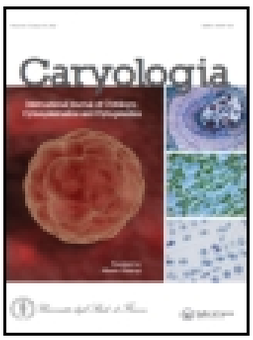

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First cytogenetic characterization of *Loricaria simillima* (Loricariidae, Siluriformes) from Paraná River (Argentina) with emphasis in cytotaxonomy of *Loricaria*

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ABSTRACT

This work aims to establish some cytogenetic characteristics of *Loricaria simillima* from Paraná River. The obtained results show that *L. simillima* has a $2n = 64$ diploid number, with 12 metacentric, 12 sub-metacentric and 40 sub-telo/acrocentric chromosomes. No chromosomal differences were found between sexes. The nucleolus organizer region (NOR) revealed by Ag-NOR was located next to the centromeric region in the short arm of pair 8. The C-bands were pale and detected in pericentromeric regions of the most chromosomes and interstitially on a sub-metacentric pair, coincident with secondary and NORs constriction. A conspicuous interstitial band on an acrocentric chromosome was also observed. Finally DAPI and CMA_3 fluorescent bandings showed G-C richness at the NOR region. Present data evidence a great similarity among *L. simillima*, *L. cataphracta* and *L. carinata*, reinforcing the hypothesis that they constitute a closely related phylogenetic group.

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Neotropical fish; Siluriformes; karyotypic characterization; chromosome banding; fluorescent staining

Introduction

The Neotropical family Loricariidae is one of the most diversified groups among the Siluriformes.There is incomplete knowledge about this family due to its great diversity and taxonomic complexity. Although cytogenetics is an effective tool for generating basic information, only ~1000 Neotropical fish species have been karyotypically characterized (Nirchio and Oliveira 2006). Only around 17% of Loricariidae have been cytogenetically studied (Bitencourt et al. 2012). These studies reveal an accentuated variation in the diploid number and variable chromosome banding patterns, suggesting that different chromosomal rearrangements and/or polyploidization events could have occurred along the speciation process of this family (Artoni and Bertollo 2001).

The genus *Loricaria* is one of the smallest genera in the subfamily Loricariinae, with 17 valid species (Eschmeyer et al. 2016). These species exhibit very similar morphological features and great taxonomic complexity. Liotta (2006) suggested that *L. simillima* is a synonym of *L. carinata*, while Reis et al. (2003), Ferraris (2007) and Eschmeyer et al. (2016) consider *L. simillima* a valid species and *L. carinata* as a junior synonym of *L. cataphracta*.

Cytogenetic studies of the genus *Loricaria* are scarce and only three nominal species have been characterized: *L. cataphracta* shows $2n = 64$ with polymorphism in localization and number of NORs (Porto et al. 2014a); *L. carinata* with $2n = 64$ (Fenocchio et al. 2003); and finally *Loricaria* sp., which also exhibits $2n = 64$ with 1–5 supernumerary chromosomes (Scavone and Ferreira-Julio 1994).

In view of the scarce available data for *Loricaria* and the potential of chromosome markers as additional tools for taxonomical identification, this work aims to characterize cytogenetically *L. simillima* and compare it with its studied congeners.

Materials and methods

Were analyzed 11 specimens of *L. simillima*, five males and six females. All of them were sampled from Paraná River (Argentina) from Corpus, Misiones (27°06′22.5″S; 55°31′20″W) to Ituzaingó, Corrientes (27°29′08.4″S; 56°40′35.35″W). The chromosome preparations were obtained by means of direct techniques (Moreira Filho and Bertollo 1991) and kidney cells cultures (Fenocchio et al. 1991). Slides were stained with Giemsa (10%, 10 min), analyzed with a Olympus CX-31 trinocular (Tokyo, Japan) and photographed with a Olympus C-5000 zoom digital camera. The diploid number was established by

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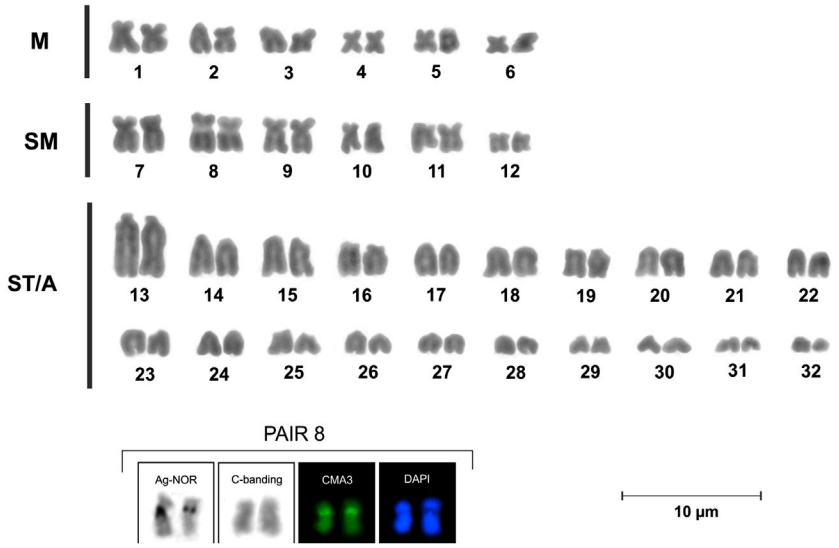

Figure 1. Giemsa stained karyotype of *Loricaria simillima*. The boxes show Ag-NOR, C-banding, CMA_3 and DAPI staining of the eighth pair.

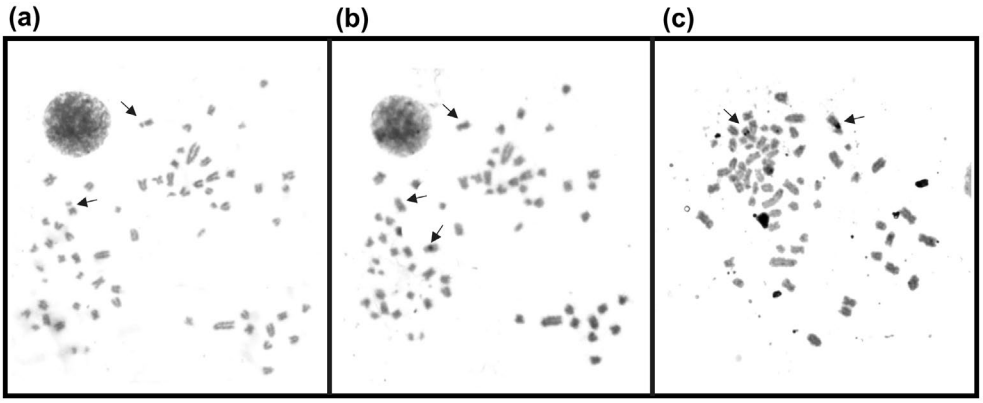

Figure 2. *Loricaria simillima*: (a) conventional Giemsa staining; arrows indicate the eighth pair. (b) C-banding technique; arrows indicate the NOR bearing pair and an acrocentric chromosome with conspicuous interstitial band. (c) Silver staining; arrows indicate the NOR bearing pair.

the analysis of 416 metaphases. Chromosomes were measured using the free software MicroMeasure 3.3 (Reeves 2001) and classified as suggested by Levan et al. (1964) with modifications of Artoni and Bertollo (2001). Karyotypes were organized in metacentrics (m), sub-metacentrics (sm) and sub-telo/acrocentric (st/a) types according to chromosome size and morphology (Klinkhardt 1998). The following were applied: differential staining, Ag-NORs according Howell and Black (1980), C-banding (Sumner 1972), chromomycine A_3 (CMA_3) and 4–6-diamino-2-phenilindol (DAPI) (Schweizer 1976).

Results

The karyotype of *L. simillima* presents $2n = 64$ with 12 metacentric, 12 sub-metacentric and 40 sub-telo/acrocentric chromosomes (Figure 1). The calculated fundamental number was FN = 88 and no karyotypic differences between males and females were found. The sub-metacentric eighth pair is characterized by the presence of a conspicuous secondary constriction at the short arm (p), near to the centromere that could be seen clearly with conventional staining (Figure 2(a)).

The C-banding revealed weak pericentromeric bands (Figure 2(b)) with the exception of an acrocentric chromosome with a conspicuous interstitial band. The NOR regions were also positive to C banding.

Staining with silver salts (Ag-NORs) revealed a pair of active nucleolus organizer regions (Figure 2(c)). They were located at the short arm of sub-metacentric eighth pair that is also characterized by the presence of a secondary constriction. In some cases only one chromosome of the pair showed argentic impregnation.

Fluorescent staining with CMA_3 and DAPI was used to detect G-C and A-T rich regions. The CMA_3 detected just a pair of bright marks located at the same chromosome pair than the NOR (Figure 3(a)). When the chromosome preparations were stained with DAPI, as expected, the bright CMA_3 band show a depletion

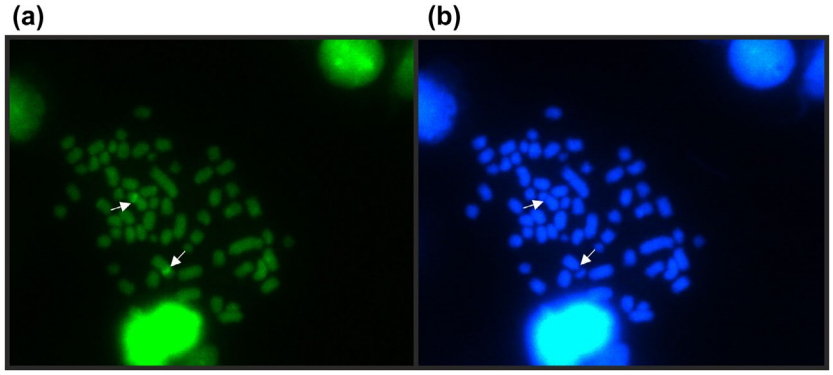

Figure 3. *Loricaria simillima* sequential fluorescent staining with: (a) CMA_3 and (b) DAPI. Arrows indicate the NOR bearing pair.

Table 1. Cytogenetic studies in genus *Loricaria*.

Species	Locality	2*n*	Karyotypic formulae	NORs	Reference
Loricaria sp.	Parana river, PR	64	10 m+6sm+ 4st+44a (B)	10p	1
L. carinata	Parana river, ARG	64	12 m-sm + 52st-a	–	2
L. cataphracta	Onça stream, MS	64	12 m+8sm+2st+42a	12q (IN)	3
	Onça stream, MS	64	12 m+8sm+2st+42a	8q (IN)	3
	Onça stream, MS	64	12 m+8sm+2st+42a	8q; 12q (IN)	3
	Onça stream, MS	64	12 m+8sm+2st+42a	9p;12q (IN)	3
	Onça stream, MS	64	12 m+8sm+2st+42a	12q; 13q (IN)	3
L. simillima	Parana river, ARG	64	12 m+12sm+40st/a	8p (IN)	4

2*n*: diploid number; NORs: nucleolus organizer regions; PR: Parana; MS: Mato Grosso do Sul; SP: Sao Paulo; ARG: Argentina; m: metacentric; sm: sub-metacentric; st: sub-telocentric; a: acrocentric; IN: interstitial; (B): supernumerary chromosomes; p: short arm; q: long arm.
References: (1) Scavone and Ferreira-Julio (1994) (2) Fenocchio et al. (2003); (3) Porto et al. (2014a); (4) this study.

of coloration, appearing as a gap in the chromosome 8 (Figure 3(b)). No other bright marks were detected with DAPI.

Discussion

Cytogenetic studies in genus *Loricaria* are scarce and reveal the presence of 64 chromosomes in three nominal species: *L. simillima* (present study), *L. cataphracta* (Porto et al. 2014a) and *L. carinata* (Fenocchio et al. 2003). The karyotypic formulae and fundamental number is distinct among these species (Table 1). *Loricaria simillima* presents the highest number of metacentric and sub-metacentric chromosomes (m/sm 24), similar to *L. cataphracta* (m/sm = 20) but distinct from *Loricaria* sp. and *L. carinata* which present 16 and 12 m/sm chromosomes respectively. A hypothesis to explain the differences in karyotype macrostructure but conservative diploid number within the species of the genus is based on the role of pericentric inversions. Meiosis analysis combined with telomeric hybridization probes will be useful to corroborate the hypothesis that inversion takes place in the karyotypic evolution of *Loricaria*.

Another feature reported is the presence of supernumerary chromosomes in one population of *Loricaria* sp. from Upper Parana River basin (Scavone and Ferreira Julio 1994); this numeric polymorphism is rare in Loricariinae and described only for *Rineloricaria pentamaculata* (Porto et al. 2011) and *Harttia longipinna* (Blanco et al. 2012).

The heterochromatin distribution pattern observed in *L. simillima* is characterized by a few blocks in pericentric regions and coincident with secondary constriction at NOR bearing pair. This pattern is similar to that described in *Loricaria* sp. (Scavone and Ferreira-Julio 1994), the only species with C-banding characterization. Porto et al. (2014a) claim that NOR bearing chromosomes were due to positive C-banding but they do not make comments on the heterochromatin distribution at the whole complement of *Loricaria cataphracta*. According to Takagui et al. (2014) the presence of a few heterochromatic blocks, mainly in the pericentric region, is a remarkable feature of armored catfishes of the subfamily Loricariinae, present in species of the basal genus *Harttia* (Kavalco et al. 2005; Blanco et al. 2012) as well as in the derived genus *Rineloricaria* (Rosa et al. 2011; Porto et al. 2014b). The exceptions to this pattern are *Farlowella amazonum* (Fernandes et al. 2015) and *Loricaria cataprhacta* (Porto et al. 2014a), which have additional conspicuous terminal heterochromatin blocks in different chromosomes.

In Loricariidae NORs in a terminal position do not occur frequently; however, Kavalco et al. (2005) mentioned that in the family around 25% of the studied species show interstitial NORs, as was reported in *Loricaria* sp., *L. cataprhacta* and in the present study (Table 1). According to Ziemniczak et al. (2012), NOR sites in only one chromosome pair in an interstitial position is a plesiomorphic condition in Loricariidae, because

this pattern is common in the sister group of the superfamily Loricarioidea (Trichomycteridae), as well as in Neoplecostominae and Hypoptopomatinae, which are basal subfamilies of Loricariidae (Andreatta et al. 1994, Armbruster 2004, Alves et al. 2005). In Loricariinae interstitial NORs occur in a few groups, included basal species of genus *Harttia* (Centofante et al. 2006, Blanco et al. 2012, Blanco et al. 2013, Blanco et al. 2014), *Loricariichthys anus* (Takagui et al. 2014), *Rineloricaria latirostris* (Giuliano-Caetano 1998) and *Loricaria* (Porto et al. 2014a; present study).

Comparisons of CMA_3 and DAPI banding with other studied species of the genus *Loricaria* are not possible, because there is no reference of this analysis in them. However, correspondence among Ag-NORs, CMA_3 positive marks and DAPI negative marks was observed in *L. simillima*. This is expected due the guanine and cytosine richness in the NOR region in vertebrates.

Taxonomists currently agree that *L. carinata* is a synonym of *L. cataphracta.* Although *L. cataphracta* is considered to be present in the Upper Paraguay River, according to Mirande and Koerber (2015) this species is not reported in Argentina. Differences on karyotypic formulae between *L. cataphracta* from Paraguay River and *L. carinata* reported by Fenocchio et al. (2003) from Paraná River are probably due a misidentification of the specimens. Two other sympatric species of *Loricaria* are cited in the Paraná River, so it would be possible that what Fenocchio et al. (2003) reported as *L. carinata* is instead *L. tucumanensis* or *L. luciae*.

Present data show a great similarity among *L. simillima*, *L. cataphracta* and *L. carinata*, reinforcing the hypothesis that they constitute a closely related phylogenetic group.

Disclosure statement

No potential conflict of interest was reported by the authors.

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