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Karyotype analysis of three species of *Corydoras* (Siluriformes: Callichthyidae) from southern Brazil: rearranged karyotypes and cytotaxonomy

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The genus *Corydoras* comprises a diversity of species with different diploid numbers. We compared cytogenetic data among *Corydoras* species from different rivers of the Ponta Grossa Arch region in southern Brazil. *Corydoras ehrhardti* and *C.* aff. *paleatus* have a similar karyotype formula and the same diploid number (2n = 44). *Corydoras lacrimostigmata* has a higher diploid number, with 2n = 58 chromosomes. Fluorescence *in situ* hybridization using 5S and 18S ribosomal DNA probes suggests that these ribosomal DNA sequences are involved in chromosomal rearrangements in these *Corydoras* species. 5S rDNA is a chromosomal marker that is considered to be unique to the species analyzed in this study. Signals of interstitial telomeric sites are seen in a chromosome pair of *C. lacrimostigmata*, suggesting chromosomal rearrangements via fusions or translocations. This study revealed that *C. ehrhardti* and *C.* aff. *paleatus* have exclusive chromosomal markers associated with chromosome differentiation, which we speculate to prevent genetic introgression.

Keywords: Cytosystematics, Heterokaryotypes, Karyotype description, rDNA, Vicariance.

Corydoras compreende um gênero diversificado com espécies de diferentes números diploides. Nós comparamos dados citogenéticos de espécies de *Corydoras* de diferentes rios da região do Arco de Ponta Grossa no sul do Brasil. *Corydoras ehrhardti* e *C.* aff. *paleatus* tem fórmula cariotípica similar e o mesmo número diploide (2n = 44). *Corydoras lacrimostigmata* tem um número diploide maior, com 2n= 58 cromossomos. A hibridação *in situ* fluorescente (FISH) com sondas de DNA ribossomal 5S e 18S sugere que estas sequências de DNA ribossomal estão envolvidas em rearranjos cromossômicos nestas espécies de *Corydoras*. A marcação do DNAr 5S foi considerada espécie-específico para as espécies analisadas neste estudo. Sinais de sítios teloméricos intersticiais foram vistos em um par de cromossomos de *C. lacrimostigmata* sugerindo a ocorrência de rearranjos cromossômicos como fusões ou translocações. Este estudo revelou que as espécies *C. ehrhardti* e *C.* aff. *paleatus* têm marcadores cromossômicos exclusivos associados à diferenciação cromossômica, os quais, em nossa hipótese, podem prevenir a introgressão gênica.

Palavras chave: Citossistemática, Descrição cariotípica, Heterocariótipos, rADN, Vicariância.

Introduction

Corydoras Lacépède, 1803 (Callichthyidae: Corydoradinae), is a species-rich genus that comprises 216 species (Eschmeyer, Fong, 2016). Chromosome diploid numbers in this genus vary widely, ranging from 2n = 40 in *Corydoras nattereri* Steindachner, 1876 (Oliveira *et al*., 1990), to 2n = 134 in *Corydoras aeneus* (Gill, 1858) (Turner *et al*., 1992). This variation is thought to be the consequence of chromosomal rearrangements caused by polyploidization, inversions, and Robertsonian fusion and fission events (Oliveira *et al*., 1990, 1992, 1993; Shimabukuro-Dias *et al*., 2004). Chromosomal rearrangements are usually associated with reduced recombination by producing unbalanced chromosomes via meiotic crossing over in the rearranged segment. The resulting heterokaryotypes fail to fully segregate during meiotic pairing, and as a result, prevent genetic introgression in the rearranged regions. This may play a role in reproductive isolation and speciation (Navarro, Barton, 2003; Faria, Navarro, 2010).

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To date, only six species of *Corydoras*, from disparate river basins of southern Brazil, have been investigated cytogenetically. Like the genus as a whole, these species show considerable variation in diploid number (2n = 44, 2n = 46, 2n = 58, and 2n = 66), chromosome structure, and karyotype formula (Oliveira *et al*., 1993; Artoni *et al*., 2006; Rocha *et al*., 2016). Although these chromosome variations make *Corydoras* a promising model for the study of karyotype evolution, molecular analyses of the genus are relatively lacking. Nucleolar organizer regions (NORs) sites have been mapped in all six southern Brazilian species for which karyotypes are available (Oliveira *et al*., 1993; Artoni *et al*., 2006). *In situ* localization of both 5S and 18S rDNA sequences has been performed in a single species, *Corydoras carlae* Nijssen & Isbrücker, 1983 (Rocha *et al*., 2016).

Here, we set out to collect and compare cytogenetic and associated molecular data for several species of *Corydoras* from a geographical region near the Ponta Grossa Arch (Fig. 1). This region includes the headwater boundaries of the Tibagi River (Upper Paraná River), the Ribeira River (Atlantic drainage), the Iguaçu River (Lower Paraná River), and a sub-tributary of the Ivaí River (Upper Paraná River) located near the left bank of the Tibagi headwater. The Ponta Grossa Arch is characterized by elevation changes resulting from adjustments at faults along the eastern margin of the underlying platform. Reactivation of these ancient rifts has led to vertical movements between blocks, promoting the capture of adjacent upland drainages and faunal interchange (Ribeiro, 2006). This is reflected by the occurrence of species typical of lowland Atlantic coastal rivers in upland regions of the upper Paraná, such as *Mimagoniates microlepis* (Steindachner, 1877), *Hyphessobrycon griemi* Hoedeman, 1957 (Characidae) and *Trichomycterus davisi* (Haseman, 1911) (Trichomycteridae).

In this study, we focus on three southern Brazilian *Corydoras* species: *C. ehrhardti* Steindachner, 1910, *C. lacrimostigmata* Tencatt, Britto & Pavanelli, 2014, and *C.* aff. *paleatus* (Jenyns, 1842). *Corydoras ehrhardti* occurs in rivers of the Atlantic basins of the Paraná and Santa Catarina states, including the Iguaçu River and Tibagi River. The geographical range of *Corydoras lacrimostigmata* is limited to the Ivaí River basin (Tencatt *et al*., 2014). *Corydoras* aff. *paleatus* occurs throughout the Lower Paraná River, where the species was scientifically described, including the Iguaçu River. *Corydoras* aff. *paleatus* is also found in the Tibagi River, presumably as a result of former headwater river capture events (Artoni *et al*., 2006, 2009).

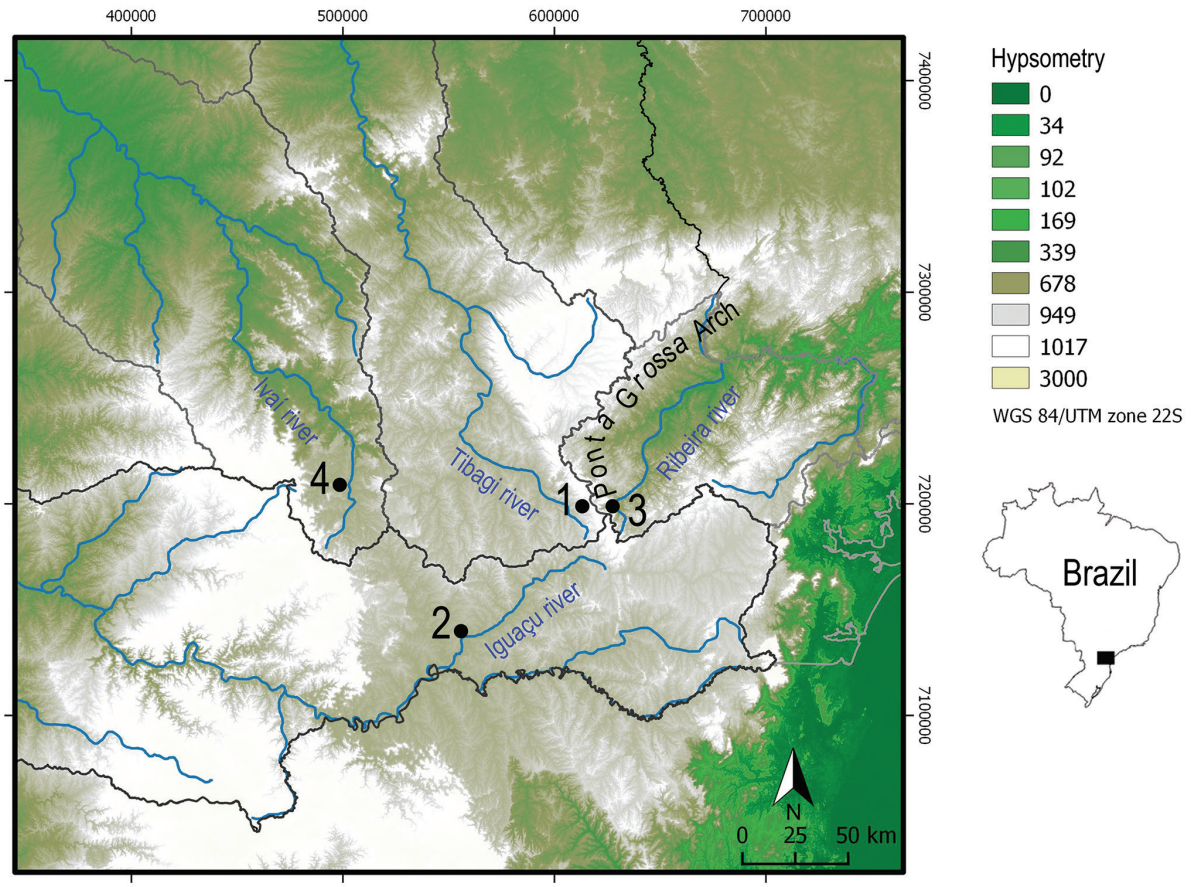

Fig. 1. Map of the Ponta Grossa Arch region in the eastern Paraná State, Brazil (inset). The map shows elevation and hydrographic basins. Details of sampled locations: (1) Verde River, Tibagi basin; (2) Iguaçu River, Iguaçu basin; (3) Areia stream, Ribeira River, Atlantic basin; and (4) Barra Grande River, Ivaí River basin. Source: Miranda (2005).

Comparison of karyotypes associated with chromosomal markers has become the method of choice for the analysis of structural chromosome reorganization (Oliveira *et al*., 2016). Here, we combine classical cytogenetic methods with molecular approaches to describe the cytogenetic characteristics of *C. ehrhardti*, *C. lacrimostigmata*, and *C.* aff. *paleatus*, and to seek evidence for chromosome rearrangements. Classical cytogenetic methods utilized herein include Giemsa staining, C-banding (which exposes the heterochromatic regions), and karyotyping techniques to describe the karyotype arrangement of the species. We also utilize Fluorescence in situ hybridization (FISH), which permits the mapping of molecular markers by localizing DNA probes with a specific sequence within a target sequence (Pinkel *et al*., 1986). FISH techniques are a powerful tool for the localization of chromosomal rearrangements, especially with site-specific probes that can identify rearranged regions when it is difficult to detect banding patterns in a karyotype (Pucci *et al*., 2014).

Material and Methods

This study is based on cytogenetic data from 68 specimens of the three species of *Corydoras* from the region of the Ponta Grossa Arch (Fig. 1). These include 24 specimens of *C. ehrhardti* from the Verde River (Tibagi basin - Upper Paraná River, Ponta Grossa, PR, 25°04'40"S 50°04'12"W); 14 specimens of *C.* aff. *paleatus* from the Iguaçu River (Iguaçu basin - Lower Paraná River, São Mateus do Sul, PR, 25°53'27.46"S 50°21'47.94"W); 12 specimens of *C.* aff. *paleatus* from the Areia stream (Ribeira River - Atlantic basin, Ponta Grossa, PR, 25°08'31"S 49°51'55"W); and 18 specimens of *C. lacrimostigmata* from the Barra Grande River (Ivaí basin - Upper Paraná River, Prudentópolis, PR, 24°58'40.72"S 51°7'34.25"W). Specimens were deposited in the Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia) of the Universidade Estadual de Maringá, Maringá, Brazil (voucher numbers: *C. lacrimostigmata*, NUP 17835; *C. ehrhardti*, NUP 17836; *C.* aff. *paleatus*, NUP 17837, Iguaçu River, and NUP 17838, Ribeira River). Research was conducted in accordance with the Ethical Committee for Animal Use (process number: 13/2014) of the Universidade Estadual de Ponta Grossa, Brazil.

Chromosomes were extracted from kidney cells following air-drying procedures (Bertollo *et al*., 1978), applying the modifications of Blanco *et al*. (2012). C-banding was performed using the Sumner (1972) technique. We used three types of repetitive probes as chromosomal markers: (1) an 18S rDNA probe (approximately 1,800 bp), which was synthetized by polymerase chain reaction (PCR) according to Hatanaka, Galetti Júnior. (2004); (2) a 5S rDNA probe synthetized by PCR according to Martins, Galetti Júnior. (1999); and (3) a probe targeting the universal vertebrate telomere sequence, the minisatellite (TTAGGG)*n,* synthetized by PCR according to Ijdo *et al*. (1991). The 18S rDNA probe was labeled with biotin-11-dUTP (Roche Applied Science), while the 5S rDNA and (TTAGGG)*n* probes were labeled with digoxigenin 11-dUTP by nick translation.

FISH was performed under very stringent conditions (2.5 ng/μL of probe, 50% formamide, 2xSSC, and 10% dextran sulfate for 18 h at 37°C), according to the method of Pinkel *et al*. (1986). Signal detection was enabled using an anti-streptavidin antibody conjugated to Alexa Fluor 488 (Molecular Probes, Carlsbad, CA, USA) and an anti-digoxigenin antibody conjugated to rhodamine (Roche Applied Science). The chromosomes were counterstained with 0.2 $\mu g \cdot mL^{-1}$ of 4′,6-diamidino-2-phenylindole (DAPI) in Vectashield mounting medium (Vector, Burlingame, CA, USA) and analyzed using an Olympus BX41 epifluorescence microscope equipped with the DP71 digital image capture system (Olympus, Tokyo, Japan). Chromosomes were identified using the system proposed by Levan *et al*. (1964) and classified as either metacentric (m) or submetacentric (sm).

Results

Conventional cytogenetic analysis showed that the populations/species of *C. ehrhardti* (Tibagi River basin), *C.* aff. *paleatus* (Iguaçu River basin), and *C.* aff. *paleatus* (Ribeira River basin) have a diploid number (2n) of 44 chromosomes, a karyotype formula of 18m + 26sm, and a fundamental number of chromosome arms (FN) of 88 (Figs. 2a-c, respectively). In contrast, *C. lacrimostigmata* (Ivaí basin) has a diploid number of 58 chromosomes, a karyotype formula of 22m + 36sm, and an FN of 116 (Fig. 2d).

C-banding exposed heterochromatic blocks, including around the centromere, on the chromosomes of all three species/populations (Fig. 2). *Corydoras ehrhardti* presented large blocks of pericentromeric heterochromatin on metacentric pairs 3, 5, 6, and 8 and submetacentric pairs 10, 14, and 17 (Fig. 2a). In the Iguaçu River basin population of *C.* aff. *paleatus*, constitutive heterochromatin was observed in the pericentromeric region of the metacentric pairs 2, 3, 7, and 9 and submetacentric pairs 10 and 14 (Fig. 2b). In the Ribeira River basin population of *C.* aff. *paleatus*, pericentromeric heterochromatic blocks were observed on metacentric pairs 2, 3, 5, 7, and 9 and submetacentric pairs 10, 14, and 16 (Fig. 2c). In *C. lacrimostigmata*, pericentromeric heterochromatic blocks were observed on metacentric pairs 2, 4, 6, and 11 and submetacentric pairs 14 and 19. In addition, a small interstitial band was detected on the long arm of pair 17 (Fig. 2d).

In *Corydoras ehrhardti*, FISH with 18S and 5S rDNA probes showed a syntenic location on the terminal and proximal positions of the long arm of the metacentric pair 3 (Fig. 3a). In *C.* aff. *paleatus* (Iguaçu basin), the 18S rDNA sites were located on the metacentric pair 5 and submetacentric pair 11, both on the terminal position of the long arm, while the 5S rDNA site was revealed to be at the proximal region of the short arm of the submetacentric pair 20 (Fig. 3b). In *C.* aff. *paleatus* (Ribeira basin), the 18S rDNA was found at the terminal end of the long arm of the metacentric pair 5, while the 5S rDNA was detected at the

proximal region of the long arm of the submetacentric pair 12 (Fig. 3c). Finally, in *C. lacrimostigmata,* the 18S rDNA was at the terminal region of the short arm of submetacentric pairs 20 and 25, while the 5S rDNA was revealed to be at the terminal end of the long arm of metacentric pair 4 (Fig. 3d).

The telomeric probe (TTAGGG)*n* showed uniform signals on the telomeres of all chromosomes of all species analyzed here (data no shown). Additionally, in *C. lacrimostigmata*, the (TTAGGG)*n* probe showed small signals of interstitial telomeric sites (ITS) on the submetacentric pair 17, which matches the location of a heterochromatic block (Fig. 3, in detail).

Discussion

We analyzed the karyotype organization of three *Corydoras* species. We found that *C. ehrhardti* and both populations of *C.* aff. *paleatus* have identical diploid chromosome numbers and karyotype formulae; however, heterochromatin (Fig. 2) and 18S and 5S rDNA FISH analyses (Fig. 3) revealed divergences in the location of chromosome regions. Such divergence has also been observed in both allopatric and sympatric populations of other species (Tab. 1, Fig. 4).

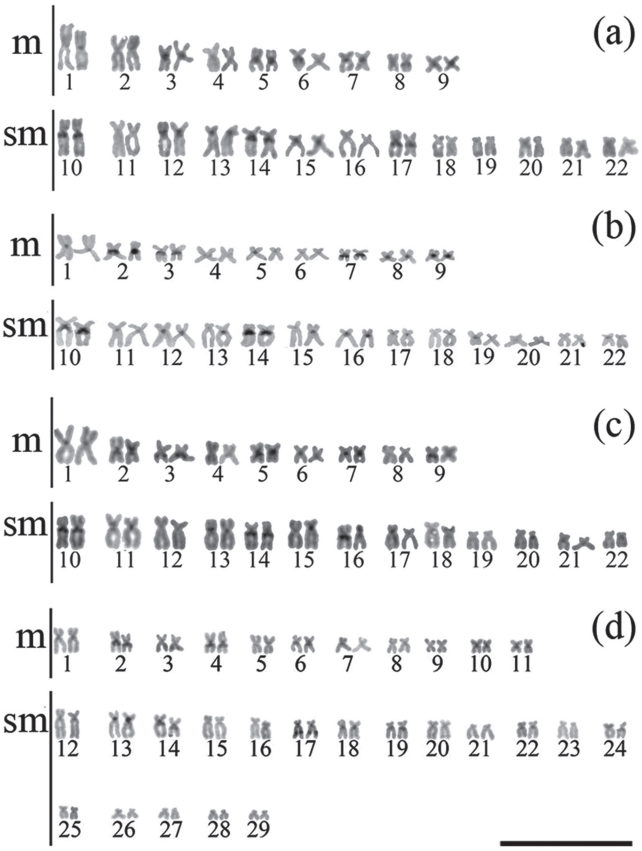

Fig. 2. Karyotypes of the *Corydoras* species subjected to C-banding: (a) *C. ehrhardti* (Verde River), (b) *C.* aff. *paleatus* (Iguaçu River), (c) *C.* aff. *paleatus* (Areia stream), and (d) *C. lacrimostigmata* (Barra Grande River). Scale bar = 10 µm.

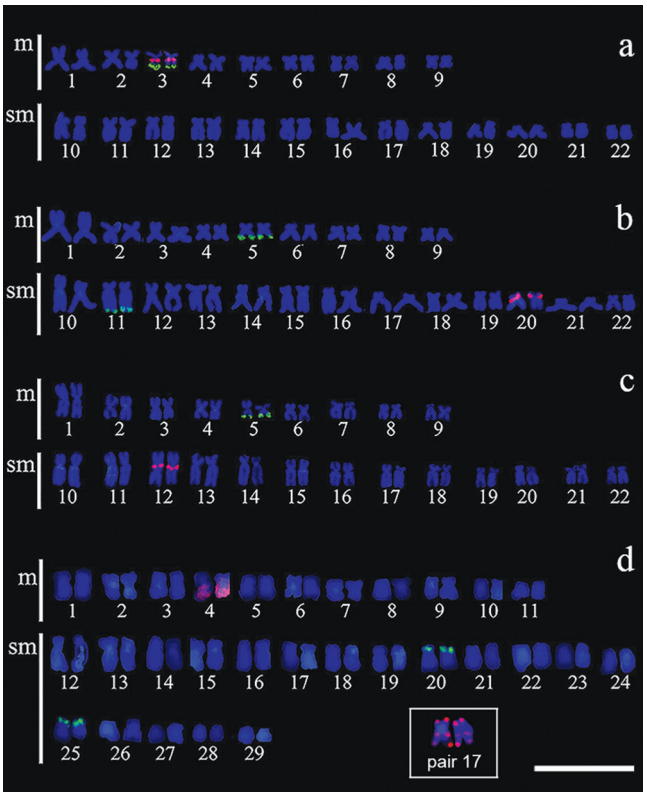

Fig. 3. Karyotypes of the *Corydoras* species subjected to two-color FISH with *18S* rDNA probes (green) and 5S rDNA probes (red): (a) *C. ehrhardti* (Verde River), (b) *C.* aff. *paleatus* (Iguaçu River), (c) *C.* aff. *paleatus* (Areia stream), and (d) *C. lacrimostigmata* (Barra Grande River). In detail, the chromosome pair of *C. lacrimostigmata* (Barra Grande River), carrier of ITS. Scale bar = 10 µm.

In the Tibagi population of *C. ehrhardti*, the constitutive heterochromatin blocks show a similar distribution to that found in a previous study of the Upper Tibagi (Artoni *et al*., 2006). Likewise, a similar pattern of heterochromatin has also been reported from an allopatric Atlantic drainage population from the coastal region of the Santa Catarina State, Brazil (Oliveira *et al*., 1993). *Corydoras* aff. *paleatus* populations of the Ribeira and Iguaçu river basins (this study) and populations from the Atlantic drainage of southern Brazil (Oliveira *et al*., 1993) and the Tibagi River basin (Artoni *et al*., 2006) present consistent differences in the number and localization of the heterochromatin blocks in their karyotypes.

Corydoras lacrimostigmata (Ivaí River basin) is morphologically similar (albeit with some diagnostic differences) to its congeneric species *Corydoras flaveolus* Ihering, 1911 (Tencatt *et al*., 2014). Its chromosomal heterochromatin blocks and the 18S rDNA sites on two chromosomal pairs are similar to those visualized with Ag-NORs in a previous study of *C. flaveolus* (Oliveira *et al*., 1992). From a geological viewpoint, *C. lacrimostigmata* of the Ivaí basin is unlikely to have been involved in the headwater capture events of Ponta Grossa Arch. It might,

Tab. 1. Cytogenetic data for *Corydoras* species from southern Brazil. 2n= diploid number; PR = Paraná State; SC = Santa Catarina State; RS = Rio Grande do Sul State; M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric; 18S = number of 18S rDNA sites; 5S = number of 5S rDNA sites; NOR = number of 18S rDNA sites detected by Ag-NOR staining. * The river was not identified in the original paper.

Species	Locality	2n	Karyotypic Formula	Number of rDNA Sites	References
C. ehrhardti	Verde river, Tibagi basin, Upper Paraná	44	18m+26sm	18S/1 pair; 5S/1 pair	Current study
C. ehrhardti	Lagoa Dourada, Tibagi basin, Upper Paraná	44	18m+26sm	18S/1 pair; -	(Artoni *et al*., 2006)
C. ehrhardti	*Jaraguá do Sul, Brazil, Atlantic basin	44	18m+26sm	18S/2 pairs; -	(Oliveira *et al*., 1993)
C. paleatus	Iguaçu river, Iguaçu basin, Lower Paraná	44	18m+26sm	18S/1 pair; 5S/1 pair	Current study
C. paleatus	Areia stream, Ribeira basin, Atlantic basin	44	18m+26sm	18S/2 pairs; 5S/1 pair	Current study
C. paleatus	Lagoa Dourada, Tibagi basin, Upper Paraná	44	18m+26sm	18S/3 chromosomes; -	(Artoni *et al*., 2006)
C. paleatus	*Curitiba, PR, Brazil, Iguaçu basin	44	20m+24sm	NOR/3 pairs; -	(Oliveira *et al*., 1993)
C. paleatus	*São Leopoldo, RS Brazil, Atlantic basin	44	20m+24sm	NOR/2 pairs; -	(Oliveira *et al*., 1993)
C. paleatus	Rio Grande, RS, Brazil, Atlantic basin	44	22m+22sm	NOR/2 pairs; -	(Oliveira *et al*., 1993)
C. lacrimostigmata	Barra Grande river, Ivaí basin, Upper Paraná	58	22m+36sm	18S/2 pairs; 5S/1 pair	Current study
C. nattereri	*Morretes, PR, Brazil, Atlantic basin	44	18m+26m	NOR/1 pair; -	(Oliveira *et al*., 1993)
C. barbatus	*Morretes, PR, Brazil, Atlantic basin	66	38m+22sm+4st+2a	NOR/4 pairs; -	(Oliveira *et al*., 1993)
C. barbatus	*Jaraguá do Sul, SC, Brazil, Atlantic basin	66	38m+22sm+4st+2a	NOR/ 3 pairs; -	(Oliveira *et al*., 1993)
C. carlae	Capanema river, Iguaçu basin, Lower Paraná	46	22m+22sm+2st	18S/1 pair; 5S/1 pair	(Rocha *et al*., 2016)

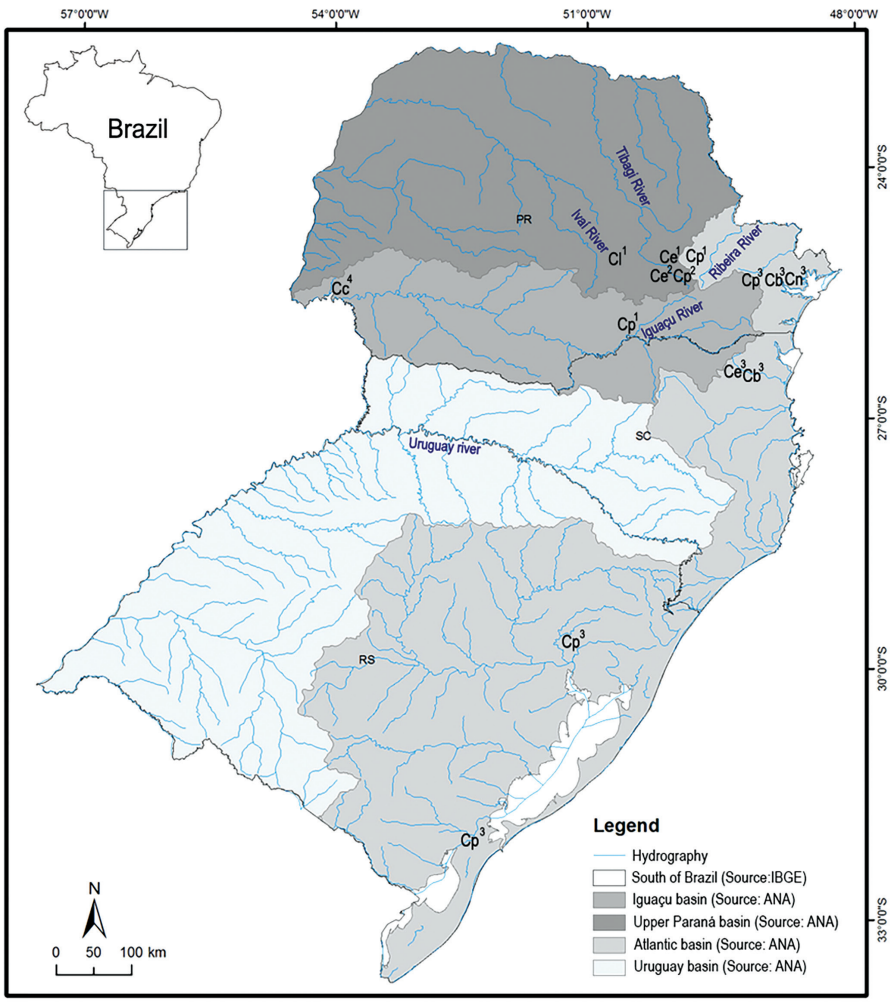

Fig. 4. Map of southern Brazil, highlighting the hydrographic basin boundaries and the location of cytogenetically studied *Corydoras* species. PR = Paraná State; SC = Santa Catarina State; RS = Rio Grande do Sul State; Cb = *S. barbatus*; CC = *C. carlae*; Ce = *C. ehrhardti*; Cl = *C. lacrimostigmata*; Cn = *C. nattereri*; Cp = *C.* aff. *paleatus*; [1] = Present study; [2] = Artoni *et al*. (2006); [3] = Oliveira *et al*. (1993); [4] Rocha *et al*. (2016); ANA= Agência Nacional das Águas and; IBGE = Instituto Brasileiro de Geografia e Estatística.

however, have differentiated in the headwaters of the Ivaí River, a sub-basin of the Upper Paraná River. In line with this, the karyotype organization of *C. lacrimostigmata* found here is similar to that described for *C. flaveolus,* a species located in another sub-basin of the upper Paraná River far from the Ponta Grossa Arch (Oliveira *et al*., 1992), albeit with some differences in the karyotype formula. A comparison of the karyotypes of *C. ehrhardti*, *C.* aff. *paleatus*, and *C. lacrimostigmata* shows some variation in the localization of heterochromatin. These are likely due to population-specific repetitive DNA accumulation in the absence of gene flow, a process previously suggested for other fish taxa (*e.g.*, Vicari *et al*., 2010; Pucci *et al*., 2014).

FISH analyses revealed a differentiated pattern of both location and number of 18S and 5S rDNA sites among the populations studied here. *Corydoras ehrhardti* was the only species in which the 18S and 5S ribosomal sites were syntenic. Previous studies of *C. ehrhardti* from the upper Tibagi River found a single chromosome pair carrying 45S rDNA (Artoni *et al*., 2006). However, the location of Ag-NORs in allopatric populations of *C. ehrhardti* from the Atlantic basin (Tab. 1) suggests additional chromosomal pairs bearing 45S rDNA (Oliveira *et al*., 1993), consistent with the occurrence of rearranged karyotypes. Chromosomal rearrangements, such as inversions, translocations, and transposon-mediated transpositions (Symonová *et al*., 2013), could explain the diversification of the major rDNA clusters in *Corydoras*.

A comparison of our observations in *C.* aff. *paleatus* with previous studies indicates some differences in the number of 18S rDNA sites. The occurrence of two chromosome pairs bearing 18S rDNA in the Iguaçu population of *C.* aff. *paleatus* is similar to what has been found in other populations from the Lower Paraná River (Tab. 1), while the Ribeira population of *C.* aff. *paleatus* only has a single 18S rDNA site. An analysis of *C.* aff. *paleatus* specimens from Lagoa Dourada (Upper Tibagi River) using an 18S rDNA probe revealed signals on three chromosomes (Artoni *et al*., 2006). Based on the premise of a historical interchange of the ichthyofauna in adjacent basins on the Ponta Grossa Arch, the specimen analyzed by Artoni *et al*. (2006) may represent an intraspecific hybrid between Tibagi and Ribeira populations of *C.* aff. *paleatus*.

In contrast, the 5S rDNA is a chromosomal marker that is specific to the species analyzed in this study. The localization of the 5S rDNA sites is divergent among *C. ehrhardti*, *C.* aff. *paleatus*, and *C. lacrimostigmata*. In *C. ehrhardti*, the *in situ* analysis of the 5S and 18S rDNA sequences revealed a syntenic localization of both rDNA sites on chromosome pair 3, albeit in independent clusters. In *C.* aff. *paleatus* (Upper Iguaçu and Upper Ribeira rivers) and in *C. lacrimostigmata*, 5S and 18S rDNA sequences were found to be non-syntenic and on different chromosomes, giving rise to heterokaryotypes due to chromosome rearrangements, such as translocations, inversions, and transpositions. According to Symonová *et al*. (2013), there is evidence to suggest that ribosomal DNA spreading is involved in chromosome rearrangements, thereby affecting recombination rates in both genomes and ultimately leading to a rapid genome divergence. Thus, the detection of the rearranged rDNA chromosome sites is an important source of evidence for mechanisms that could prevent genetic introgression.

Based on molecular data and variation in the diploid number, the genus *Corydoras* has been divided into five groups (Oliveira *et al*., 1992). *Corydoras ehrhardti* and *C. paleatus* have been classified as part of the same group (group 4), the origin of which Oliveira *et al.* (1992) attribute to polyploidization and chromosome number reduction. It has been suggested that in separate species maintaining the same diploid number and similar karyotypes, genetic introgression is limited by geographical barriers (Oliveira *et al.*, 1992). However, *C. ehrhardti* and *C.* aff. *paleatus* are found in sympatry in the Tibagi River basin (Artoni *et al*., 2006). The occurrence of different *Corydoras* species in sympatry could be a result of the historical headwater capture events and ichthyofaunal interchange of the Ponta Grossa Arch (Ribeiro, 2006). *Corydoras* aff. *paleatus* has not previously been reported to occur in the Ribeira River basin; its distribution across the Ponta Grossa Arch might be the recent result of headwater capture events between the Iguaçu and Ribeira basins.

Chromosome segregation failure and the ensuing production of unviable gametes due to the accumulation of chromosomal rearrangements might play an important role in speciation (Navarro, Barton, 2003; Faria, Navarro, 2010). In the same way, the genetic differences accumulated in the two divergent populations of *C.* aff. *paleatus* studied here could act as a meiotic barrier: A lack of synteny within the rearranged chromosome regions of heterokaryotypes inhibits crossover during meiosis, leading to irregular meiotic segregation and the production of genetically unbalanced germ cells. This leads to a reduced gene flow even after restoring contact between the two species.

It has previously been suggested that *C. ehrhardti* and *C. paleatus* have originated through polyploidization, inversion, and chromosome fusion events (Oliveira *et al*., 1992). Use of a telomeric probe failed to detect any vestiges of ITS in the chromosomes of *C. ehrhardti* and *C.* aff. *paleatus*, although it is possible that telomeric sequences have deteriorated or even been completely lost at the points of chromosomal rearrangements (Slijepcevic, 1998; Rosa *et al*., 2011; Primo *et al*., 2016). *Corydoras lacrimostigmata* has a diploid chromosome number of 58 (m/sm) and shows a weak ITS signal on a submetacentric pair, corroborating the occurrence of chromosomal rearrangements in *Corydoras* (Oliveira *et al.*, 1992). The ITS site is localized on pair 17 and corresponds to a heterochromatic block at the same position. It can be classified as heterochromatic-ITS (het-ITS), meaning that it might represent possible fission points due to rearrangements, in which the telomeric

repeats were retained after the reorganization. Furthermore, these het-ITS are considered sites of spontaneous and induced chromosome breakage, with the capacity to lead to further chromosome reorganization (Ruiz-Herrera *et al*., 2008; Barros *et al*., 2017).

In conclusion, we observed specific differences in chromosomal markers among *C. ehrhardti*, *C.* aff. *paleatus*, and *C. lacrimostigmata* with respect to heterochromatin distribution, chromosome mapping of 18S and 5S rDNA, and the distribution of telomeric sequences and presence of ITS. Our cytogenetic assessments revealed the accumulation of differences between populations of *C.* aff. *paleatus* from the Ribeira, Upper Paraná, and Lower Paraná River basins, which we hypothesize to be associated with rearrangements under conditions of reduced gene flow. To determine if these differences act as a mechanism of reproductive isolation, further analyses are needed. These could include tests for reduced gene flow and recombination within chromosomal rearrangements, in particular near the breakpoints, or a test for signatures of selection within chromosome rearrangements where adaptation drives the process. The mapping of genes in rearranged regions remains a challenge, especially in unknown genomes.

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