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Cytogenetic analysis in catfish species of the genus *Peckoltia* Miranda Ribeiro, 1912 (Teleostei: Siluriformes: Loricariidae)

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Abstract. This study describes the karyotypes of three species of the genus *Peckoltia* (Loricariidae: Ancistrini). Fishes were collected in the Jari (*Peckoltia* sp. 1 and *Peckoltia* sp. 2) and Xingu rivers (*Peckoltia vittata* (Steindachner, 1881)) in the Amazon rainforest. Karyotypes were 2n = 52 for *Peckoltia vittata* (FN=102: 16 metacentrics (m), 20 submetacentrics (sm), 14 subtelocentrics (st), 2 acrocentrics (a)) and *Peckoltia* sp. 2 (FN=102: 32 m + sm, 18st, 2a). *Peckoltia* sp. 1 (FN=102: 44 m + sm, 6st, 2a, 1B) had 2n = 53 due to a B chromosome. The species differ in chromosomal morphology. Nucleolar Organizer Regions (NOR) was identified within a distal region of the long arm of pair 9 in *Peckoltia vittata*, in pair 10 and in a homologue of pair 25 in *Peckoltia* sp. 1, as well as in pair 17 and in a homologue of pair 18 in *Peckoltia* sp. 2. Chromomycin A3 banding agreed with the location of the NORs. C-banding patterns revealed large non-centromeric heterochromatic blocks, probably of a common origin. Our results suggest a higher level of similarity between *Peckoltia vittata* and *Peckoltia* sp. 1.

Key words: phylogeny, fish, Loricariidae, cytogenetics, C-banding, NOR.

Introduction

Loricariidae are the largest family in the order Siluriformes, encompassing more than 700 species of Neotropical fish (Armbruster, Sabaj, 2002, http://silurus.acnatsci.org/ACSI/taxa/Genera_by_Family/Genera_Loricariidae.html, on 06 April 06, 2009). There has been some controversy regarding the number of families in Siluriformes and the taxonomic relationships among them. Armbruster (2004) classified Loricariidae as comprising five subfamilies: Hypoptopomatinae, Loricariinae, Neoplecostominae, Upsilodine and Hypostominae, with the tribes Corymbophanini, Rhinelepini, Hypostomini, Pterygoplichthini, and Ancistrini. Later Reis et al. (2006) proposed a sixth subfamily, Delturinae, to include the loricariid genera *Delturus* Eigenmann et Eigenmann, 1889 and *Hemipsilichthys* Eigenmann et Eigenmann, 1889. Loricariidae are

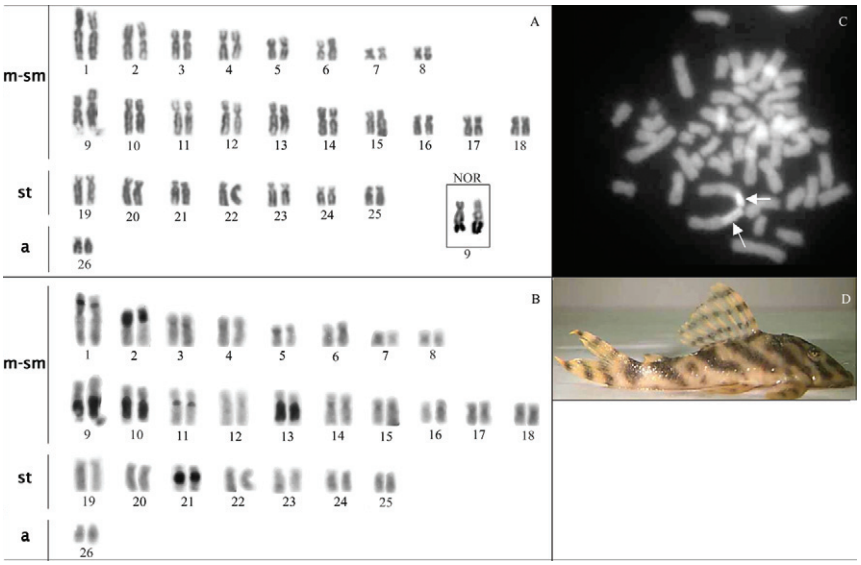

Fig. 1. Karyotype of a *Peckoltia vittata*, sequentially Giemsa-stained (**A**) and C-banded (**B**) chromosomes. The CMA_3-stained metaphase of the same specimen (**C**) revealed CMA_3-positive signals in the end-to-end associated NOR-bearing chromosome pair (arrows). The specimen was obtained from Xingu River (**D**).

found exclusively in Neotropical areas, ranging from Panama to Uruguay. The genus *Peckoltia* Miranda Ribeiro, 1912 (tribe Ancistrini) includes 19 species found in Brazil, Venezuela, Colombia and Peru (Burgess, 1989).

The relatively few cytogenetic studies of the tribe Ancistrini, including our own, have found that the diploid numbers of these fish range from 38 to 53, while the FN ranges from 68 to 104. The Table 1 shows this information as compiled by us. The aim of this paper was to study the karyotypes of three species in the genus *Peckoltia*. To our knowledge, this is the first description of karyotypes in this genus.

Material and methods

We studied four males and three females of the species *Peckoltia vittata* (Steindachner, 1908) (Fig. 1, D), which were caught in the Xingu River, in the town of Altamira and Para state (03°12′4″ N/52°12′41.7″ W). In addition, we studied one female of the taxon *Peckoltia* sp. 1 (Fig. 2, D) and one female of *Peckoltia* sp. 2 (Fig. 3, D), which were both caught in the Jari River, located in the town of Monte Dourado and Para state, at Cachoeira Santo Antonio (03°18′14.9″ N/52°03′29.3″ W). Fish specimens were vouchered at the Museu Paraense Emilio Goeldi (MPEG) with reference number MPEG 13427 to *Peckoltia vittata*, 1 MPEG 3420 to *Peckoltia* sp. 1 and MPEG 13426 to *Peckoltia* sp. 2.

Fish were processed using a yeast treatment (Cole, Leavens, 1971). Specimens were injected with 0.5 ml of a 0.025% colchicine solution per 100 g of body weight. After 30 minutes, metaphase chromosomes were prepared using the method described by Bertollo et al. (1978).

Chromosomes were classified morphologically as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a) according to the criteria established by Levan et al. (1964). Fundamental numbers (FNs) were de-

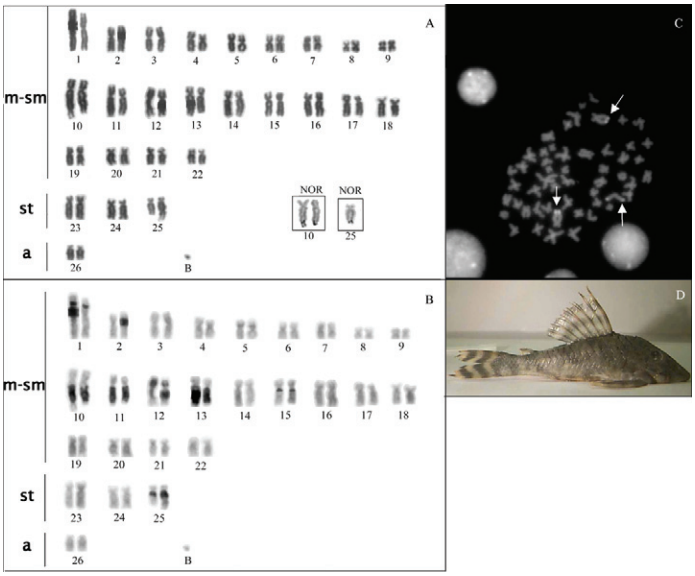

Fig. 2. Karyotype of a *Peckoltia* sp. 1, sequentially Giemsa-stained (A) and C-banded (B) chromosomes. The CMA_3-stained metaphase of the same specimen (C) revealed CMA_3-positive signals in the end-to-end associated NOR-bearing chromosome pair. The specimen was obtained from Jari river (D).

termined considering m, sm and st as bi-armed and acrocentric as one-armed chromosomes.

For each individual thirty metaphases were examined in order to determine the diploid number (2n), the FN, and to perform the banding analyzed.

C-banding was performed according to Sumner (1972). Nucleolar Organizer Regions (NOR) was performed according to Howell, Black (1980). Chromomycin A3 (CMA_3) banding was performed as described by Schmid (1980), with modifications in the pH of the Mcllvaine buffer (Lima, 1986) and use of methyl green as a counterstain (Donlon, Magenis, 1983).

For bright field microscopy analysis (Giemsa conventional staining, C-banding, Ag-NOR staining) we used a Zeiss Axiophot 2 microscope, 10x ocular lens and 100x objective lens. The images were captured with an Axiocam Mrm camera, controlled by the Zeiss AxioVision 3.1 software in a PC computer. For metaphases stained with CMA_3 we used the same microscope, camera and software, but illuminated with a HBO-100 Osram Mercury lamp. We used Zeiss filters BP 436, FT 460 and LP 470. Representative metaphases were captured digitally, using the Zeiss AxioCam MRm and AxioVision software.

Results

Peckoltia vittata has 2n = 52 chromosomes and a FN of 102 (36 m + sm, 14st, 2a) (Fig. 1, A). C-banding analysis revealed an interstitial block in the short arm of pair 1 and a large block that spanned most of the short arm of pair 2, the pericentromeric region of pair 11 and most of the long arms of pairs 9, 10, 13 and 21 (Fig. 1, B). NORs were identified in a distal position on the long arm of a large submetacentric chromosome of pair 9 (Fig. 1, A). Size heteromorphism of the NORs was revealed (Fig. 1, A). CMA_3 banding revealed a pair of chromosomes having rich G-C base

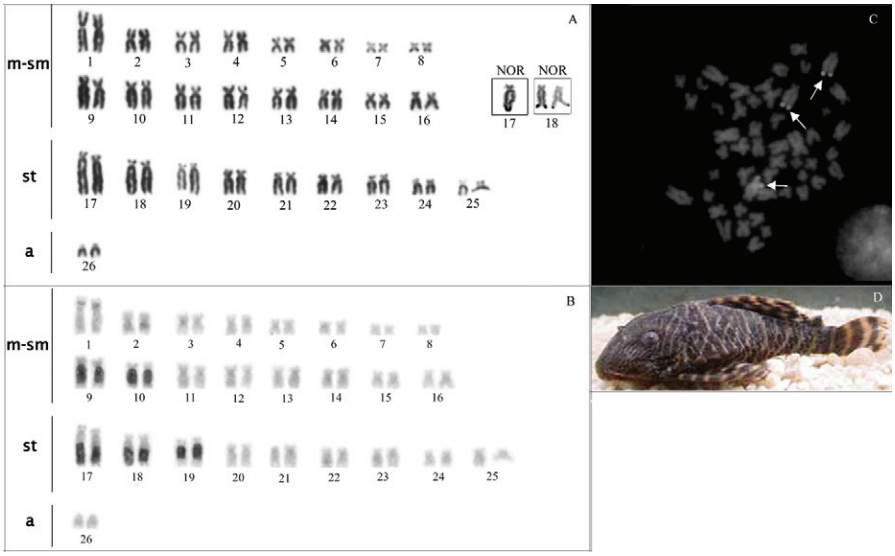

Fig. 3. Karyotype of a *Peckoltia* sp. 2, sequentially Giemsa-stained (**A**) and C-banded (**B**) chromosomes. The CMA_3-stained metaphase of the same specimen (**C**) revealed CMA_3-positive signals in the end-to-end associated NOR-bearing chromosome pair. The specimen was obtained from river Jari (**D**).

composition, in agreement with the location of the NORs (Fig. 1, C).

Peckoltia sp. 1 obtained from the Jari river has 2n = 52 chromosomes and a FN of 102 (44 m + sm, 6st, 2a; +1B). In addition, a small supernumerary B chromosome was observed in all metaphases of this specimen (Fig. 2, A). C-banding revealed an interstitial block that spanned the short arm of chromosome pair 1, most of the long arms of pairs 10, 11 and 13, most of the short arm of pair 12 and the long arms (i.e., close to the centromeres) of pairs 15 and 25 (Fig. 2, B). NORs were identified in a distal position on the long arm of pair 10 and in a homologue of pair 25. All NORs were C-band negative (Fig. 2, A). As we found in *Peckoltia vittata*, the NOR shows a size heteromorphism. CMA_3 banding was positive in three chromosomes, in agreement with the NOR findings, confirming that only one of the 25 homologues contains a NOR (Fig. 2, C).

Peckoltia sp. 2 obtained from the Jari river has 2n = 52 chromosomes and a FN of 102 (32 m + sm, 18st, 2a) (Fig. 3, A). C-banding revealed an proximal block in the short arm of pair 1 and in the long arm of pair 2 and in most of the long arm of pairs 9, 10, 17, 18 and 19 (Fig. 3, B). The heterochromatin block on the pair 19 has a size heteromorphism. NORs were identified in the distal region of the long arm of pair 18 and in a homologue of pair 17 (Fig. 3, A). CMA_3 banding was positive in three chromosomes, in agreement with the NOR findings, confirming that only one of the homologues of pair 17 has a NOR (Fig. 3, C).

Discussion

Our study revealed that *Peckoltia* has a diploid number of 52, consistent with most other members of the tribe Ancistrini. The number and size of ribosomal sites vary, but all are located in the distal regions of the long arms of specific chromosomes. The absence of NORs in one of the homologues may reflect a genetic deletion or a translocation. Each of the three

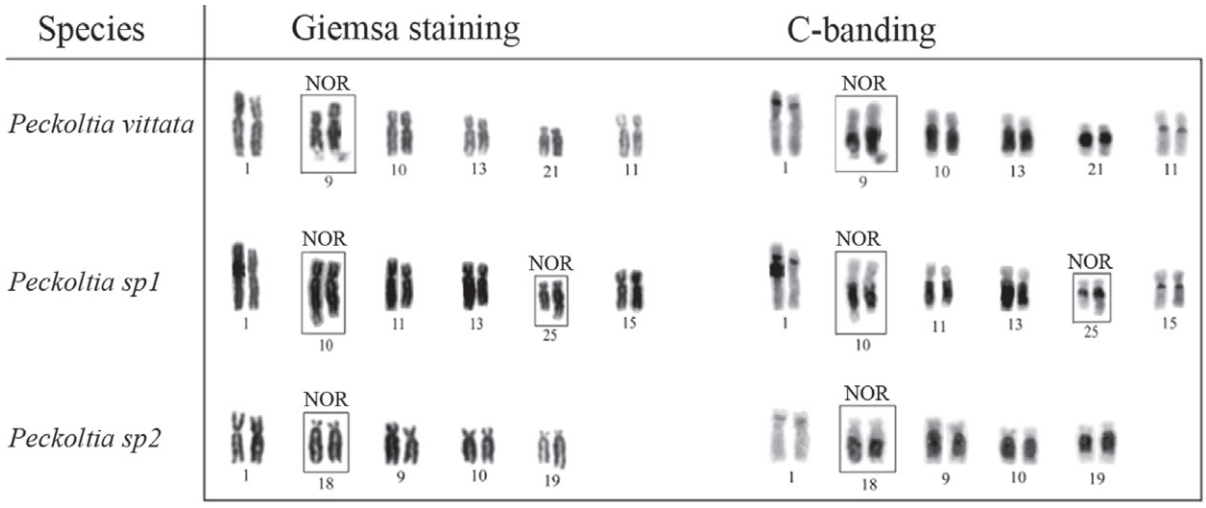

Fig. 4. Possible homologies among species of the *Peckoltia* genus, as determined via conventional staining, C-banding and NOR staining.

species had large heterochromatic blocks, probably of a common origin that covered almost all of the long arms of some submetacentric and subtelocentric chromosomes. The heterochromatic short arm identified in pair 2 of *Peckoltia vittata* may be a good marker for this species, as it was specific to this taxon. The analysis of chromosome morphology and heterochromatin distribution suggested that *Peckoltia vittata* chromosome pairs 1, 9, 10, 13, 21 and 11 correspond to *Peckoltia* sp. 1 chromosomes 1, 10, 11, 13, 25 and 15, and *Peckoltia* sp. 2 chromosomes 1, 18, 9, 10 and 19 (Fig. 4). Thus, the large amount of heterochromatin in *Peckoltia* probably originated after this genus split from its ancestor.

Analysis of constitutive heterochromatin distribution and NOR morphology revealed a higher level of similarity between *Peckoltia vittata* and *Peckoltia* sp. 1 than between either of them and *Peckoltia* sp. 2. This similarity is probably not related to geographical distribution, as *Peckoltia* sp. 1 and sp. 2 were collected in the Jari river while *Peckoltia vittata* was collected in the Xingu river. Considering the allopatric relationship among the species from the Xingu and Jari rivers, it is possible that a channel once connected these rivers and allowed the dispersion of species, which were later subjected to vicariance. Alternatively, a large population from one river may have been transferred to the other and then was later subjected to fragmentation via geological movements within in the Amazon. These movements might have created new geographical barriers, which would have limited the gene flow in this population.

After conducting an extensive review of Loricariidae karyotypes, Kavalco et al. (2005) suggested that the 2n = 54 karyotype be considered a plesiomorphic condition in this family. Armbruster (2004) published a simplified Loricariidae phylogeny, which is available at http://www.auburn.edu/academic/science_math/res_area/loricariid/fish_key/tree/tree.html. If we put on the phylogeny the diploid number already known for each genus we will find that the 2n = 52 karyotype is found al-

Table 1. Cytogenetic data on fish of the tribe Ancistrini (2n - diploid number of chromosomes, M – metacentric, SM – submetacentric, ST – subtelocentric, A – acrocentric, B - supernumerary chromosome).

Genus and species	2n	Karyotype	References
Ancistrus sp.	48	32m/sm, 16st/a	Artoni (1996)
Ancistrus sp.	50	26m/sm, 24st/a	Tchaicka, Margarido (1999)
Ancistrus sp.	52	32m/sm, 20st/a	Alves (2000)
Ancistrus sp.	52	28m/sm, 24st/a	Alves (2000)
Ancistrus sp. nov. 1	38	30m/sm, 8st	Alves et al. (2003)
Ancistrus multispinnis	52	28m/sm, 24st/a	Alves et al. (2003)
Ancistrus sp. nov. 2	52	32m/sm, 20st/a	Alves et al. (2003)
Ancistrus sp. nov. 1	40 39	40m/sm 39m/sm	Alves et al. (2006)
Ancistrus sp. nov. 2	52	26m/sm, 26st/a	Alves et al. (2006)
Ancistrus ranunculus	48	20m, 8sm, 6st, 14a	Oliveira et al. (2007)
Ancistrus sp.	52	16m, 8sm, 2st, 26a 16m, 9sm, 2st, 25a	Oliveira et al. (2007)
Hemiancistrus sp.	52	40m/sm, 12st/a	Artoni, Bertollo (2001)
Panaque prope *nigrolineatus*	52	46m/sm, 6st/a	Artoni, Bertollo (2001)
Baryancistrus prope *nivitatus*	52	48m/sm, 4st	Souza et al. (2004)
Hemiancistrus sp.	52	40m/sm, 12st/a	Artoni (1996)
Megalancistrus aculeatus	52	52m/sm	Artoni (1996)
Panaque prope *nigrolineatus*	52	46m/sm, 6st	Artoni (1996)
Peckoltia vittata	52	16m, 20sm, 14st, 2a	Present study
Peckoltia sp. 1.	53	18m, 26sm, 6st, 2a, 1b	Present study
Peckoltia sp. 2.	52	16m, 16sm, 18st, 2a	Present study

most exclusively in the most derived branches of the phylogeny which supports the Kavalco et al. (2005) suggestion.

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