wildleri Kindle, 1895 (Siluriformes) and their relevance in doradids chromosomal evolution. Genetica 130:99-103
Felicetti D, Haerter CAG, Baumgärtner L, Paiz LM, Takagui FH, Margarido VP, Blanco DR, Feldberg E, da Silva M and Lui RL (2021) A new variant B chromosome in Auchenipteridae: the role of (GATA)n and (TTAGGG)n sequences in understanding the evolution of supernumeraries in Trachyoposterus. Cytogenet Genome Res 181:1-12.
Fenocchio AS, Jorge LC, Venere PC and Bertollo LAC (1993) Karyotypic characterization and nucleolus organizer regions in three species of Doradidae (Pisces, Siluriformes). Braz J Genet 4:1097–1101.
Fernandes CA, Paiz LM, Baumgärtner L, Margarido VP and Vieira MMR (2017) Comparative cytogenetics of the Black Ghost Knifefish (Gymnotiformes: Apterontoridae): evidence of chromosomal fusion and pericentric inversions in karyotypes of two Apterontus species. Zebrabrief 14:471-76
Ferraris CJ (2007) Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalog primary types. Porto Alegre: Zootaxa 1:628.
Ferreira M, Garcia C, Matoso DA, Jesus IS, Cioffi MB, Bertollo LAC, Zuanon J and Feldberg E (2017) The Bunocephalus coracoideus species complex (Siluriformes, Aspredinidae) as a speciation process through chromosomal, genetic and ecological diversity. Front Genet 8:120.
Ferreira M, de Jesus IS, Viana PF, Garcia C, Matoso DA, Cioffi MB, Bertollo LAC and Feldberg E (2020) Chromosomal evolution in Aspredinidae (Teleostei, Siluriformes): insights on intra- and interspecific relationships with related groups. Cytogenet Genome Res 160:539-553.
Frade LFS, Almeida BRR, Milhomem-Paixão SSR, Ready JS, Nagamachi CY, Pieczarka JC and Noronha RCR (2019) Karyoevolution of Chorizza hickel 1840 (Cichlidae, Perciformes): a process mediated by inversions. Biol Open 8:bio41699
Fricke R and Eschmeyer WN (2020) Species by family/subfamily in the catalog of fishes, http://researcharchive.calacademy.org//research/ichthyology/catalog/collections/SpeciesByFamily. asp (accessed 26 February 2020).
Gouveia JG, Wolf TR, Vilas Boas L, Hespolar-Harrison P, Schwarzacher T and Dias AL (2017) Repetitive DNA in the catfish genome: rDNA, microsatellites and Tc1-Mariner transposon sequences in Imparipinnis species (Siluriformes, Haplopteridae). J Hered 108:650-657
Goulding M (1980) The Fishes and the Forest: Explorations in Amazonian natural history. University of California Press, Berkeley, 280 p.
Graphodatsky AS, Trifonov VA and Stanyon R (2011) The genome diversity and karyotype evolution of mammals. Mol Cytogenet 4:22.
Hashimoto DT, Ferguson-Smith MA, Rens W, Foresti F and Porto-Foresti F (2011) Chromosome mapping of H1 histone and SS rRNA gene clusters in three species of Astyanax (Teleostei, Characiformes). Cytogenet Genome Res 134:64-71.
Hatanaka T and Galetti Jr PM (2004) Mapping 18S and 5S rDNA, microsatellites and Tc1-Mariner transposon sequences in Imparipinnis species (Siluriformes, Haplopteridae). J Hered 108:650-657
Hirai H (2020) Chromosome dynamics regulating genomic dispersion and alteration of nucleolar organizer regions (NORs). Cells 9:971.
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Kim J, Farré M, Auvi L, Capitanu B, Larkin DM, Ma J and Lewin HA (2017) Reconstruction and evolutionary history of eutherian dimensions. Proc Natl Acad Sci U S A 114:E5379–E5388.

King M (1993) Species evolution: The role of chromosomal change. Cambridge University Press, Cambridge, 360 p.

Levan A, Fredga K and Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52: 201-220.

Lui RL, Blanco DR, Margarido VP and Moreira-Filho O (2009) First description of B chromosome in the family Auchenipteridae, Paracharaciphilus gutatus (Siluriformes) of the Sao Francisco river basin (MG, Brazil). Micron 40:552-559.

Lui RL, Blanco DR, Margarido VP and Moreira-Filho O (2010) Chromosome characterization and biogeographic relations among three populations of the drifted catfish *Paracharaciphilus gutatus* (Linnaeus, 1766) (Siluriformes: Auchenipteridae) in Brazil. Biol J Linn Soc 99:648–656.

Lui RL, Blanco DR, Moreira Filho O and Margarido VP (2012) Propidium iodide for making heterochromatin more evident in the C-band technique. Biotech Biochem Systems 87:433-438.

Maddison WP and Maddison DR (2011) Mesquite: a modular system for phylogenetic analysis. Version 2.75, http://mesquiteproject.org.

Matile AM, Watanabe Y and Jadali AH (2005) Taxonomy assessment of Lepodotarca (Siluriformes: Doradidae) with description of three new species. Neotrop Ichthyol 14:101-113.

Maddison WP and Maddison DR (2011) Mesquite: a modular system for phylogenetic analysis. Version 2.75, http://mesquiteproject.org.

Sumner AT (1972) A simple technique for demonstrating centromeric dispersion and the evolution of C-band patterns. In: Stahl A, Luciani JM, Vagner-Capodano AM (eds) Chromosomes Today. Springer, Cham, pp 61–74.

Silva DMZA, Utsunomia R, Pansonato-Alves JC, Oliveira C and Foresti F (2015) Chromosomal mapping of repetitive DNA sequences in five species of *Astyanax* (Characiformes, Erythrinidae). Genet Res 146:144-152.

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75:304-306.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post analysis of large phylogenies. Bioinformatics 30:1312–1313.

Swarca AC, Sanchez S, Dias AL and Fenocchio AS (2013) Cytogenetics of the porthole Shovelnose catfish, *Hemisorubin platyrhynchos* (Valenciennes, 1840) (Siluriformes, Pimelodidae), a widespread species in South American rivers. Comp Cytogenet 7:103–110.

Takagui FH, Venturrelli NB, Dias AL, Swarca AC, Vicari MR, Fenocchio AS and Giuliano-Caetano L (2014) The importance of pericentric inversions in the karyotypic diversification of the species *Loricariichthys anis* and *Loricariichthys platymeron*. Zebrabfish 11:300–305.

Takagui FH, Dias AL, Birindelli JLO, Swarca AC, Rosa R, Lui RL and Giuliano-Caetano L (2017a) First report of B chromosomes in three neotropical thorny catfishes (Siluriformes, Doradidae). Comp Cytogenet 14:191-206.

Takagui FH, Moura LF, Ferreira DC, Centofante L, Vittorino CA, Bueno V and Venere P (2017b) Karyotype diversity in Doradidae (Siluriformes, Doradidae) and presence of the hemorhomorphic ZZ/ZW sex chromosome system in the family. Zebrabfish 14:236–243.
Takagui et al.

Chromosomal similarity between two species of *Apteronotus albifrons* complex (Apteronotidae-Gymnotiformes) implications in citotaxonomy and karyotypic evolution. Caryologia 70:147-50.

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

Takagui FH, Venturelli NB, Baumgärtner L, Paiz LM, Viana P, Dionisio JF, Pompeo LRS, Margarido P, Fenocchio AS, Rosa R and Giuliano-Caetano L (2020) Unraveling the karyotypic evolution and cytotaxonomy of armored catfishes (Loricariinae) with emphasis in *Sturisoma, Loricaria, Proloricaria, Pyxiloricaria* and *Rineloricaria*. Zebrafish 17:319-332.

Terra MC, Takagui FH, Baldissera JN, Feldberg E and Dias AL (2019) The karyotypic diversification of Calophysines and the *Exallodontus-Propimelodus* Clade (Pimelodidae, Siluriformes): A cytotaxonomic and evolutionary approach in Pimelodidae based on ancestral state reconstruction. Zebrafish 16:527-541.

Viana PF, Ezaz T, de Bello Cioffi M, Liehr T, Al-Rikabi A, Goll LG, Rocha AM and Feldberg E. (2020). Landscape of snake’ sex chromosomes evolution spanning 85 MYR reveals ancestry of sequences despite distinct evolutionary trajectories. Sci Rep 10:12499.

Viana PF, Ezaz T, de Bello Cioffi M, Jackson Almeida B and Feldberg E (2019) Evolutionary Insights of the ZW Sex Chromosomes in Snakes: A new chapter added by the amazonian Puffing Snakes of the genus *Spilotes*. Genes 10:288.

Viana PF, Ezaz T, Marajó L, Ferreira M, Zuanon J, Cioffi MB and Feldberg E (2017) Genomic organization of repetitive DNAs and differentiation of an XX/XY sex chromosome system in the the Amazonian puffer fish, *Colomesus asellus* (Tetraodontiformes). Cytogenet Gen Res 153:96-104.

Voltolin TA, Penitente M, Mendonça BB, Senhorini JÁ, Foresti F and Porto-Foresti F (2013) Karyotypic conservatism in five species of *Prochilodus* (Characiformes, Prochilodontidae) disclosed by cytogenetic markers. Genet Mol Biol 36:347-352.

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