Chromosomal Radiation: a model to explain karyotypic diversity in cryptic species

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ABSTRACT

We present a concept that summarizes the pattern of occurrence of widely distributed organisms with large chromosomal diversity, low molecular divergence, and the absence of morphological identity. Our model is based on cytogenetic and molecular data of four populations of the siluriform *Hypostomus ancistroides* presented in this study in comparison with those of 15 other previously described populations but is applicable to any group of sister species, chronospecies, or cryptic species. Through the evaluation of karyotype macrostructure and physical location of genes, in addition to phylogenetic reconstructions from mitochondrial and nuclear genes, we observed morphologically indistinguishable individuals presenting different locally fixed karyomorphs with phylogeographic discontinuity. The process behind this pattern seen in many groups of cryptic species, in which variation lies mainly in the organization of their genomes but not necessarily in the ecosystems they inhabit or in their external morphology, is similar to the processes behind other events observed in the distribution of lineages. In this work we explain this pattern through the hypothesis of the existence of a process analogous to ecological-evolutionary radiation, the chromosomal radiation, which can be adaptive or non-adaptive, and explore the possibility of applying the concept of chromosomal radiation to different groups of organisms.

Keywords: Adaptive radiation, Chromosomal diversity, Karyotypic polymorphism, Chromosomal speciation.

INTRODUCTION

Several models have been proposed to explain the relationship between chromosome evolution and speciation, particularly regarding the mechanisms of fixation of polymorphisms (for a review see Rieseberg 2001; Navarro and Barton 2003; Hoffmann and Rieseberg 2008; Jackson et al. 2016). However, these models have not addressed a practical problem arising from chromosomal speciation: the formation of groups of cryptic species, usually with an intense karyotypic variation. Genomic polymorphisms from hybridisms, as well as events of haploid genome changes, may lead to reorganization of karyotypes, culminating in speciation. In the absence of these events (hybridization and euploidy), the existence of large genomic diversity and karyotype plasticity in the ancestral lineage could explain the emergence of different karyomorphs. These karyomorphs, in turn, can be fixed by evolutionary processes or demographic events, contributing to the formation of new evolutionary units.

Groups with independent evolutionary units with wide geographic distribution usually constitute informative examples of radiation, since adaptive radiation refers to those evolutionary groups that exhibit exceptional extension of adaptive diversification in a variety of ecological niches (Schluter 2000) and non-adaptive radiation can be described as an evolutionary diversification from a common ancestor not accompanied by relevant niche differentiation, but by isolation for competition (Gittenberger 1991). Basic mutation and selection processes alone are sufficient to promote the rapid proliferation of new forms, which supports the theory that compensations in the competitive capacity drive adaptive radiation (Gravilets and Vose 2005). On the contrary, niche conservatism can contribute to the rapid accumulation of lineages by promoting the isolation of derived forms and the multiplication of species through a spatially and temporally floating environment (Kozak et al. 2005).

Plecos of the subfamily Hypostominae (Loricariidae) constitute a large group of fish, megadiverse and with complex taxonomies, organized in approximately 40 genera (Lujan et al. 2015) and 477 valid species (Eschmeyer and Fong 2019). The large variation in characters such as coloration and external morphology (Oyakawa et al. 2005; Zawadzki et al. 2008) and wide distribution in South American rivers means that the genus *Hypostomus* Lacépède 1803 presents the largest number of species already karyotyped. The group of cryptic species known as *Hypostomus ancistroides* is one of the best-represented species in the literature.

In the 15 natural populations of *H. ancistroides* already studied, there are 15 different karyomorphs, usually with 2n = 68 chromosomes (Artoni and Bertollo 1996; Alves et al. 2006; Rubert et al. 2011; Alves et al. 2012; Bueno et al. 2012; Endo et al. 2012; Fernandes et al. 2012; Pansonato-Alves et al. 2013; Traldi et al. 2013). However, different diploid numbers (Maurutto et al. 2012) and even the presence of differentiated systems of sex chromosomes of the type XX/XY (Rocha-Reis et al. 2018), and ZZ/ZW (Lara-Kamei et al. 2017) have been observed. Markers, such as C-banding and the location of ribosomal cistrons, present significant variations, although for fish, especially Siluriformes, the available cytogenetic data is quite limited.

Despite these limitations, the classical chromosomal analysis indicates that *Hypostomus ancistroides* constitute a group of morphologically indistinguishable (cryptic) forms/species, in which allopatric populations are chromosomally differentiated without the presence of clines. In this study, we present molecular and chromosomal data of four new populations of the group and propose a concept based on the idea of radiation of the karyomorphs to explain this population-specific pattern, common in several megadiverse groups of animals and plants.

MATERIAL AND METHODS

The material analyzed in this study came from four sampling points within the basins of the rivers Tietê, Paranapanema, and Grande, all in the state of São Paulo, Southeastern Brazil. The geographical coordinates of the sampling points are shown in Table 1 and illustrated in Figure 1.

After sampling, living specimens were forwarded to the laboratory, where they were euthanized according to the technical norms of CONCEA (National Council for Control of Animal Experimentation). Subsequently, samples were deposited in the Tissue and Cell Suspension Bank and the specimens were deposited in the Vertebrate Collection of the Laboratory of Ecological and Evolutionary Genetics of the Federal University of Viçosa, Rio Paranaíba Campus (UFV-CRP), Brazil. Taxonomists at the Zoology Museum of the University of São Paulo (MZUSP) identified all lots as being specimens of *Hypostomus ancistroides*.

Mitotic chromosomes were obtained from samples from the renal tissue of individuals by air drying (Gold et al. 1990). In addition to the conventional Giemsa staining, C-banding was performed to detect constitutive heterochromatin (Sumner 1972) and Fluorescent in situ Hybridization (FISH) (according to Pinkel et al. 1986 and

Hamkalo and Elgin 1991, adapted by Pazza et al. 2006), using rDNA 5S and 18S probes obtained from PCR (polymerase chain reaction) using *H. ancistroides* DNA as a template. The primers used in this study are listed in Table 2. The probes were labeled with biotin-14-dATP by Nick Translation using the BioNick Labeling System kit (Invitrogen), following the manufacturer's recommendations. Hybridizations were detected with Cy3 and the slides mounted with antifade and DAPI (4'-6-diamino-2-phenyl indole). High stringency washes (20% formamide / 0.1xSSC) were performed for 15 minutes and the signal was amplified with biotin-conjugated to anti-avidin, incubated in NFDM-PBS (non-fat dry milk) buffer.

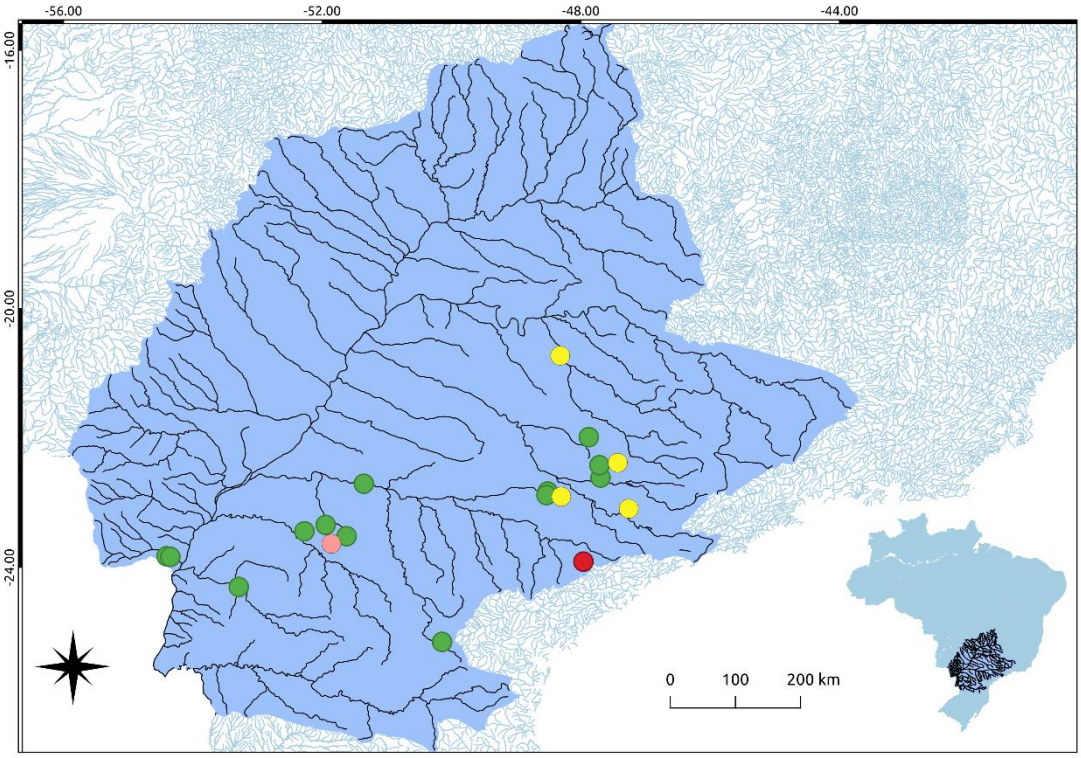

Figure 1: Sampling points along the Tietê, Paranapanema and Grande river basins, highlighting in purple the upper Paraná river system in South America. Data from the literature: in green dots, sampled populations of *Hypostomus ancistroides*; in red dot, sampled population of *Hypostomus* aff. *ancistroides* possessing XX/XY sex chromosomal system; in pink dot, sampled population of *Hypostomus* aff. *ancistroides* possessing ZZ/ZW sex chromosomal system; in yellow dots, populations presented in this paper.

Table 1. Sampling localities and chromosomal data of the *Hypostomus ancistroides* populations under study.

Population /City	Geographic coordinates	Hydrographic basin	Specimens sampled			2n	KF	FN	CB+	18S+	5S+
			M	F	Total						
Araras	22°22’59.64” S 47°55’46.75” W	Mogi-Guaçu	7	9	16	68	12m + 12sm + 16st + 28a	108	2, 4, 7, 16, 26, 33	13, 19	6
Botucatu	22°52’29.15” S 48°22’27.50” W	Tietê	4	5	9	68	12m + 10sm + 16st + 30a	106	4, 7, 20, 26	12, 17	31
Indaiatuba	23°05’39.12” S 47°15’38.16” W	Tietê	6	4	10	68	12m + 10sm + 14st + 32a	104	1, 4, 7, 10, 13, 26	14, 18	3
Terra Roxa	20°43’34.00” S 48°19’15.80” W	Grande	2	3	5	68	12m + 8sm + 16st + 32a	104	4, 7, 21, 26, 32	11, 18	27
Total = N			19	21	40						

M – Males; F – Females; 2n – Diploid Number; KF – Karyotypical Formula (m - metacentric; sm - submetacentric; st - subtelocentric; a - acrocentric chromosome); FN – Fundamental Number; CB+ Chromosome pairs bearing Constitutive Heterochromatin blocks; 18S+ Chromosome pairs bearing 18S rDNA sites; 5S+ Chromosome pairs bearing 5S rDNA sites.

Cytogenetic analysis was accomplished through optical microscopy, and the best metaphases were captured using a camera coupled to an epifluorescence microscope OLYMPUS BX41 with the QCapture Pro 6.0 software. The images were edited to assemble the karyotypes using the software Photoshop 9.0 and GIMP 2.8.14. Chromosomal types were classified taking into account the ratio of the chromosomal arms (Levan et al. 1964).

Mitochondrial (Cytochrome c Oxidase I - mt-co1; Cytochrome b - mt-cyb), and nuclear regions (recombination activation gene 1 - rag1; recombination activation gene 2 - rag2) were sequenced for the phylogenetic analysis. The primers used are listed in Table 2.

Table 2. Primers used for amplification of ribosomal, mitochondrial and nuclear genes.

Locus	**Primer**	**Sequence (5’ 3’)**	**Tm (°C)**	**Reference**
rDNA 5S	5SA	F - TACGCCCGATCTCGTCCGATC	52	Pendás *et al.* (1994)
	5SB	R - CAGGCTGGTATGGCCGTAAGC		
rDNA 18S	NS1	F - GTAGTCATATGCTTGTCTC	53	White *et al.* (1990)
	NS8	R - TCCGCAGGTTCACCTACGGA		
mt-co1	FishF1	F - TCAACCAACCACAAAGACATTGGCAC	54	Ward *et al.* (2005)
	FishR1	R - TAGACTTCTGGGTGGCCAAAGA		
mt-cyb	CytbFc	F - CGCCCTAATTGATCTCCCCG	57	Lujan *et al.* (2015)
	CytbRc	R - CTCCGGATTACAAGACCGGC		
rag1	RAG1Fa	F - CCTGGTTTTCATGCATTTGAGTGGCA	53	Lujan *et al.* (2015)
	RAG1R1186	R - ACGCTCTTCTGARGGAACTA		
rag2	RAG2Fc	F - ATGGAGGCCGAACACCCAACA	58	Lujan *et al.* (2015)
	RAG2R961	R - CGCTGCTGWACTCCATTT		

Tm (°C) - Melting temperature displayed in degrees Celsius

Using the IQ-TREE 1.5.6 software (Nguyen et al. 2015; Chernomor et al. 2016), maximum likelihood phylograms were constructed for each gene sequence obtained. The best nucleotide substitution patterns for each gene were then estimated according to the

Bayesian Information Criterion (BIC). Analyses were performed with 1,000 bootstrap replicas.

Bayesian analysis of the concatenated sequences was carried out using the software MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003). For each gene, an independent search was made for the best nucleotide substitution model using PartitionFinder 1.1.1 software (Lanfear et al. 2012). After a 50-million-generation run, we evaluated the length of the sampling chain every thousand generations with the Tracer 1.7 software (Rambaut et al. 2018), verifying the effective sample size (ESS) and strand convergence. 25% of the first trees were discarded as burn-in with the Tree Annotator software v.1.8. All phylogenetic trees were visualized with FigTree 1.4.2 software (Rambaut et al. 2012).

RESULTS

All specimens analyzed presented a diploid number of 2n = 68 chromosomes, with different karyotypes in each population, but without supernumerary elements or heteromorphisms related to sex. The results, which demonstrate that each karyomorph presents unique characteristics, but with traces of homology between them, are summarized in Table 1 and illustrated in figures 2 (karyotypes stained with Giemsa and rDNA 18S FISH), 3 (C-banding), 4 (rDNA 5S FISH) and 5 (population idiograms including all markers obtained).

The karyotypes are fairly symmetrical and, although different, have a similar conformation in all populations (Figures 2, 5). In contrast, the 18S rDNA cistrons are always located on subtelocentric chromosomes, in two pairs of very similar size, which probably correspond to homeologous chromosomes between the different populations (Figures 2 - detail, 5).

Similarly, probable homologous pairs with heterochromatic blocks can be observed in the four karyomorphs observed: pairs 4, 7, and 26 (Figures 3, 5). In these pairs, the heterochromatic block may have a different size or be in a different position along the chromosome arm. Other blocks restricted to a given population were also observed, which may constitute population markers. For example, only the population of Indaiatuba has C-bands in the first metacentric pair, whereas only a few individuals of the Terra Roxa population have a large C+ block in one of the chromosomes of the acrocentric pair 21, thus forming a heteromorphism (Figures 3, 5).

Generally, we observed two phenotypes concerning the location of the rDNA 5S cistrons, although they occur in just one chromosome pair in the four populations studied.

The karyomorphs from Araras and Indaiatuba presented the 5S rDNA sites in an interstitial position in the short arm of metacentric chromosomes. The karyomorphs from Botucatu and Terra Roxa presented them in the terminal position on the long arm of the medium acrocentric chromosomes - chromosome pairs (Figures 4, 5).

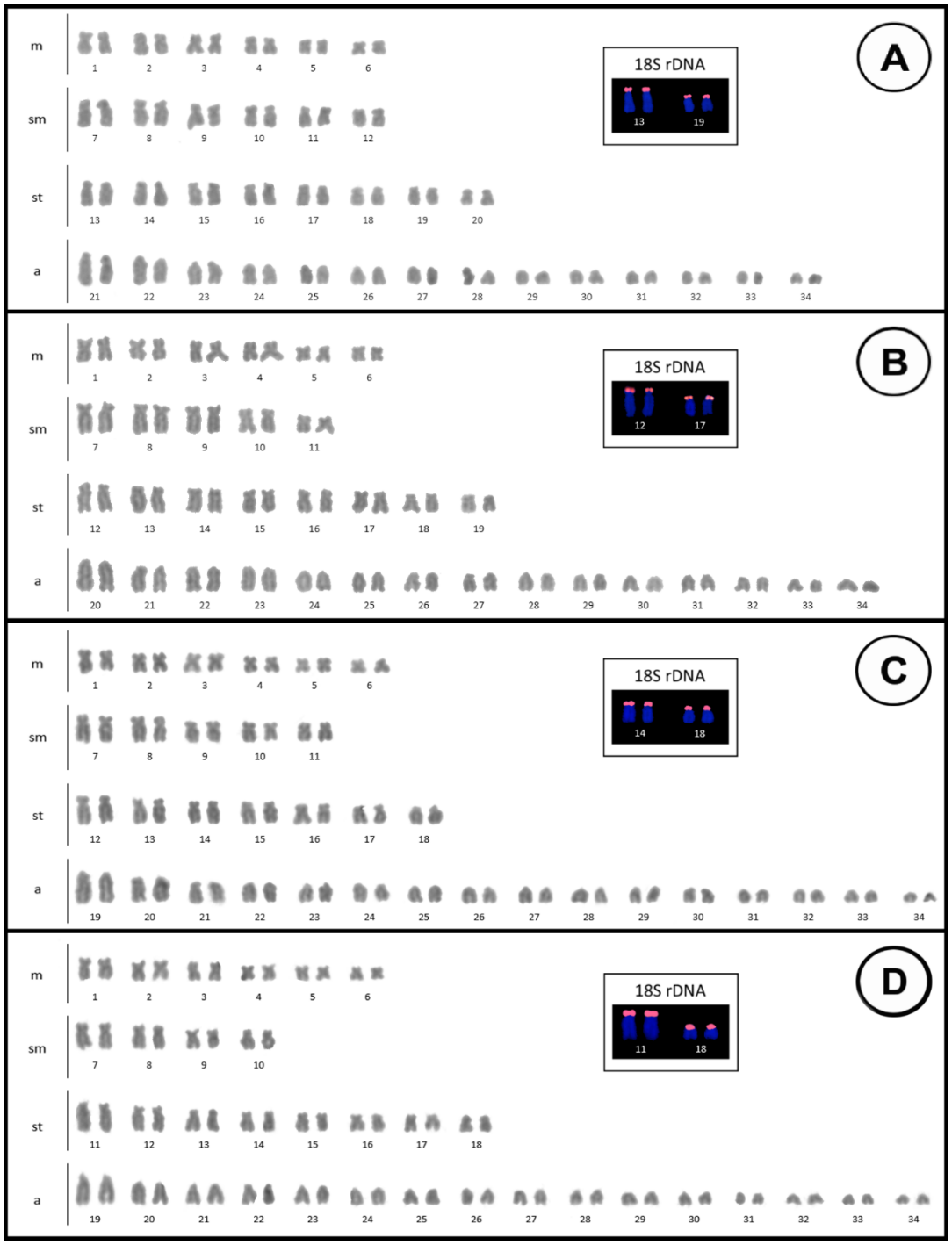

Figure 2: Karyotypes and rDNA 18S gene location (boxes) observed by FISH for the four populations of *Hypostomus ancistroides* presented in this paper: A - Araras, B- Botucatu, C - Indaiatuba, and D - Terra Roxa.

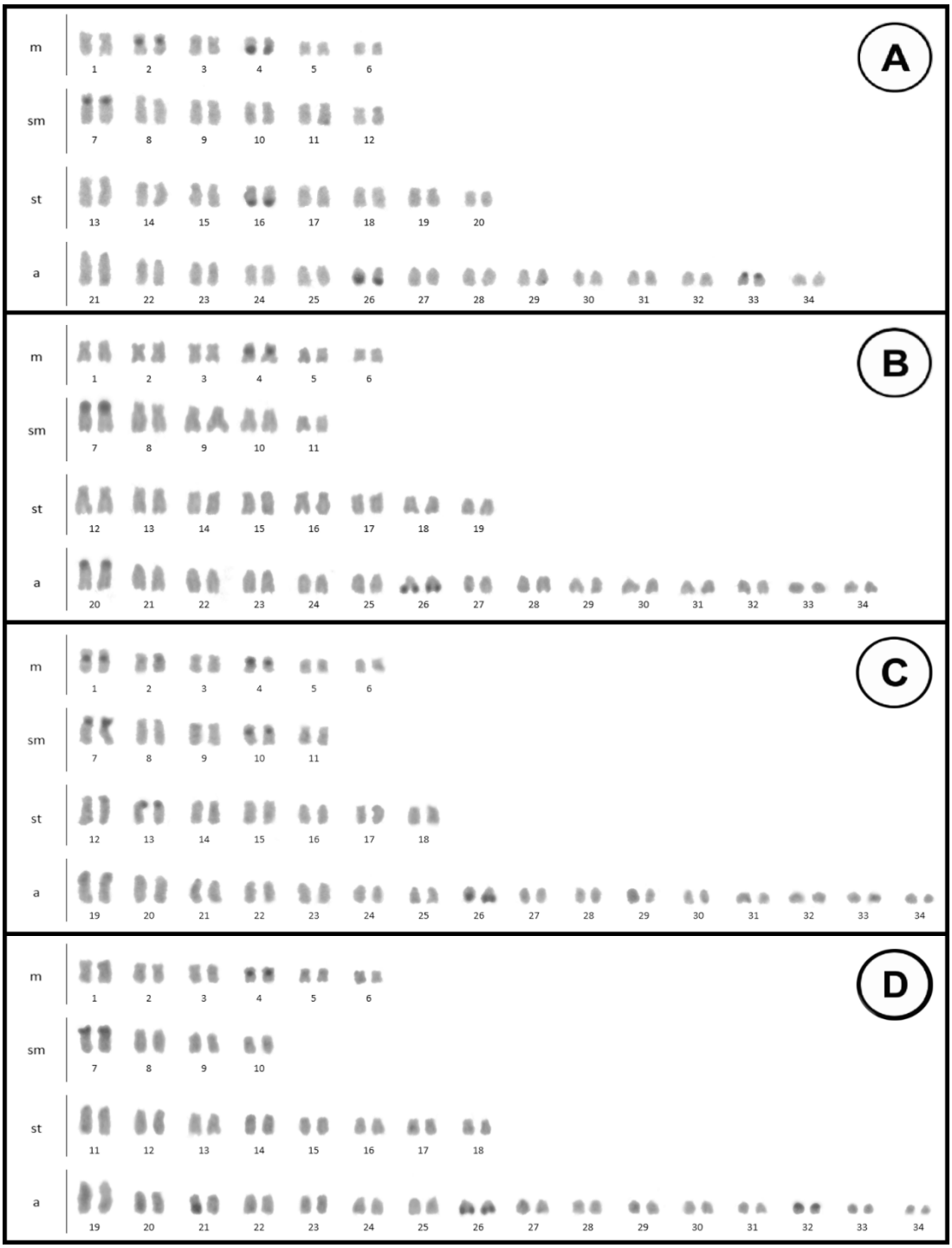

Figure 3: Karyotypes showing the constitutive heterochromatin pattern obtained by C-banding for the four populations of *Hypostomus ancistroides* from: A - Araras, B- Botucatu, C - Indaiatuba, and D - Terra Roxa.

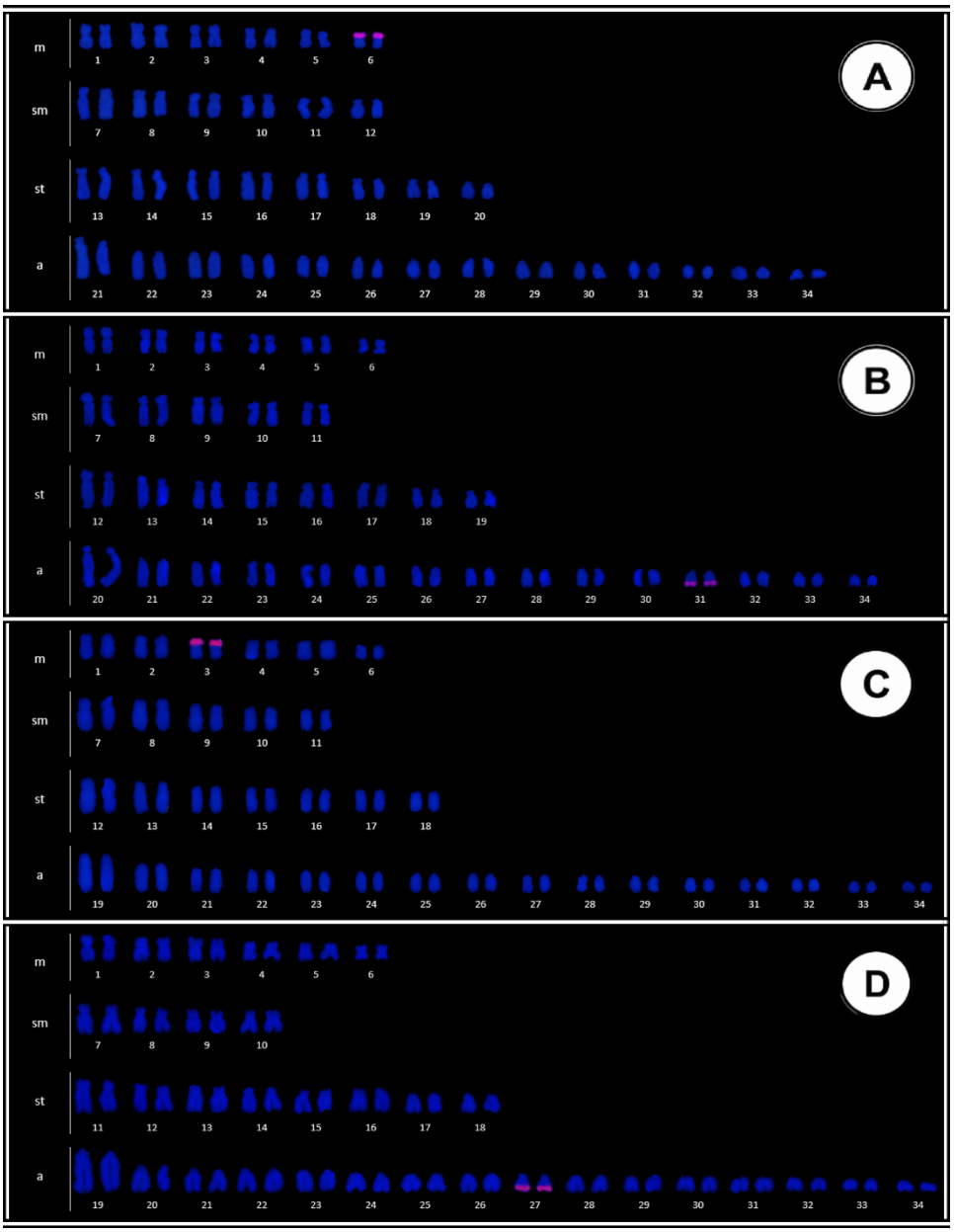

Figure 4: Karyotypes showing the rDNA 5S gene location observed by FISH for the four populations of *Hypostomus ancistroides* presented in this paper: A - Araras, B- Botucatu, C - Indaiatuba, and D - Terra Roxa.

The clusters verified by the configuration of the 5S rDNA sites (Figures 4, 5) are not supported by the molecular data. The tree generated by the Bayesian analysis of the concatenated genes (Figure 6), as well as the other phylogenetic analyses performed, always display two clusters, one formed by individuals from the populations of Araras and Terra Roxa and another by individuals from the Botucatu and Indaiatuba populations. The analyses also show the sharing of haplotypes within each group, but not between the two clusters. The populations of Botucatu and Indaiatuba both live in the Tietê river basin.

However, Araras and Terra Roxa populations are from several kilometers away and different river basins, without any connection.

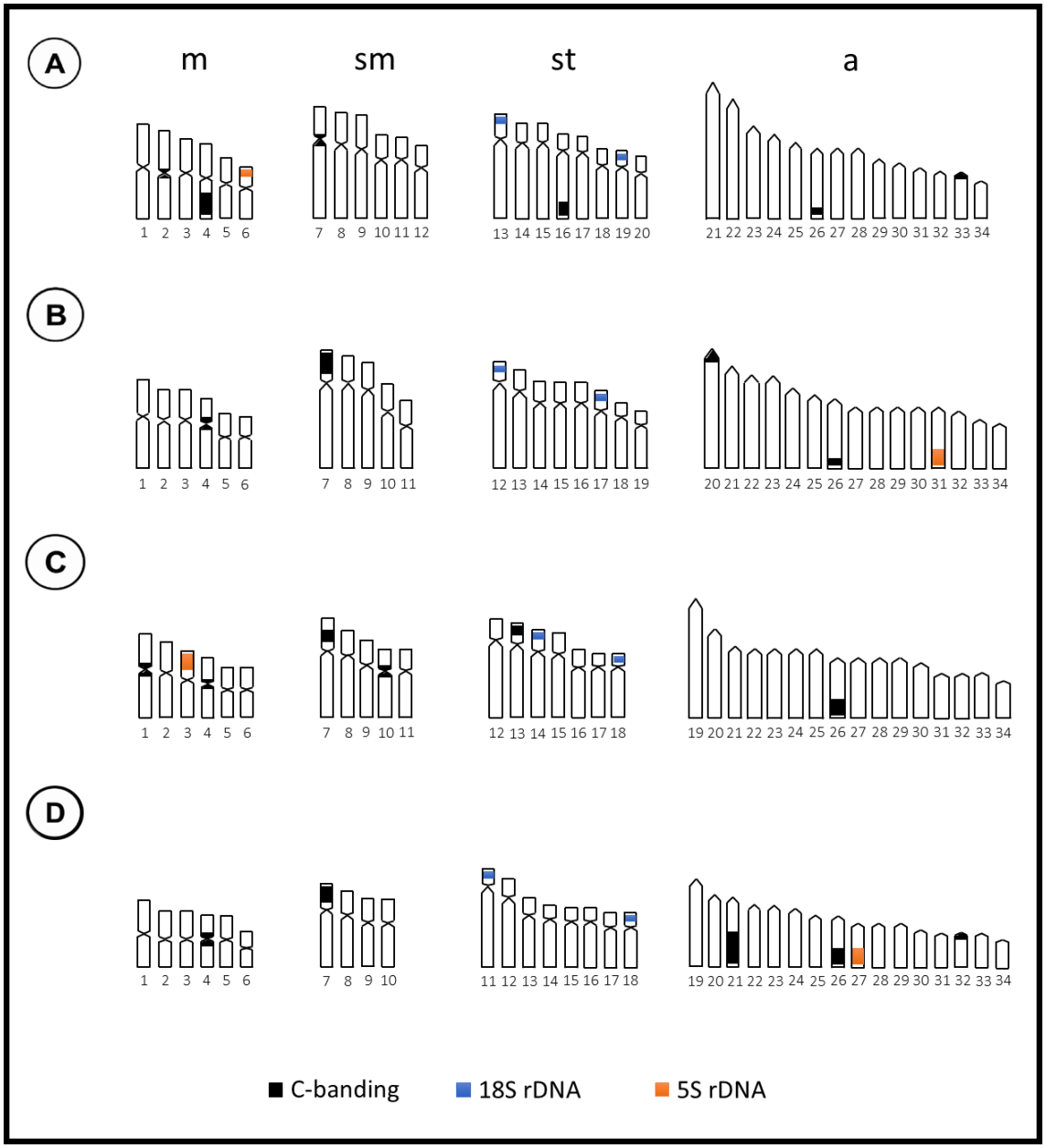

Figure 5: Ideograms summarizing the cytogenetic data observed for the four populations of *Hypostomus ancistroides* presented in this paper: karyotype constitution with karyotypic formula, C-banding, and rDNA 18S and 5S location by FISH. A - Araras, B- Botucatu, C - Indaiatuba, and D - Terra Roxa.

DISCUSSION

We herein introduce the concept of "Chromosomal Radiation," which explains not only the karyotype diversity observed in different populations of *Hypostomus*

ancistroides but also the diversity patterns observed for many years in the chromosomal studies of various organisms. This concept can be applied to the study of any group of cryptic species, chronospecies, or sister-species, which display low molecular and morphological divergence and large karyotype diversity, i.e., a rapid chromosome evolution.

We believe, therefore, that chromosomal radiation consists of a pattern in which the common ancestor of the group has the potential for karyotype plasticity, enabling the expansion of forms, with new populations presenting different karyotypes, which can be set independently by evolutionary and/or demographic processes. Under this explanation, chromosomal differentiation currently found in populations that do not always show signs of homology would be the result of two main processes: (i) the emergence of rearrangements that generate intrapopulation genetic variability in different lineages, without affecting the reproductive performance of individuals; and (ii) the fixation of different rearrangements independently in different populations over time, leading to the interpopulation variability observed. The pattern observed among populations caused by chromosomal radiation would be similar to that seen in cryptic species of *H. ancistroides* (from the literature and the present study), in which there is a diversity of karyomorphs with markers showing apparently homoplastic phenotypes, without necessarily occurring continuously across geographic distribution and between haplogroups. One explanation for this pattern is that the period of diversification and the time spent during population divergence were so brief that some karyomorphs and haplotypes may have been lost. Such losses prevent one from understanding the entire evolutionary panorama of a group, mainly due to the lack of intermediate forms. Thus, cryptic species complexes where there is a great diversity of karyomorphs with autapomorphies seem to have originated due to plasticity in the ancestral karyotype karyomorph. The multiple karyotypic forms found in cryptic species could constitute, therefore, important examples of non-adaptive or adaptive chromosomal radiation, depending on the relation of the character with the environment.

The role of chromosomal rearrangements in the diversification of species has been a subject of debate for many years, and there is evidence that unbalanced rearrangements can interfere in gametogenesis, reinforcing the reproductive isolation of karyomorphs by reduction of gene flow, as some species have increased tolerance to chromosomal rearrangements, maintaining polymorphic populations or possessing large karyotype plasticity (Pazza et al. 2018). Different models try to explain the fixation of chromosomal

rearrangements in populations with more or less “adaptation,” arguments involving demographic events and evolutionary processes (Rieseberg 2001; Navarro and Barton 2003; Hoffmann and Rieseberg 2008; Jackson et al. 2016). Regardless of the explanations for the appearance and fixation of the existing variation, there is a consensus that chromosomes play an important role in the speciation of several groups of organisms.

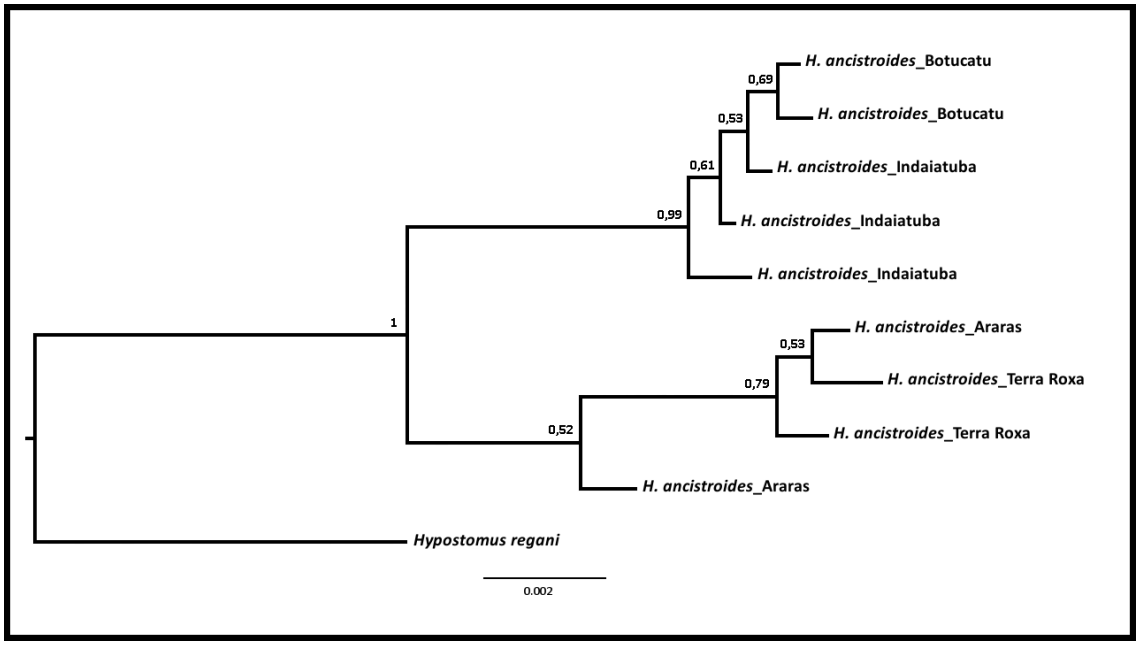

Figure 6: Bayesian tree obtained by concatenation of mitochondrial and nuclear genes sequenced from the four populations of *Hypostomus ancistroides*. Numbers in nodes represent posterior probability values. Sequences from *Hypostomus regani* was used as outgroup.

In theory, although frequently a causal factor of differentiation, the accumulation of chromosomal rearrangements can also be the result of evolutionary processes in natural populations. In groups where gene flow persists for a long time between different forms of a lineage, it is very difficult to assign reproductive isolation to chromosomal rearrangements. In species with large karyotype plasticity, for example, equids (Jónsson et al. 2014), the maintenance of gene flow between different karyomorphs appears to be possible, at least for some time. This may occur more often than we estimate, which would explain the discontinuity between molecular and karyotypic evolution seen in some groups with significant chromosomal diversity (Kavalco et al. 2016).

Almost all living organisms possess some karyotype plasticity, although, in certain groups, large plasticity can be considered a characteristic biological trait. This is the case

in several groups of fish, especially in the tetras of the genus *Astyanax* (Characiformes) and in the *Hypostomus* (Siluriformes) plecos, which may have led to the chromosomal radiation present in these groups (for a review see Pazza and Kavalco 2007; Rocha-Reis, et al. 2018). This was also observed in species of *Chromaphyosemion* (Cyprinodontiformes), especially in *C. riggenbachi* (Völker et al. 2006). The authors emphasize that karyotypic and haplotypic differentiation suggests speciation in its initial stages and that the karyotype differentiation in *C. riggenbachi* is an ongoing process, in which the rearrangements may be fixed by several processes such as natural selection, genetic drift, or meiotic impulse.

In other vertebrates, such as amphibians, reptiles, and mammals, large diversity associated with specious groups and with wide distribution can also be observed. Rodents are known to constitute a group with a large chromosomal diversity and several polymorphisms. At least seven cryptic species are observed in the African gerbil *Taterillus* spp., (Dobigny et al. 2001), distinguishable only by their chromosomal characteristics, with karyotypes displaying extensive chromosomal rearrangements (Dobigny et al. 2002a, 2002b). In neotropical Rodentia, extensive Robertsonian chromosome rearrangements, tandem fusions, fissions, and peri and paracentric inversions, besides heterochromatin polymorphisms, have been described. A recent review compiles data on the role of rearrangements in speciation and cytotaxonomy of South American species and demonstrates that the richness of distinct chromosomal forms makes it possible to delimit cryptic species in *Akodon*, *Calomys*, *Cerradomys*, *Euryoryzomys*, *Delomys*, *Hylaeamys*, *Juliomys*, *Neacomys*, *Oecomys*, *Oligoryzomys*, *Ctenomys*, *Thrichomys*, and *Trinomys* (Di Nizo et al.,2017). It is undeniable that the ancestors of these rodents had the potential for karyotypic plasticity since so many different species arose from the preexisting chromosome variation of natural populations. Thus, we see clear radiation of karyomorphs, most probably due to the pre-zygotic isolation that the chromosomal alterations may have caused.

One group in which there is significant chromosomal diversity and where radiation probably played an important evolutionary role is the genus *Drosophila*. Dobzhansky had already described polymorphisms in Drosophila chromosomes in the 1930s. In the 1970s more than 80 different karyomorphs were recognized as acting in the diversification of Hawaiian *Drosophila* spp., having been proposed phylogenies based on chromosomal rearrangements of the group since then. It seems a lot, but it is possible that this number is several-fold higher, since the adaptive radiation that led to the speciation in these

islands has generated over 700 species and the main chromosomal markers emerged after the 1970s. Similarly, the chromosomal variations described in groups of sister species or cryptic species of many other insects, including beetles, locusts, crickets and mosquitoes (for a historical overview see Dobzhansky 1947; White 1973) are historically extensive.

Chromosomal diversity is not exclusive to animals. In plants, besides hybridisms, numerical and structural polymorphisms and polyploidy events are implicated in the rapid speciation of various groups, with polyploidy considered a paramount adaptive mechanism. In Orchidaceae, one of the most numerous angiosperm families, groups of sister-species and cryptic species feature a large number of karyomorphs, such as *Epidendrum* (Nóbrega et al. 2017). Similarly, chromosomal diversity in the Asteraceae is substantial and seen in several genera, especially in the patterns of heterochromatin, where differences in the sizes and quantities of C+ bands appear to be related to the presence of rearrangements (see Marinho et al. 2016). In Liliaceae, there is evidence for the occurrence of fusions, fissions, translocations and inversions that generate a great diversity of karyomorphs in several genera, altering karyotypic symmetry (Peruzzi et al. 2009). Karyomorph radiation from an ancestor of high intrinsic diversity, naturally selected or fixed by genetic drift, explains all these patterns.

Several diversifications may contain elements of both adaptive and non-adaptive radiation (Rundell and Price 2009). Similarly, we do not rule out the possibility that there may be an adaptive character in certain chromosomal features of organisms, including *H. ancistroides*, although much of the variation observed appears to be non-adaptive. The maintenance of the diploid number in the plecos in this study, for example, seems to be a group tendency, since the great majority of the studied populations present 2n = 68 chromosomes (Artoni and Bertollo 1996; Alves et al. 2006; Bueno et al. 2012; Rubert et al. 2011; Endo et al. 2012; Alves et al. 2012; Fernandes et al. 2012; Pansonato-Alves et al. 2013; Traldi et al. 2013; Lara-Kamei et al. 2017) and this could represent an adaptive and ancestral state. The exception for this character is an isolated population in the Tibagi River (Maurutto et al. 2012) and a case of a new species of the *H. ancistroides* complex carrying a differentiated sexual chromosomal system, which is very divergent from the others (Rocha-Reis et al. 2018).

Another characteristic that seems to be conservative in the group of *Hypostomus ancistroides* cryptic species and may be adaptive is the presence of at least two sub-telocentric or acentric chromosome pairs carrying the 18S rDNA cistrons, a trend observed in several studies (see Rubert et al. 2011; Pansonato-Alves et al. 2013; Traldi et

al. 2013; Bueno et al. 2014; Lara-Kamei et al. 2017; this study). Thus, it seems quite plausible that we can identify adaptive and non-adaptive traits in karyotypes of groups of sister-species and accept that chromosomal radiation can cover both adaptive and non-adaptive characters, not being mutually exclusive.

Chromosomal variations in *Hypostomus* are not restricted only to the karyotype formulae, occurring also in patterns of location of the constitutive heterochromatin. Although variable when observing the totality of the karyotype macrostructure, the presence of C-banding in some chromosomes of all karyomorphs may indicate the existence of some homology (Rubert et al. 2011; Fernandes et al. 2012; Maurutto et al. 2012; Pansonato-Alves et al. 2013; Traldi et al. 2013; Lara-Kamei et al. 2017; Rocha-Reis et al. 2018), and may even be considered a phylogenetic sign. In this study, we observed the presence of conserved C+ blocks in pairs of chromosomes 4 (m), 7 (sm) and 26 (a), in a pericentromeric or subterminal region (Figures 2 and 4). Other markings, however, were quite autapomorphic. Individuals of *H. ancistroides* from Terra Roxa displayed polymorphisms related to heterochromatin distribution (Figure 2D - pair 21). The absence of blocks in one of the homologs reveals the likely occurrence of unequal exchanges during cell division, where a part or the entire heterochromatin block is translocated to another chromosome. It is interesting that such polymorphisms are observed, especially in heterokariotypes, since the major part of the polymorphisms appears in heterozygosity in populations, and, depending on demographic events and evolutionary processes, can be fixed or eliminated over generations, usually after overcoming subdominance (Hoffmann and Rieseberg 2008; Kirkpatrick 2010) or by selection or genetic drift in small populations (Spirito 1998). This indicates not only that there is variation in the population, but it is in overt process of chromosome evolution.

The dynamics of dispersion of repetitive sequences in chromosomes is often associated with the presence of active transposable elements (TE) in the genomes (Silva-Neto et al. 2015), which could explain the different patterns in the distribution of heterochromatin blocks and rDNAs 5S cistrons in different pairs of chromosomes. rDNA sequence analyses have demonstrated the existence of these elements in spacer regions, disseminating gene families in functional copies or pseudogenes (Drouin et al. 1995; Gornung 2013; Rebordinos et al. 2013; Symonová et al. 2013). The dispersion of TEs (and consequently of ribosomal DNA) could then affect the rate of recombination in the genomes and lead to rapid divergence of the karyotype/genome, as observed in the salmonids *Coregonus albula* and *Coregonus fontanae* (Symonová et al. 2013).

The discontinuity between phylogeography constructed from the sequence of four genes (mitDNA and nDNA) and 5S rDNA phenotypes in *H. ancistroides* could be a result of convergence in the location of ribosomal cistrons, generated by translocations in both clusters (Araras + Terra Roxa and Botucatu + Indaiatuba). However, the idea that several derived karyomorphs can be produced from one pluripotent karyomorph as a result of the intrinsic karyotype plasticity of the species is more parsimonious, there being multiple possible forms for each type of chromosome character, and the reality that the chromosomes carrying the sites of rDNA 5S are not necessarily homologous to that of the ancestral karyomorph. That is, each new arrangement would be independent in the analyzed forms since they do not appear to be lineages of the same direct ancestor. This would explain not only the distribution pattern of this gene but also the existence of different karyotype formulas and heterochromatic blocks not shared between populations. Chromosomal radiation would, therefore, have generated different karyomorphs in allopatric populations, which may or may not share a haplotype lineage, depending on whether they originated at different times or distributed differently during the genesis of the hydrographic basins

The data presented in this study reinforce the idea that there is a genome-intrinsic karyotype plasticity in some groups. Together with the wide distribution of usually cryptic evolutionary units, chromosomal diversity shows a pattern similar to that of a species radiation, which we conceptualized as chromosomal radiation, which can be both adaptive and non-adaptive.

The recognition of the concept developed in our article may facilitate the discussion of topics related not only to evolution and karyotypic speciation among or within populations, but also to chromosomal evolution itself, including the processes of emergence of variant karyomorphs and specialized chromosomes, such as sex chromosomes, in an eco-evolutionary context.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflict of interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

DATA ACCESSIBILITY STATEMENT

DNA sequences will be deposited in GenBank after acceptance of the manuscript.

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