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First cytogenetic record for a species of *Otothyropsis* Ribeiro, Carvalho & Melo, 2005 (Loricariidae, Hypoptopomatinae)

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Hypoptopomatinae is a monophyletic subfamily that includes 147 species, distributed in 20 genera. *Otothyropsis* is a genus of Hypoptopomatinae, recently described. Here, we provided the first cytogenetic information of *Otothyropsis*. The specimens were collected from córrego Dourado, a small tributary of rio Iguatemi, which flows into rio Paraná. The specimens of *Otothyropsis* cf. *polyodon* were analyzed with respect to diploid number, C-Band and Ag-NOR patterns. The diploid number was 54 chromosomes, distributed in 18 metacentric, 28 submetacentric, and 8 subtelocentric chromosomes, with single Ag-NOR and conspicuous heterochromatic blocks on the short and long arms of the 24^{th} pair of chromosomes. Our study highlights the conservation trend of the diploid number (2n=54) and fundamental number (FN = 108) among the species of Hypoptopomatinae. However, the karyotype formula (18m+28sm+8st) seems to be specific to *O.* cf. *polyodon*, considering the other Hypoptopomatinae species already analyzed.

Hypoptopomatinae é uma subfamília monofilética que inclui 147 espécies distribuídas em 20 gêneros, sendo *Otothyropsis* um gênero recentemente descrito. Aqui, fornecemos a primeira informação citogenética do gênero *Otothyropsis*. Espécimes foram coletados no córrego Dourado, um pequeno tributário do rio Iguatemi, o qual deságua no rio Paraná. Espécimes de *Otothyropsis* cf. *polyodon* foram analisados em relação ao número diploide e padrões de Banda-C e Ag-NOR. O número diploide foi de 54 cromossomos, distribuídos em 18 metacêntricos, 28 submetacêntricos e 8 subtelocêntricos, com Ag-NOR simples e blocos heterocromáticos evidentes no braços curto e longo do par de cromossomos 24. Nosso estudo destaca a tendência de conservação do número diploide (2n=54) e número fundamental (NF=108) entre as espécies de Hypoptopomatinae. Entretanto, a fórmula cariotípica (18m+28sm+8st) parece ser específica para *O.* cf. *polyodon,* considerando as outras espécies de Hypoptopomatinae já analisadas.

Keywords: Ag-NOR, Chromosomal evolution, Freshwater fishes, Heterochromatin constitutive, Pericentric inversions.

Introduction

Among the Siluriformes, Loricariidae, one of the most specious families of Neotropical freshwater fish (Albert & Reis, 2011), has 906 valid species distributed in seven subfamilies: Hypoptopomatinae, Loricariinae, Hypostominae, Neoplecostominae, Lithogeninae, Delturinae, and Ancistrinae (Eschmeyer & Fong, 2015). Hypoptopomatinae is a monophyletic subfamily, that includes 147 species (Eschmeyer & Fong, 2015), distributed in 20 genera (Froese & Pauly, 2015). These species, popularly known as "cascudinhos", present small body sizes and are widely distributed in cis-Andean South America from Venezuela to Argentina, occurring in small to moderate-sized streams and rivers (Schaefer, 2003).

Otothyropsis is a genus of Hypoptopomatinae recently described by Ribeiro *et al*. (2005), currently including five species: *O. alicula, O. marapoama* and *O. polyodon* which occur in the upper rio Paraná drainage, and *O. biamnicus* and *O. piribebuy* which occur in tributaries of Iguaçu and Paraguai rivers, respectively. *O.* cf. *polyodon*, which is the focus of this study, differs from all congeners mainly by having a greater number of premaxillary and dentary teeth and lower caudal peduncle (Calegari *et al*., 2013).

Cytogenetic studies carried out on twenty two species belonging to ten genera of Hypoptopomatinae (Table 1) showed that this group has a relatively constant diploid number (2n=54), except to *Hisonotus gibbosos*, with 2n=58 chromosomes (Andreata *et al*., 2000) and *Otocinclus* aff. *vestitus*, with 2n=72 chromosomes (Andreata *et al*., 1994).

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Although most fish species do not display differentiated sex chromosomes, two systems involving male heterogamety (XY) in *Pseudotocinclus tietensis* (Andreata *et al*., 1992) and female heterogamety (ZW) in *Hisonotus leucofrenatus* (Andreata *et al*., 1993) and *Otocinclus* aff. *vestitus* (Andreata *et al*., 1994) were described in this subfamily.

Despite the absence of cytogenetic studies in *Otothyropsis* genus, this study defines the number of chromosomes, location of the Ag-NOR sites and C-positive heterochromatin in *Otothyropsis* cf. *polyodon*. General considerations about the chromosome evolution in the Hypoptopomatinae subfamily were also provided.

Material and Methods

Thirty-seven specimens of *Otothyropsis* cf. *polyodon* (10 males and 27 females) from córrego Dourado, Mato Grosso do Sul State, Brazil (23°51'04,9"S 54°25'13,9"W) were analyzed. This stream is a tributary of the right margin of the rio Iguatemi, which belongs to the upper rio Paraná basin (Fig. 1). Voucher specimens were deposited in the fish collection of the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia), Universidade Estadual de Maringá, PR Brazil, as *Otothyropsis* cf. *polyodon* (NUP 16171) (Fig. 2).

Before the evisceration process, the specimens were anesthetized by an overdose of clove oil (Griffiths, 2000). Metaphase chromosomes were obtained from anterior kidney cells using the air-drying technique (Bertollo *et al*., 1978). Analysis of the C-positive heterochromatin (C-bands) followed the basic procedure of Sumner (1972), with some minor adaptations. The NORs were detected by means of silver nitrate staining (Ag-NORs), according to Howell & Black (1980). The chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a) according to their arm ratio (Levan *et al*., 1964). For the determination of the fundamental number (FN), or number of chromosome arms, the m, sm and st chromosomes were considered as bearing two arms and the acrocentric chromosomes only one arm.

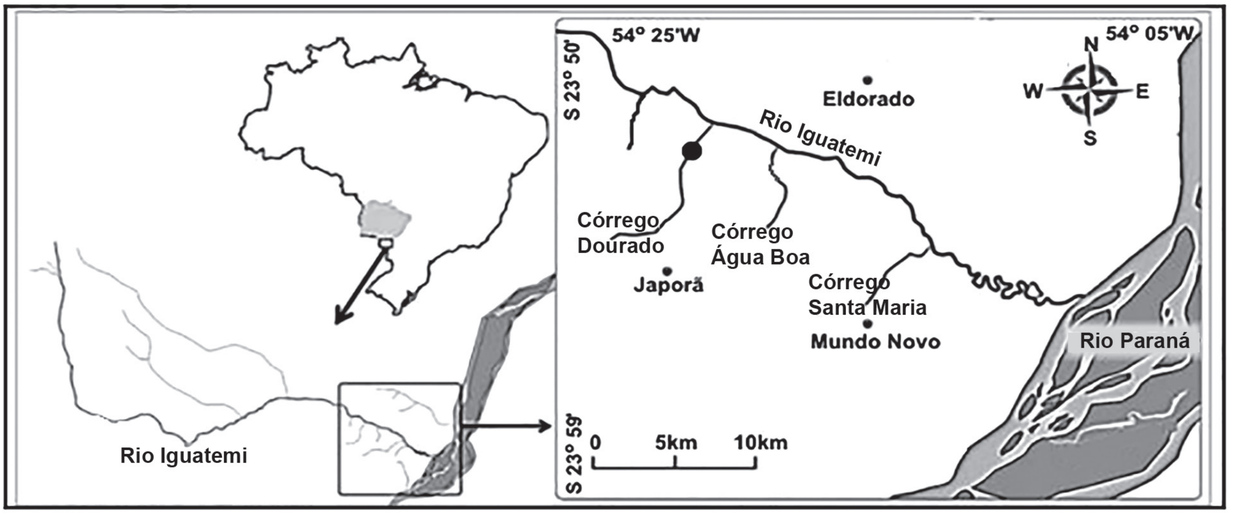

Fig. 1. Localization of córrego Dourado in the upper rio Paraná basin where specimens were captured. Black dot indicates the sampled point.

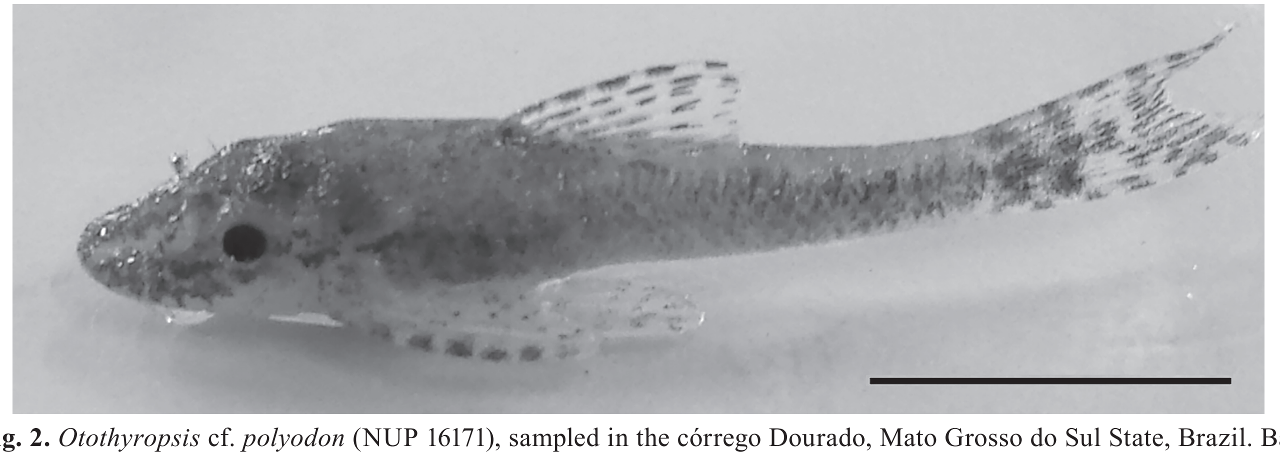

Fig. 2. *Otothyropsis* cf. *polyodon* (NUP 16171), sampled in the córrego Dourado, Mato Grosso do Sul State, Brazil. Bar = 10 mm.

Results

Otothyropsis cf. *polyodon* presented a modal diploid number of 54 chromosomes in males and females, and the karyotype contained 18 metacentric, 28 submetacentric, and 8 subtelocentric chromosomes (18m+28sm+8st), yielding a FN of 108 in both sexes (Fig. 3a). Heteromorphic sex chromosomes were not identified. A secondary constriction was evident in the median region of the long arm of the subtelocentric pair 24, which corresponds to the Ag-NORs location (Fig. 3a). Heterochromatic blocks were evident in the centromeric region of the pairs 1, 2, 5 and 25 and also in the short and long arms of the pair 24 (Fig. 3b).

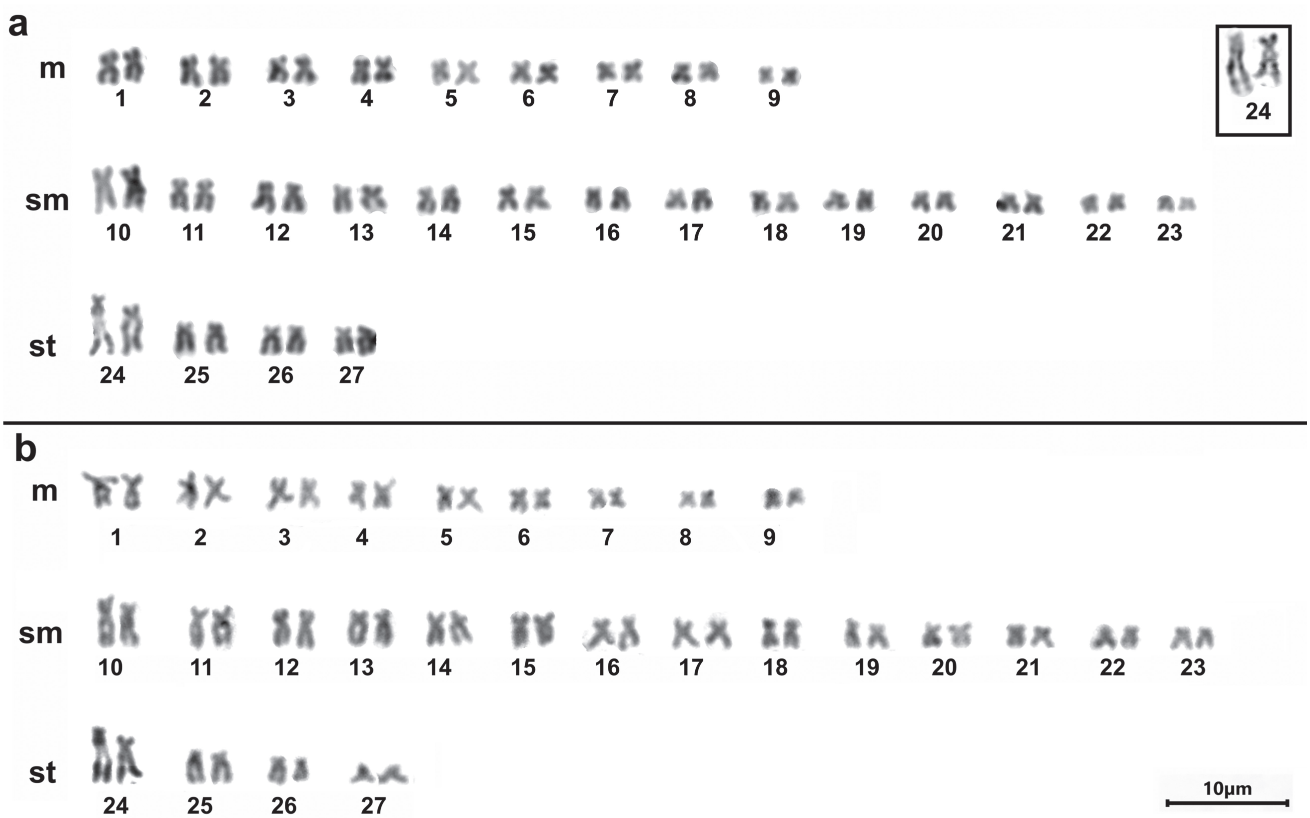

Fig. 3. Karyotypes stained with Giemsa (a) and C-banding (b) of *Otothyropsis* cf. *polyodon* from the córrego Dourado. Box: pair 24, bearing the NOR.

Discussion

The diploid number (2n=54) of *Otothyropsis* cf. *polyodon* is coincident to the diploid number of the most Hypoptopomatinae species. Likewise, the FN=108 is also found in approximately 75% of the investigated species and populations of this subfamily (Table 1). However, the karyotype formulae of *Otothyropsis* cf. *polyodon* (18m+28sm+8st) differs from the others Hypoptopomatinae and, so far, appears to be a unique feature of this species. In fact, despite the maintenance of the diploid number, rearrangements modifying the chromosomal morphology, such as pericentric inversions, have played a major role in the karyotypic evolution of the Hypoptopomatinae species.

This conservatism of the diploid number in the Hypoptopomatinae subfamily differs from the pattern of broad variation of diploid number observed in other subfamilies of Loricariidae. For example, for Hypostominae subfamily the diploid number ranges from 2n = 34 in *Ancistrus cuiabae* (Mariotto *et al*., 2009) and *Ancistrus* sp. *purus* INPA-25625 (Oliveira *et al*., 2009) to 2n = 84 in *Hypostomus* sp. 2 (Cereali *et al*., 2008); and for Loricariinae the diploid number range from 2n = 36 in *Rineloricaria latirostris* (Giuliano-Caetano, 1998) to 2n = 74 in *Sturisoma* cf. *nigrirostrum* (Artoni & Bertollo, 2001).

A single Ag-NOR pair is located on the first subtelocentric pair (no. 24), the largest chromosome in the karyotype of *O.* cf. *polyodon*, thus characterizing a simple NOR system. Simple NOR system was also detected in the others Hypoptomatinae species, except in *Otocinclus vittatus* and *Hisonotus* sp. A, which present multiple NORs (Table 1). It is noteworthy the big heteromorphism in size of the two homologous NORs, which may be due to unequal crossing-over between these chromosome regions or some other chromosomal rearrangements. Interestingly, this characteristic appears to be common for Hypoptomatinae species, as already reported by Andreata *et al*. (1994) and Camilo & Moreira Filho (2005).

Table 1. Summary of the cytogenetic data available for Hypoptopomatinae. 2n = diploid number; FN = fundamental number; m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric; NORs =nucleolar organizer regions. * Species with supernumerary chromosomes.

Species	Locality	2n	Karyotypic Formulae	FN	Pairs with NORs	Sex chromosomes	References
Corumbataia cuestae	Alambari River, São Paulo	54	34m+20sm	108	1		Cristina *et al*. (2005)
Corumbataia cuestae	Lapa Stream, São Paulo	54	28m+20sm+6st	108	1		Camilo & Moreira Filho (2005)
Corumbataia tocantinensis	Vermelho River, Goiás	54	28m+26sm	108	1		Cristina *et al*. (2005)
Hisonotus depressicauda	Santo Inácio River, São Paulo	54	14m+28sm+2st+10a	98	1		Andreata *et al*. (1994)
Hisonotus gibbosos	Betari River, São Paulo	58					Andreata *et al*. (2000)
*Hisonotus leucofrenatus**	Poço Grande River, São Paulo	54-56	♀24m+25sm+5st ♂24m+26sm+4st	108 108	1	ZW	Andreata *et al*. (1993)
*Hisonotus leucofrenatus**	Marumbi River, Paraná	54-56	♀24m+25sm+5st ♂24m+26sm+4st	108 108	1	ZW	Andreata *et al*. (1993)
Hisonotus leucofrenatus	Cavalo Stream, Santa Catarina	54	22m+24sm+6st+2a	106	1		Andreata *et al*. (2006)
Hisonotus sp. A	Alambari River, São Paulo	54	30m+20sm+4st	108	2		Andreata *et al*. (1993)
Hisonotus sp. A	Paraitinga River, São Paulo	54	26m+26sm+2st	108	1		Andreata *et al*. (2006)
Hisonotus sp. B	Moia Stream, São Paulo	54	22m+28sm+4st	108	1		Andreata *et al*. (1993)
Hisonotus sp. D	Grande Stream, São Paulo	54	26m+26sm+2st	108	1		Andreata *et al*. (2006)
Hisonotus nigricauda	Guaíba River, Rio Grande do Sul	54	26m+20sm+8st	108	1		Andreata *et al*. (2006)
Hypoptopoma guentheri	Piraí River, Mato Grosso	54	10m+18sm+8st+18a	90	1		Cristina *et al*. (2005)
Otocinclus affinis	Biguá River, São Paulo	54	46m+8sm	108	1		Andreata *et al*. (1994)
Otocinclus affinis	Bonito River, Rio de Janeiro	54	40m+12sm+2st	108	1		Andreata *et al*. (1994)
Otocinclus flexilis	Santo Antônio da Patrulha River, Rio Grande do Sul	54	36m+18sm	108	1		Cristina *et al*. (2005)
Otocinclus aff. *vestitus*	Livramento River, Pará	72	22m+12sm+4st+34a	110	1	ZW	Andreata *et al*. (1994)
Otocinclus vittatus	Cuiabá River, Mato Grosso	54	12m+10sm+14st+18a	90	1		Cristina *et al*. (2005)
Otocinclus vittatus	Taquari River, Mato Grosso do Sul	54	36m+18sm	108	2		Cristina *et al*. (2005)
Otothyris juquiae	Rio Preto Stream, São Paulo	54	32m+10sm+12st	108	1		Cristina *et al*. (2005)
Otothyris travassosi	Ribeira da Terra Firme River, Bahia	54	26m+16sm+12st	108	1		Cristina *et al*. (2005)
Otothyropsis cf. *polyodon*	Stream Dourado, Mato Grosso do Sul	54	18m+28sm+8st	108	1		Present study
Parotocinclus maculicauda	Poço Grande Stream, São Paulo	54	20m+32sm+2st	108	1		Andreata *et al*. (1994)
Parotocinclus maculicauda	Açungui River, Paraná	54	20m+20sm+14st	108	1		Ziemniczak *et al*. (2012)
Pseudotocinclus tietensis	Paranapiacaba River, São Paulo	54	♀28m+20sm+6st ♂27m+21sm+6st	108 108	1	XY	Andreata *et al*. (1992)
Pseudotocinclus n. sp	Juquiá River, São Paulo	54	22m+24sm+8st	108	1		Cristina *et al*. (2005)
Pseudotothyris obtusa	Itanhém River, São Paulo	54	26m+18sm+4st+6a	102	1		Andreata *et al*. (1994)
Schizolecis guentheri	Parati-Mirim Stream, Rio de Janeiro	54	30m+18sm+6a	102	1		Cristina *et al*. (2005)
Schizolecis guentheri	Sítio do Meio Stream, São Paulo	54	30m+18sm+6a	102	1		Cristina *et al*. (2005)
Schizolecis guentheri	Descoberto Stream, Paraná	54	30m+18sm+6a	102	1		Cristina *et al*. (2005)
Schizolecis guentheri	Garuva Stream, Santa Catarina	54	30m+18sm+6a	102	1		Cristina *et al*. (2005)

C-banding highlighted a small number of C-positive segments in the bearing chromosomes of *O.* cf. *polyodon*, with exception of the 24th pair where some conspicuous blocks are evident. Similar results were also observed in some other Hypoptopomatinae species, such as *Corumbataia cuestae*, *Hisonotus nigricauda*, *Hisonotus* sp. A and *Hisonotus* sp. D (Camilo & Moreira Filho, 2005; Andreata *et al*., 2006). In turn, *Hisonotus leucofrenatus* has a contrasting pattern, with a large number of positive C-bands spread over several chromosome arms (Andreata *et al*., 1993, 2006). In this sense, the C-banding pattern appears to be a useful marker for some species of Hypoptopomatinae (Andreata *et al*., 1993).

In conclusion, our study provides new cytogenetic information on the chromosomal characteristic of the Hypoptopomatinae fishes, with results corroborating a highly conserved macrostructural karyotype pattern in this subfamily. However, additional cytogenetic studies in the *Otothyropsis* genus are needed to the better understanding its evolutionary relationships with other Hypoptopomatinae genera.

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