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Original article

Contributions to the systematic of Pimelodidae (Osteichthyes, Siluriformes): basic and molecular cytogenetics on seven species of *Pimelodus* from three Brazilian hydrographic systems

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Pimelodidae harbors several species and is widely distributed throughout the Neotropical region. *Pimelodus* is the genus with the largest number of species, however it is a polyphyletic group. Cytogenetic analyzes of the valid species still covers less than half of them. Herein, seven *Pimelodus* species from three Brazilian hydrographic systems were analyzed through basic (Giemsa, AgNORs and C banding) and molecular (5S and 18S rDNA-FISH) cytogenetic methods. All species had 2n=56 chromosomes with different karyotype formulas observed among the species. AgNORs were corresponding to 18S rDNA and localized on long arm of one chromosome pair in all species. Heterochromatin distribution follows the pattern commonly verified in the family and allows to identify each one of the studied species. 5S rDNA marker was interspecifically variable in number and position of cistrons. *Pimelodus ortmanni* had B chromosomes varying intra and inter-individually. We performed a discussion on our own and available cytogenetic data for Pimelodidae, and the associating of them with available phylogeny enable us identifying features that distinguish subgroups within Pimelodidae, such as NORs location (terminal/long arm for species belonging to "*Iheringichthys-Parapimelodus*" and "*Pimelodus maculatus*" subclades) and location of 5S rDNA sites (pericentromeric/interstitial/ long arm for species belonging to *Pimelodus* group).

Keywords: 5S rDNA, 18S rDNA, B chromosome, *Calophysus-Pimelodus* clade, Citotaxonomy.

Pimelodidae abriga várias espécies e é amplamente distribuída ao longo da região Neotropical. *Pimelodus* é o gênero com o maior número de espécies, porém é um grupo polifilético. Análises citogenéticas foram realizadas em menos da metade das espécies válidas. Aqui, sete espécies de *Pimelodus* de três sistemas hidrográficos brasileiros foram estudadas através das técnicas citogenéticas básicas (Giemsa, AgRONs e banda C) e moleculares (FISH-DNAr 5S e 18S). Todas as espécies apresentaram 2n=56 cromossomos, sendo observadas variações na fórmula cariotípica entre algumas espécies. As AgRONs correspondentes ao DNAr 18S foram localizadas no braço longo de um par de cromossomos em todas as espécies. A heterocromatina segue o padrão comumente observado na família e permite identificar cada uma das espécies estudadas. O DNAr 5S apresentou variação interespecífica em número e na posição dos cístrons. Cromossomos B foram evidenciados em *P. ortmanni* com variação intra e interindividual. Nós discutimos os nossos resultados com os dados citogenéticos válidos para Pimelodidae, e a associação desses dados com a filogenia válida nos permitiu identificar características que distinguem subgrupos dentro de Pimelodidae, tais como a localização das RONs (terminal/braço longo para espécies pertencentes aos subclados "*Iheringichthys-Parapimelodus*" e "*Pimelodus maculatus*") e localização dos sítios de DNAr 5S (pericentromérico/ intersticial no braço longo para espécies pertencentes ao grupo *Pimelodus*).

Palavras-chave: Citotaxonomia, Clado *Calophysus-Pimelodus*, Cromossomo B, DNAr 5S, DNAr 18S.

Introduction

Pimelodidae is an endemic fish family from the Neotropical region belonging to the order Siluriformes, and comprises 114 valid species (Eschmeyer, Fong, 2018), presents a greater diversity of species in the basins of Amazonas, Paraná and Orinoco, and greats rivers of the Guianas (Lundberg, Littman, 2003). Pimelodidae includes widely distributed species as well as locally endemic in the region of large rivers in northwestern Colombia and eastern Panamá, in Magdalena, Maracaibo, and in southwestern Brazil (Lundberg, Littman, 2003).

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Pimelodus Lacepède, 1803 is largely distributed throughout the Neotropical region, being the most diversified genus of Pimelodidae, with approximately 34 valid species (Eschmeyer *et al*., 2018). This genus does not present a monophyletic hypothesis, and the inclusion of species in this genus followed the non-cladistics characters, proposed even in the 19th century, which probably will lead many species to be relocated after phylogenetic studies (Ribeiro *et al*., 2008; 2011). This non-monophyletism is evident in the phylogenetic study of Lundberg *et al*. (2011), where the analyzed *Pimelodus* species were allocated in four clades with different genus. The *Pimelodus* group *sensu* by Lundberg *et al*. (2011) includes *Pimelodus* species and a sister clade formed by *Iheringichthys* and *Parapimelodus*, not being part of this group *Pimelodus ornatus* Kner, 1858 and *Pimelodus* cf. *altissimus* Eigenmann & Pearson, 1942.

Cytogenetic studies in Pimelodidae are restricted to 34 valid species (Tab. 1). These studies show the prevalence of 2n=56 chromosomes with variations in the species of the Calophysines group, which have 2n=54 and 2n=50 chromosomes (Ramirez-Gil *et al*., 1998; Swarça *et al*., 1999; Vasconcelos, Martins-Santos, 2000; Swarça *et al*., 2001c; Sanchez *et al*., 2010; Carvalho *et al*., 2011), and in *Pimelodus fur* (Lütken 1874) with 2n=54 chromosomes (Garcia, Moreira-Filho, 2005, 2008). The pattern of simple telomeric nucleolar organizer regions (NORs) is common to all studied Pimelodidae, showing variation in position, short or long arm. Only fifteen species of this family have data on the location of 5S and 18S rDNA, which show variations in number and position (Tab. 1).

Although cytogenetic data are relatively scarce in Pimelodidae, they have contributed to the differentiation and identification of sympatric species, besides make possible the establishment of cytotaxonomic relationships among Pimelodidae species (Swarça *et al*., 2007). In this perspective, the increase of cytogenetic information can contribute to the classification and elucidation of uncertain relations in species of problematic genera, such as *Pimelodus*.

We present basic and molecular cytogenetic analyses of all the five species of *Pimelodus* with color pattern of black spots from the Brazilian part of the La Plata basin: *Pimelodus britskii* Garavello & Shibatta, 2007 and *Pimelodus ortmanni* Haseman, 1911 are endemic of the Iguaçu River (Baumgartner *et al*., 2012); *Pimelodus maculatus* Lacèpede, 1803 is widely distributed, being found in the La Plata and São Francisco rivers basins; *Pimelodus microstoma* Steindachner, 1877 and *Pimelodus paranaensis* Britski & Langeani, 1988 are present in the upper Paraná River (Eschmeyer *et al*., 2018). In addition, we carried out the same analyses in two species with the same coloration pattern that occur in adjacent basins: *Pimelodus mysteriosus* Azpelicueta, 1998 is located in the La Plata, the lower Uruguay, middle Paraná and Paraguay River, whereas *Pimelodus absconditus* Azpelicueta, 1995 on the La Plata River to the confluence with the Paraná and the Paraguay and Uruguay Rivers (Rocha, 2012).

This study reveals the first cytogenetic data of *Pimelodus maculatus* and *P. absconditus* of the Ijuí River population; first location of 5S and 18S rDNA in *P. ortmanni* and *P. mysteriosus* from the Iguaçu River downstream from the Cataratas do Iguaçu, and in *P. paranaensis* and *P. microstoma* from the Piquiri River, upper Paraná River basin. In addition, a review of cytogenetic studies in Pimelodidae is given with possible inferences about chromosome evolution in *Pimelodus* as well as in the family.

Material and Methods

Specimens of *Pimelodus* were collected and deposited in the Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (NUPELIA) of the Universidade Estadual de Maringá, Brazil. The collecting sites and the voucher numbers are summarized in Tab. 2. This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, approved by the Committee on the Ethics of Animal Experiments of the Universidade Estadual do Oeste do Paraná (License Number: Protocol 13/09 – CEEAAP/Unioeste).

All specimens were anesthetized and euthanized by an overdose of clove oil according to Griffiths (2000). Chromosome preparations were obtained from cells of the anterior kidney by technique proposed by Bertollo *et al*. (1978). AgNORs were revealed by silver impregnation according to Howell, Black (1980) and C-banding following Sumner (1972), with modifications suggested by Lui *et al*. (2012). Physical mapping of the 5S rDNA and 18S rDNA was carried out by fluorescence *in situ* hybridization (FISH) according to Pinkel *et al*. (1986) and modifications suggested by Margarido, Moreira-Filho (2008), using DNA probes obtained from *Megaleporinus elongatus* (Valenciennes, 1850) (Martins, Galetti-Junior, 1999) and from *Prochilodus argenteus* Spix & Agassiz, 1829 (Hatanaka, Galetti-Junior, 2004), respectively. Probes were labeled by nick translation method with digoxigenin-11-dUTP (5S rDNA) and biotin-16-dUTP (18S rDNA) (Roche®). Detection of signals was performed with antidigoxigenin-rhodamine (Roche®) for probe of 5S rDNA and amplified avidin-FITC with biotinylated anti-avidin (Sigma-Aldrich) for probe of 18S rDNA, with the chromosomes counterstained with 4',6-diamidino-2-phenylindole (DAPI, 50 µg/mL). Metaphases were photographed using a BX 61 epifluorescence microscope, coupled with Olympus DP 71 digital camera (Olympus America, Inc.) with the Olympus DP Controller software 3.2.1.276. Chromosomes were classified and organized in accordance with Levan *et al*. (1964) in metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a).

Tab. 1. Summary of cytogenetics studies in Pimelodidae. p = short arm; q = long arm; t = terminal; c = centromeric; m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric; para = paracentromeric; peri = pericentromérica; inter = intersticial; subt = subterminal; t = telomeric. Updated species names: **Pimelodus heraldoi*; ***Pimelodus maculatus*, ****Paulicea luetkenii*. In bold are the species of the group Calophysines.

Species	Locality (all from Brazil, except where otherwise mentioned)	2n	Karyotypic formula	B	18S rDNA	AgNORs	5S rDNA	Heterochromatin	Reference
Bergiaria westermanni	São Francisco River, Minas Gerais State	56	42m/sm+14st	0-5		q-t, st/a			Dias, Foresti (1993)
Brachyplatystoma filamentosum	Araguaia River, Goias State	56	24m+12sm+10st+10a		p-t, st	p-t, st	p-peri, st	peri and t	Gonçalves *et al.* (2014)
Calophysus macropterus	Negro and Solimões River, Amazonas State	50	22m+18sm+10a			p-t, a			Ramirez-Gil *et al.* (1998)
Hemisorubim platyrhynchos	Paraná River, Paraná State,	56	22m+18sm+6st+10a			p-t, sm		inter, peri and t	Martins-Santos *et al.* (1996)
H. platyrhynchos	Paraná River, Corrientes State, Argentina	56	22m+16sm+10st+8a		p-t, st	p-t, st		peri, p and q	Swarça *et al.* (2013)
H. platyrhynchos	Miranda River, Mato Grosso do Sul State	56	22m+16sm+10st+8a		p-t, st	p-t, st		peri, p and q	Swarça *et al.* (2013)
Iheringichthys labrosus	Tibagi River, Paraná State	56	32m+8sm+6st+10a	0-3	q-t, st	q-t, st	q-inter, st	t	Carvalho *et al.* (2010; 2004)
I. labrosus	Capivara Reservoir, Paraná State	56	26m+12sm+6st+12a	0-1	q-t, st	q-t, st	q-t, st/a	t	Carvalho, Dias (2005; 2007)
I. labrosus	Jurumirim Reservoir, São Paulo State	56	22m+18sm+10st+6a	0-2				inter and t	Vissotto *et al.* (1999a)
I. labrosus	Guaraúna River, Paraná State	56	14m+32sm+4st+6a			q-t, sm		c and t	Ribeiro *et al.* (2008)
I. labrosus	Paraná River, 7 localities, Argentina	56	42m/sm+14st/a			q-t, st		c and t	Sanchez *et al.* (2014)
Luciopimelodus pati	Paraná River, Corrientes Province, Argentina	50	16m+14sm+8st+12a			p-t, a			Sanchez *et al.* (2010)
Megalonema platanum	Paraná River, different localities, Argentina	54	24m+16sm+2st+12a		p-t, sm	p-t, sm		inter and t	Carvalho *et al.* (2011)
M. platanum	Tibagi River, Paraná State	54	24m+16sm+2st+12a	0-1	p-t, sm	p-t, sm		inter and t	Carvalho *et al.* (2011)
Parapimelodus nigribarbis	Guaíba lake, Rio Grande do Sul State	56	20m+20sm+4st+12a			q-t, st		c and t	Treco *et al.* (2008)
Phractocephalus hemioliopterus	Amazon Basin	56	16m+20m+6st+14a		p- t, a	p-t, a	p-peri, st	t and inter	Swarça *et al.* (2017)
Pimelodus absconditus	Paraná River, Paraná State	56	24m+18sm+8st+6a			q-t, st		c, inter and t	Borin, Martins-Santos (2002)
P. absconditus	Ijuí River, Rio Grande do Sul State	56	24m+18sm+8st+6a			q-t, st	q-peri, sm	c, peri and t	Here
P. argenteus	Paraguai River, Mato Grosso do Sul State	56	24m+16sm+12st+4a			p-t, st		t	Souza *et al.* (2003)
P. blochii	Not mentioned	56	18m+16sm+10st+12a						Nirchio *et al.* (2014)
P. britskii	Iguaçu River, Paraná State	56	24m+18sm+8st+6a		q-t, st	q-t, st	p-inter, sm; q-t, st	c and t	Moraes Neto *et al.* (2011)
P. britskii	Iguaçu River, Paraná State	56	24m+18sm+8st+6a		q-t, st	q-t, st	q-peri, sm; q-t, st; q-t, a	peri and t	Here
P. fur	São Francisco River, Minas Gerais State	54	32m+8sm+6st+8a		q-t, sm	q-t, sm	q-inter, m; q-peri, sm		Garcia, Moreira-Filho (2005; 2008)
P. maculatus	São Francisco River, Minas Gerais State	56	32m+12sm+12st		q-t, sm	q-t, sm	q-inter, m; q-t, sm; q-peri, sm		Garcia, Moreira-Filho (2005; 2008)
P. maculatus	Congonhas Stream, Paraná State	56	20m+20sm+10st+6a						Mazzuchelli *et al.* (2007)

Tab. 1. (continued).

Species	Locality (all from Brazil, except where otherwise mentioned)	2n	Karyotypic formula	B	18S rDNA	AgNORs	5S rDNA	Heterochromatin	Reference
P. maculatus	São Francisco and Mogi-Guacu River, Minas Gerais and São Paulo State	56	40m/sm+16st/a			q-t, st/a			Dias, Foresti (1993)
P. maculatus	Tibagi River, Paraná State	56	20m+20sm+10st+6a			q-t, st		c and t	Swarça *et al.* (2001a)
P. maculatus	Paranapanema River, São Paulo State	56	20m+20sm+10st+6a			q-t, st		c, inter and t	Vissoto *et al.* (1999b)
P. maculatus	Paraná River, Paraná State	56	20m+20sm+10st+6a			q-t, st		c, inter and t	Borin, Martins-Santos (2002)
P. maculatus	Guaíba Lake, Rio Grande do Sul State	56	24m+20sm+6st+6a			q-t, st			Treco *et al.* (2008)
P. maculatus	Angatuba City, São Paulo State	56	24m +22sm+8st+2a		q-t, st	q-t, st	q-t, st	peri and t	Ferreira *et al.* (2014)
P. maculatus	Guapiara City, São Paulo State	56	28m+18sm+4st+6a		q-t, st	q-t, st	q-t, st	peri and t	Ferreira *et al.* (2014)
P. maculatus	Três Lagoas City, Mato Grosso do Sul State	56	20m+22sm+10st+4a		q-t, st	q-t, st	q-t, st	peri and t	Ferreira *et al.* (2014)
P. maculatus	Terra Roxa City, São Paulo State	56	22m+26sm+6st+2a		q-t, st	q-t, st	q-t, st	peri and t	Ferreira *et al.* (2014)
P. maculatus	Ijuí River, Rio Grande do Sul State	56	24m+20sm+6st+6a		q-t, st	q-t, st	q-t, st	c, peri and t	Here
*P. microstoma**	Tibagi River, Paraná State	56	22m+22sm+6st+6a			q-t, st		t	Souza *et al.* (2004)
*P. microstoma**	Piquiri River, Paraná State	56	18m+24sm+6st+8a			q-t, st		t	Treco, Dias (2009)
*P. microstoma**	Mogi-Guaçu River, São Paulo State	56	32m+14sm+6st+4a		q-t, st	q-t, st	q-peri, st	peri and t	Ferreira *et al.* (2014)
P. microstoma	Piquiri River, Paraná State	56	24m+18sm+8st+6a		q-t, st	q-t, st	q-peri, sm	c, peri and t	Here
P. mysteriosus	Paraguai River, Mato Grosso do Sul State	56	26m+20sm+2st+8a			p-t, st		t	Souza *et al.* (2003)
P. mysteriosus	Iguaçu River, Paraná State	56	28m+10sm+2st+16a		q-t, st	q-t, st	q-inter, m; q-peri, sm; q-peri, a	c, inter, peri and t	Here
P. ornatus	Paraná River, Paraná State	56	20m+18sm+8st+10a			p-t, st		c and t	Borin , Martins-Santos (2002)
P. ortmanni	Iguaçu River, Paraná State	56	24m+18sm+8st+6a	0-4		q-t, st		c, peri and t	Borin , Martins-Santos (2004)
P. ortmanni	Iguaçu River, Paraná State	56	24m+18sm+8st+6a		q-t, st	q-t, st	q-peri, sm	c, peri and t	Here
*P. pantaneiro***	Paraguai River, Mato Grosso do Sul State	56	22m+16sm+10st+8a			q-t, st		inter and t	Souza *et al.* (2003)
P. paranaensis	Piquiri River, Paraná State	56	22m+22sm+4st+8a			q-t, st		t	Treco, Dias (2009)
P. paranaensis	Piquiri River, Paraná State	56	22m+22sm+4st+8a		q-t, st	q-t, st	q-peri, sm; q-peri, a	c, peri and t	Here
Pimelodus sp.	São Francisco River, Minas Gerais State	56	40m/sm+16st/a						Dias, Foresti (1993)
Pimelodus sp.	São Francisco River, Minas Gerais State	56	32m+12sm+6st+6a		q-t, sm		q-inter, m; q-peri, sm; q-peri, sm		Garcia, Moreira-Filho (2005; 2008)
Pimelodus sp.	Iguaçu River, Paraná State	56	24m+26sm+4st+2a			q-t, st		t	Souza *et al.* (2004)

Tab. 1. (continued).

Species	Locality (all from Brazil, except where otherwise mentioned)	2n	Karyotypic formula	B	18S rDNA	AgNORs	5S rDNA	Heterochromatin	Reference
Pimelodus sp.	Iguaçu River, Paraná State	56	30m+14sm+8st+4a	0-4		q-t, st		c, peri and t	Borin, Martins-Santos (2004)
Pinirampus pirinampu	Paraná River, Paraná State	50	22m+12sm+4st+12a			p-t, a			Vasconcelos, Martins-Santos (2000)
P. pirinampu	Tibagi River, Paraná State	50	26m+12sm+2st+10a			p-t, st			Swarça *et al*. (1999; 2001c)
P. pirinampu	Paraná River, Corrientes province, Argentina	50	18m+14sm+4st+14a			p-t, a		t and peri	Sanchez *et al*. (2010)
Pseudoplatystoma corruscans	Paraná River, Paraná State	56	18m+16sm+10st+12a			p-t, sm		peri and t	Martins-Santos *et al*. (1996)
P. corruscans	Tres Marias River, Minas Gerais State	56	20m+12sm+12st+12a						Fenocchio, Bertollo (1992)
P. corruscans	Paraguai River, Mato Grosso do Sul State	56	20m+16sm+8st+12a		p-t, a	p-t, a	p-subt, st; 1 peri, m		Swarça *et al*. (2005b)
P. corruscans	Paraná River, São Paulo and Paraná State	56	26m+10sm+6st+14a		p-t, sm	p-t, sm	p-subt, st; 1 subt, sm		Swarça *et al*. (2005b)
P. fasciatum	Solimões River, Amazonas State	56	18m+14sm+10st+14a						Fenocchio , Bertollo (1992)
P. fasciatum	Paraguai River, Mato Grosso do Sul State	56	20m+12sm+12st+12a			p-t, st		t and peri	Porto-Foresti *et al*. (2000)
P. metaense	Orinoco River, Bolívar State Venezuela	56	42m/sm+14st/a		p-t, sm	p-t, sm	p-para, sm	t and peri	Nirchio *et al*. (2013)
P. orinocoense	Orinoco River, Bolívar State Venezuela	56	42m/sm+14st/a		p-t, sm	p-t, sm	p-para, sm	t and peri	Nirchio *et al*. (2013)
P. reticulatum	Paraguai River, Mato Grosso do Sul State	56	22m+20sm+6st+8a		p-t, sm	p-t, sm	p-inter, sm	c and t	Moraes Neto *et al*. (2011)
P. tigrinum	Solimões River, Amazonas State	56	18m+16sm+8st+14a						Fenocchio, Bertollo (1992)
Sorubim lima	Paraná River, Paraná State	56	20m+14sm+10st+12a			p-t, sm		inter, peri and t	Martins-Santos *et al*. (1996)
S. lima	Paraguai River, Mato Grosso do Sul State	56	24m+16sm+8st+8a		p-t, st	p-t, st	p-inter, sm	c and t	Moraes Neto *et al*. (2011)
S. lima	Paraguai River, Mato Grosso do Sul State	56	24m+16sm+8st+8a		p-t, st	p-t, st	p, t and peric, sm		Sczepanski *et al*. (2013)
Steindachneridion melanodermatum	Iguaçu River, Paraná State, Brazil	56	20m+24sm+2st+10a/ 21m+23sm+2st+10a		p-t, a	p-t, a	p-subt, st		Swarça *et al*. (2006; 2008; 2009)
S. melanodermatum	Iguaçu River, Paraná State, Brazil	56	14 m+22sm+12st+8[a]		p-t, a	p-t, a	p-subt, st		Matoso *et al*. (2011)
S. parahybae	Paraíba do Sul River, São Paulo State, Brazil	56	4m+22sm+12st+8a		p-t, sm	p-t, sm	p-inter, sm	c and t	Moraes Neto *et al*. (2011)
S. scriptum	Paranapanema and Tibagi River, Paraná State, Brazil	56	24m+20sm+4st+8a		p-t, a	p-t, a	p-subt, st		Swarça *et al*. (2005a; 2008; 2009)
*Zungaro zungaro****	Paraná River, Paraná State, Brazil	56	26m+10sm+6st+14a			p-t, sm			Martins-Santos *et al*. (1996)
Z. zungaro	Paraná River, São Paulo State, Brazil	56	32m+6sm+8st+10a			p-t, sm			Swarça *et al*. (2001b)

Tab. 2. Sample data of analyzed *Pimelodus*. *Population downstream from the Iguaçu Falls. ♂ = male, ♀ = female, U = Unidentified, NUP = voucher numbers of the Coleção Ictiológica do Nupélia.

Species	Locality	Basin	Geographic Coordinates	♂	♀	U	NUP
P. absconditus	Ijuí River	Upper Uruguai River	28°18'06.3"S 53°53'33.6"W	17	6	1	17259, 17264
P. britskii	Iguaçu River	Lower Iguaçu River	25°37'13.20"S 54°23'29.20"W	2	8	1	17260, 17265, 17266, 17269
P. maculatus	Ijuí River	Upper Uruguai River	28°18'06.3"S 53°53'33.6"W	1	3	-	17263
P. microstoma	Piquiri River	Upper Paraná River	24°56'54"S 52°35'49"W	4	9	-	14938
*P. mysteriosus**	Iguaçu River	Midlle Paraná River	25°39'02"S 54°27'25"W	1	-	-	16111
P. ortmanni	Iguaçu River	Lower Iguaçu River	25°37'13.20"S 54°23'29.20"W	5	4	1	17261, 17267, 17270, 17271
P. paranaensis	Piquiri River	Upper Paraná River	24°56'54"S 52°35'49"W	-	2	-	14936, 17274

Tab. 3. Cytogenetics data obtained for *Pimelodus* species. m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric; p = short arm; q = long arm; tel = telomeric; peri = pericentromeric; inter = interstitial.

Species	Karyotypic Formula	Heterochromatin (C-banding)	AgNORs/18S rDNA	5S rDNA
P. absconditus	24m + 18sm + 8st + 6a	Centromeric, pericentromeric and telomeric	tel q (24)	peri q (pair 18)
P. britskii	24m + 18sm + 8st + 6a	Pericentromeric and telomeric	tel q (23)	peri q (pair 17); tel q (pairs 23 and 28)
P. maculatus	24m + 20sm + 6st + 6a	Centromeric, pericentromeric and telomeric	tel q (23)	tel q (pair 23)
P. microstoma	24m + 18sm + 8st + 6a	Centromeric, pericentromeric and telomeric	tel q (24)	peri q (pair 18)
P. mysteriosus	28m + 10sm + 2st + 16a	Centromeric, interstitial, pericentromeric and telomeric	tel q (20)	inter q (pair 1); peri q (pair 21 and 22)
P. ortmanni	24m + 18 sm + 8st + 6a	Centromeric, pericentromeric and telomeric	tel q (24)	peri q (pair 18)
P. paranaensis	22m + 22sm + 4st + 8a	Centromeric, pericentromeric and telomeric	tel q (24)	peri q (pairs 13, 18 and 26)

Results

The results are summarized in Tab. 3 and are presented below.

***Pimelodus absconditus*.** We found a diploid number of 56 chromosomes (24 m + 18 sm + 8 st + 6 a) (Fig. 1a). The AgNORs were located in the terminal region of the long arm of a pair of subtelocentric chromosomes (24) (Fig. 1a, box). C-banding revealed the existence of pale heterochromatin in the most of the centromeres of chromosomes, with some pairs showing heterochromatin conspicuous in the telomeric region of the short arm (pars 17 and 18) and the long arm (pair 24), in both telomeres (pairs 8 and 20), in the pericentromeric region of the long arm of pair 18 and in subterminal region of the long arm of pair 17 (Fig. 2a). The 18S rDNA was located on the subtelocentric chromosome pair (24), which corresponds to the AgNORs, while the 5S rDNA was found in the pericentromeric region of the long arm of pair 18 (Fig. 3a).

***Pimelodus britskii*.** The diploid number of this species was also 56 chromosomes (24 m + 18 sm + 8 st + 6 a) (Fig. 1b). The AgNORs were located in the terminal region of the long arm of a pair of subtelocentric chromosomes 23 (Fig. 1b, box). C-banding revealed the existence of pale heterochromatin in the region of the telomeres of some chromosomes, with more conspicuous heterochromatin being found in the telomeric region of the long arm (pairs 3, 6 and 23), in the subterminal region of the long arm (pair 15), pericentromeric region of the short arm (pair 2) and in long arm in pair 17 (Fig. 2b). The 18S rDNA was located on the subtelocentric chromosome pair (23), which corresponds to the AgNORs, while the 5S rDNA was found at multiple sites, located in the pericentromeric region of the long arm of pair 17 and terminal region of the long arm of pairs 23 and 28 (Fig. 3b).

***Pimelodus maculatus*.** The diploid number of this species was also 56 chromosomes (24 m + 20 sm + 6 st + 6 a) (Fig. 1c). The AgNORs were located in the terminal region of the long arm of a pair of subtelocentric chromosomes 23 (Fig. 1c, box). C-banding revealed the existence of pale heterochromatin in the region of the telomeres of some chromosomes, with more conspicuous heterochromatin being found in the telomeric region of the short arm (pair 6) and long arm (pairs 17, 22, 23 and 24), in both telomeres (pair 8) and the pericentromeric region of the short arm (pairs 1 and 25) and in long arm in pair 21 (Fig. 2c). The 18S rDNA and 5S rDNA showed syntenic sites in the terminal region of the long arm of a pair of subtelocentric chromosomes (23), which corresponds to the AgNORs (Fig. 3c).

***Pimelodus microstoma*.** The diploid number was also 56 chromosomes (24 m + 18 sm + 8 st + 6 a) (Fig. 1d). The AgNORs were located in the terminal region of the long arm of a pair of subtelocentric chromosomes 24 (Fig. 1d, box). C-banding highlighted pale heterochromatin in the region of the centromere in the most of chromosomes, with more conspicuous heterochromatin being found in the telomeric region of the short arm (4) and long arm (pairs 16 and 24), in both telomeres (pair 8) and in the pericentromeric region of the long arm in pair 18 (Fig. 2d). The 18S rDNA was located on the subtelocentric chromosome pair (24), which corresponds to the AgNORs, while the 5S rDNA was found in the pericentromeric region of the long arm of pair 18 (Fig. 3d).

Fig. 1. Karyotypes arranged from Giemsa-stained chromosomes. **a.** *Pimelodus absconditus*; **b.** *Pimelodus britskii*; **c.** *Pimelodus maculatus*; **d.** *Pimelodus microstoma*; **e.** *Pimelodus mysteriosus*; **f.** *Pimelodus ortmanni*; **g.** *Pimelodus paranaensis*. Pairs of the AgNORs and B chromosomes are in the boxes. Scales bar = 10 μm.

Fig. 2. Karyotypes arranged from C-banded chromosomes. **a.** *Pimelodus absconditus*; **b.** *Pimelodus britskii*; **c.** *Pimelodus maculatus*; **d.** *Pimelodus microstoma*; **e.** *Pimelodus mysteriosus*; **f.** *Pimelodus ortmanni*; **g.** *Pimelodus paranaensis*. B chromosomes in the boxes. Scales bar = 10 μm.

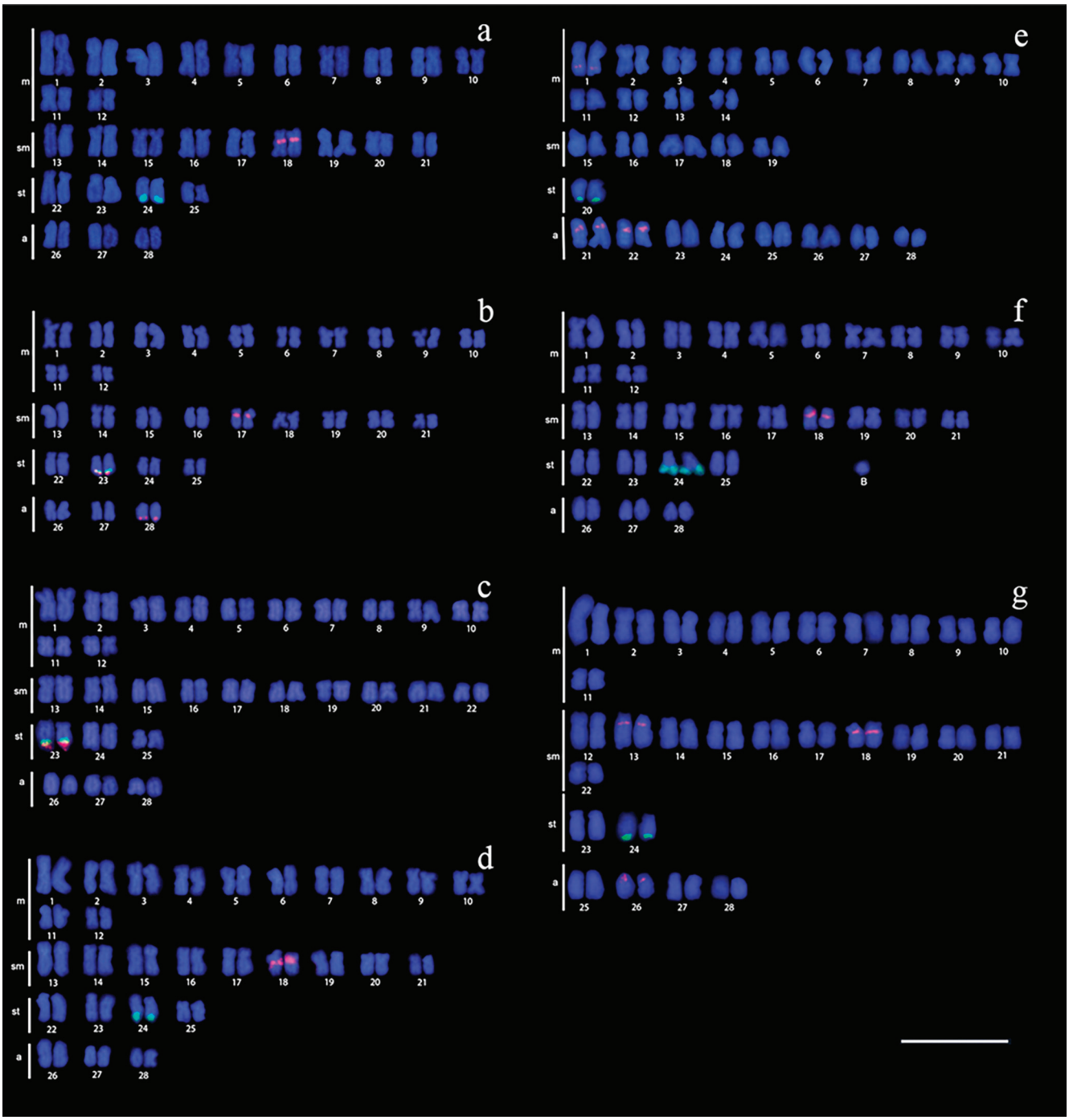

Fig. 3. Karyotypes after FISH with 5S rDNA probes (red) and 18S rDNA probe (green). **a.** *Pimelodus absconditus*; **b.** *Pimelodus britskii*; **c.** *Pimelodus maculatus*; **d.** *Pimelodus microstoma*; **e.** *Pimelodus mysteriosus*; **f.** *Pimelodus ortmanni*; **g.** *Pimelodus paranaensis*. Scales bar = 10 μm.

***Pimelodus mysteriosus*.** The diploid number was also 56 chromosomes (28 m + 10 sm + 2 st + 16 a) (Fig. 1e). The AgNORs were located in the terminal region of the long arm of a pair of subtelocentric chromosomes 20 (Fig. 1e, box). C-banding highlighted pale heterochromatin in the region of the centromere in the most of chromosomes, with more conspicuous heterochromatin being found in the telomeric region of the short arm (pair 20) and long arm (pairs 5, 17, 20, 21, 22, 23, 24, 26, 27 and 28), in interstitial region (pairs 21 and 23) and in the pericentromeric region of the long arm of pairs 21 and 22 (Fig. 2e). The 18S rDNA was located on the subtelocentric chromosome pair (20), which corresponds to the AgNORs, while the 5S rDNA was found at multiple sites, located in the interstitial region of the long arm of pair 1 and pericentromeric region of the long arm of pairs 21 and 22 (Fig. 3e).

Pimelodus ortmanni. The diploid number was also 56 chromosomes (24 m + 18 sm + 8 st + 6 a) (Fig. 1f). Among one to four B chromosomes (micro-chromosomes and acrocentric) (Fig. 1f) were observed in all specimens, with intra- and inter-individual variation (Tab. 4). The AgNORs were located in the terminal region of the long arm of a pair of subtelocentric chromosomes 24 (Fig. 1f, box). C-banding revealed the existence of pale heterochromatin in the region of the telomeres and centromeres of some chromosomes, with more conspicuous heterochromatin being found in the telomeric region of short arm (pair 16) and long arm (pairs 10, 24 and 27), in both telomere (pair 5) and in the pericentromeric region of the long arm (pairs 17 and 18), while the B chromosomes are entirely heterochromatic (Figs. 2f, 4). The 18S rDNA was located on the subtelocentric chromosome pair (24), which corresponds to the AgNORs, while the 5S rDNA was found in the pericentromeric region of the long arm of pair 18 (Fig. 3f).

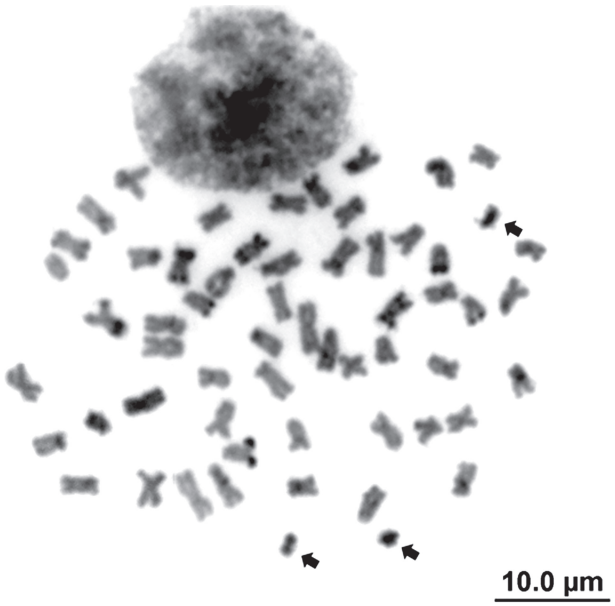

Fig. 4. C-banded metaphase of *Pimelodus ortmanni*. Arrows indicate the heterochromatic B chromosomes. Scales bar = 10 µm.

Pimelodus paranaensis. The diploid number of this species was also 56 chromosomes (22 m + 22 sm + 4 st + 8 a) (Fig. 1g). The AgNORs were located in the terminal region of the long arm of a pair of subtelocentric chromosomes 24 (Fig. 1g, box). C-banding highlighted pale heterochromatin in the region of the telomere in the most of chromosomes, with more conspicuous heterochromatin being found in the telomeric region of long arm (pairs 4, 13, 19, 20, 24 and 26), in both telomeres (pairs 7 and 23), in the pericentromeric region of the long arm (pairs 13, 18 and 26) and short arm (pairs 2 and 12) (Fig. 2g). The 18S rDNA was located on the subtelocentric chromosome pair (24), which corresponds to the AgNORs, while the 5S rDNA was found at multiple sites, located in the pericentromeric region of the long arm of pairs 13, 18 and 26 (Fig. 3g).

Discussion

The presence of 2n = 56 chromosomes in all species of *Pimelodus* studied here and in most species of Pimelodidae (Tab. 1) supports the hypothesis by Moraes-Neto *et al*. (2011) that this is the ancestral diploid number for the family. Variations in this pattern were observed in Calophysines *sensu* Lundberg *et al*. (2011) (Fig. 5), such as *Calophysus macropterus* (Lichtenstein, 1819) 2n=50 chromosomes (Ramirez-Gil *et al*., 1998), *Luciopimelodus pati* (Valenciennes, 1835) 2n= 50 chromosomes (Sanchez *et al*., 2010), *Megalonema platanum* (Günther, 1880) 2n= 54 chromosomes (Carvalho *et al*., 2011) and *Pinirampus pirinampu* (Spix & Agassiz, 1829) 2n = 50 chromosomes (Swarça *et al*., 1999, 2001c; Vasconcelos, Martins-Santos, 2000; Sanchez *et al*., 2010). The only other exception was found in the also black spotted congener, *Pimelodus fur* 2n= 54 chromosomes (Garcia, Moreira-Filho, 2008), which inhabits the São Francisco River basin. The reduction of the diploid number in those species represents a derived feature in the family possibly originated from an independently chromosome fusion process, due to the phylogenetic distance between those groups.

Tab. 4. Frequency of B chromosomes in *Pimelodus ortmanni* from the Iguaçu River. *Unidentified.

Specimems	Sex	0 B	1 B	2 B	3 B	4 B	Total	Cells with B (%)
3000	♀	-	19	-	-	-	19	100
3001	♂	4	3	-	-	-	7	42,9
3084	♂	3	5	1	-	-	9	66,7
3143	♂	-	2	-	4	9	15	100
3146	♀	-	6	-	1	-	7	100
3151	♀	-	2	13	-	-	15	100
3276	♂	-	27	17	5	18	67	100
3281	♂	13	9	3	-	-	25	48
3287	*	-	-	21	37	2	60	100
3288	♀	-	2	4	15	-	21	100
Total (%)		20 (8,2)	75 (30,6)	59 (24,1)	62 (25,3)	29 (11,8)	245	

Single terminal NORs are common to all studied Pimelodidae species (Tab. 1), indicating that this feature was conserved during the process of karyotypic evolution of the family. Swarça *et al*. (2007) suggested the delimitation of two cytotaxonomic groups based on the location of NORs in the Pimelodidae species with 2n = 56 chromosomes: Surubiminae, NORs in the short arm, and *Pimelodus*, NORs in the long arm. All species of this study show these sequences in the terminal region of the long arm (Fig. 6) agreeing with the pattern shown in most *Pimelodus* species. We correlated cytogenetic data currently available (Tab.1) with the systematic classification proposed by Lundberg *et al*. (2011) (Fig. 5). The presence of NORs in the long arm is exclusive for species belonging to subgroups formed by the subclades "*Iheringichthys-Parapimelodus*" and "*Pimelodus maculatus*", while NORs in the short arm are present in the other groups and in some species currently attributed to *Pimelodus*. The location of these sequences in the short arm of most all the studied species and in basal node genera, *Phractocephalus* and *Steindachneridion*, allows inferring that this may be the primitive form for this character in this family, whereas the presence in the long arm would indicate the derived condition. It is likely that this feature originated from chromosomal inversions, and may be an important cytotaxonomic marker to distinguish species from these groups.

Regarding the distribution of heterochromatin, the *Pimelodus* species studied here follow the pattern found in Pimelodidae, that is, a small amount of heterochromatin, distributed in the telomere and centromere region, with some interstitial/pericentromeric markings (Fig. 6). This marker allows distinguishing studied *Pimelodus* species. Although *P. absconditus* and *P. microstoma* have similar patterns, *P. absconditus* presents two pairs (8 and 20) with heterochromatin in telomere of both arms and one pair (17) with subterminal heterochromatin, while *P. microstoma* shows only one pair with heterochromatin in telomere of both arms. *Pimelodus britskii* has heterochromatin in the pericentromeric region of the short arm of the second metacentric chromosome pair, which is also observed in *P. paranaensis*; however, in *P. paranaensis* there is a large number of pairs with telomeric bands, which does not occur in *P. bristkii*. *Pimelodus mysteriosus* contains a large number of heterochromatic blocks in the acrocentric chromosomes, which enable us to differentiate it from the other analyzed species. Heterochromatins in the pericentromeric region of the short arm on the first chromosome pair are exclusively observed in *P. maculatus*. *Pimelodus ortmanni* has a heterochromatin pattern similar to *P. microstoma* and *P. absconditus*, being differentiated by not having a pair of submetacentric chromosomes with heterochromatin in both telomeres. In *P. microstoma*, *P. absconditus*, *P. ortmanni*, *P. paranaensis* and *P. maculatus* the presence of a metacentric chromosome pair with conspicuous bi-telomeric heterochromatin was observed (Fig. 6); Garcia, Moreira-Filho (2005) reported that this characteristic has been observed in several species of Pimelodidae and Heptateridae, and hypothesized it as a marker for these families. However, the absence of this pattern in many species of both families makes difficult its effective use as marker.

The 5S rDNA sites show variations in number and position. *Pimelodus microstoma*, *P. ortmanni*, *P. maculatus* and *P. absconditus* presented single sites, whereas *P. britskii*, *P. mysteriosus* and *P. paranaensis* have three chromosomes pairs with these sequences (Fig. 6). According to Martins, Galetti-Junior (1999), the presence of 5S rDNA in only one pair of chromosomes probably represents the ancestral condition for fish. In Pimelodidae most species also presented a single chromosomes pair bearing 5S rDNA sites (Tab. 1); however, in *Pimelodus* the prevalence is multiple sites, which may indicate the occurrence of chromosomal rearrangements like transposition / translocation during the speciation process of this group.

Despite the small number of species with data on the location of 5S rDNA compared to the number of species in Pimelodidae, we can realize that species of the *Pimelodus* group *sensu* Lundberg *et al*. (2011) have these sites in the long arm, whereas species of the remaining Pimelodidae are evidenced in the short arm, and this can be an important cytotaxonomic marker. However, one exemplar of *P. britskii* has these sites in both, short and long arms (Moraes-Neto *et al*., 2011), indicating that analyzes of these sequences in a greater number of species are essential to confirm that hypothesis. Regarding the position of the 5S rDNA sites, almost all the studied species in Pimelodidae have at least one pair with interstitial, pericentromeric or subterminal location, except for *P. maculatus* (Ferreira *et al*., 2014; and herein) and *Iheringichthys labrosus* (Lütken, 1874) (Carvalho, Dias, 2007) that have this sequence only in the telomeric region. According to Martins, Galetti-Junior (2001), most fish species have the rDNA sites in the interstitial position, suggesting that such pattern is not casual and may represent some advantage related to the organization of these sequences in vertebrates.

The location of 5S and 18S rDNA genes in different chromosomes is the most common arrangement in fish (Martins, Galetti-Junior, 2001; Martins, Wasko, 2004). According to Martins, Galetti-Junior (1999), when the 45S and 5S genes are linked to the same chromosome disruptive interference may occur, such as translocations from 5S to 45S, which could explain why the separate arrangement is the most common in vertebrates. However, cases of syntenic location of these sequences are reported in several Neotropical fish species (Bellafronte *et al*., 2005; Kavalco *et al*., 2004; Mariotto *et al*., 2011; Bueno *et al*., 2014; among others). In Pimelodidae, synteny of these sites are restricted to *P. britskii* (Moraes-Neto *et al*., 2011; and herein) and *P. maculatus* (herein), which may indicate a derived condition and possible phylogenetic proximity between these species, corroborating the taxonomic analyzes carried out by Rocha (2012).

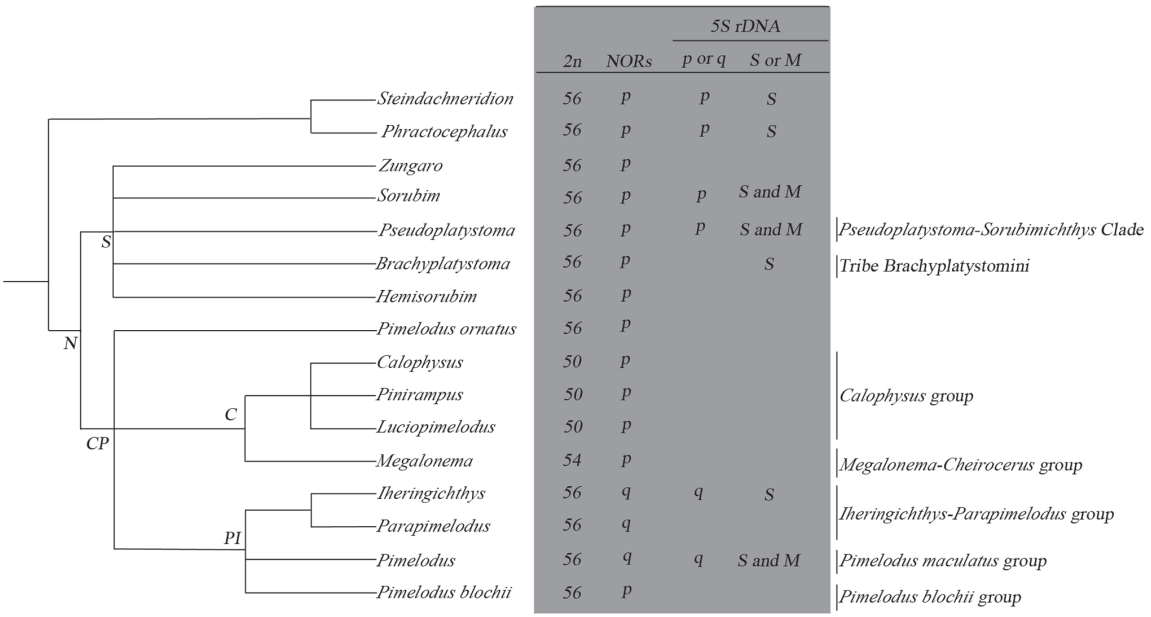

Fig. 5. Cytogenetics data and phylogenetic relationships between Pimelodidae (modified from Lundberg *et al*., 2011). N= Neopimelodines; S= Sorubimines; CP= *Calophysus-Pimelodus* Clade; C = Calophysines; PI = *Pimelodus* Group; 2n = number diploid; p = short arm; q = long arm; S = simple; M = multiple.

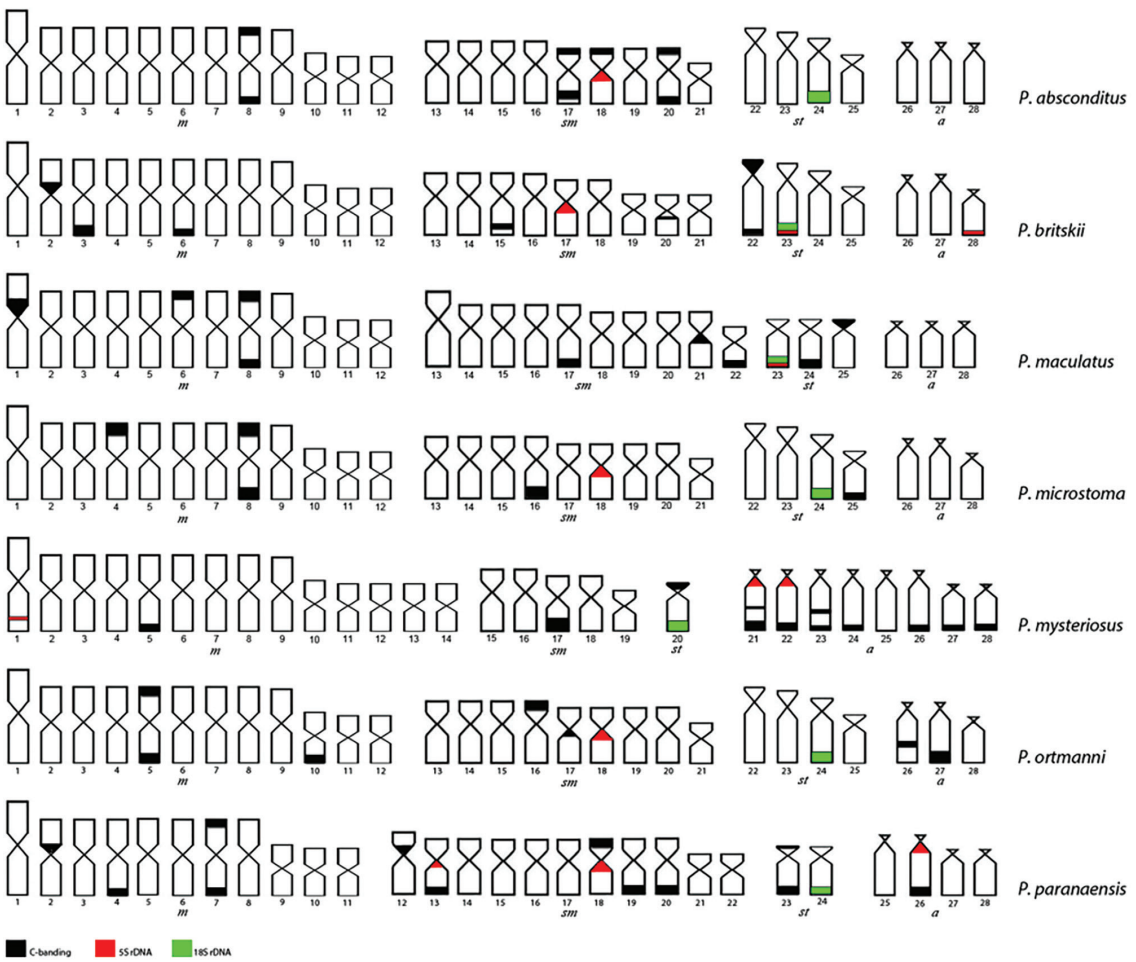

Fig. 6. Idiogram for the seven studied *Pimelodus* species with data obtained with different methodologies. Heterochromatin (black), 5S rDNA (red) and 18S rDNA (green).

Chromosomes B are present in some individuals of some populations and, in some species, they are additional dispensable chromosomes that probably originated from complementary A chromosomes, but follow an evolutionary path of their own (Beukeboom, 1994). They are found in the main groups of animals and plants (Camacho *et al*., 2000). *Pimelodus ortmanni* presents B chromosomes in form of micro chromosomes and some acrocentric (Fig. 4), varying in number from 0 to 4 chromosomes per cell, occurring in more than 91% of the analyzed cells (Tab. 4). According to Camacho *et al*. (2000), the frequency of these chromosomes in natural populations depends on how much the species can tolerate these additional elements, and the strength of the accumulation mechanism and the maximum number of B chromosomes that the species is able to tolerate depends on the intensity of selective (environmental) factors, historical (number of generations since B origin), transmission factors and random factors (genetic drift). The B chromosomes in *P. ortmanni* were completely heterochromatic, being this characteristic observed in most cases (Camacho *et al*., 2000). In Pimelodidae they have been reported in *Bergiaria westermanni* (Lütken, 1874) (Dias, Foresti, 1993), *I. labrosus* (Vissotto *et al*., 1999a; Carvalho *et al*., 2004; Carvalho, Dias 2005; 2007; Carvalho *et al*., 2010), *Megalonema platanum* (Carvalho *et al*., 2011), *Pimelodus ortmanni* and *Pimelodus* sp. (Borin, Martins-Santos, 2004).

Pimelodus maculatus is widely distributed along the rivers La Plata, São Francisco, Paraná and Uruguay (Rocha, 2012). Cytogenetic studies in several populations show maintenance of the diploid number with variation in the karyotypic formula. According to Ferreira *et al*. (2014), these variations are subtle and may be related to different patterns of condensation of the chromosomal preparations or due to the fixation of distinct chromosomal rearrangements during the evolutionary process of each population. The population of the Ijuí River studied here presents a karyotypic formula and distribution pattern of heterochromatin similar to the Lake Guaíba population studied by Treco *et al*. (2008), which may indicate close evolutionary history between them. In the Ijuí River, 5S rDNA was located in the terminal position of the long arm of a pair of subtelocentric chromosomes, and for the first time, it was observed syntenic with 18S rDNA in *P. maculatus*. Populations of the Upper Paraná River studied by Ferreira *et al*. (2014) also show a single pair with 5S rDNA sites. Garcia, Moreira-Filho (2008) performed cytogenetic studies in the *P. maculatus* population of the São Francisco River and observed 5S rDNA in three pairs of chromosomes. These differences in the amount and arrangement of the 5S rDNA sites may indicate the existence of more than one species. According to Ferreira *et al*. (2014), although the populations of the Upper Paraná and São Francisco river basins are not morphologically differentiated, PCR-RFLP data and genetic sequencing indicate the existence of genetically distinct but related groups, which corroborates the hypothesis also suggested by the authors.

We found the population of *P. mysteriosus* has 2n = 56 chromosomes (28m + 10sm + 2st + 16a), single NORs in the long arm of a subtelocentric pair, pale heterochromatic blocks in the region of the centromere with conspicuous blocks in the region of telomeres and centromeres of some chromosomes. These results, except for the diploid number, differ from that reported by Souza *et al*. (2003) for the *P. mysteriosus* population from the Paraguay River, which presents 26m + 20sm + 2st + 8a, single NORs on the short arm of a subtelocentric pair and pale heterochromatin in the telomere region. These differences suggest the existence of more than one species, evidencing the need for further studies among populations from the Paraguay and Middle Paraná rivers.

The population of *P. microstoma* (cited as *P. heraldoi*) by Treco, Dias (2009) comes from the same basin as the exemplars of that species analyzed here. Despite the difference in the karyotypic formula, this should not indicate a real alteration but rather be a result of variations in chromosome condensation, which makes it difficult to classify the chromosomes and enables such differences in the organization of karyotypes among authors (Moraes-Neto *et al*., 2011). In Neotropical fishes, cases of diploid number maintenance with variations in the karyotypic formula are recurrent, as for example in *Iheringichthys labrosus* (Carvalho, Dias 2005; Ribeiro *et al*., 2008), *Parauchenipterus galeatus* (Linnaeus, 1766) (Lui *et al*., 2010), *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829) (Swarça *et al*., 2005b) and *Rhamdia quelen* (Quoy & Gaimard, 1824) (Martinez *et al*., 2011).

The cytogenetic data of *Steindachneridion* and *Phractocephalus* species show the diploid number of 56 chromosomes, simple NORs (Ag- and 18S rDNA) and single 5S rDNA in telomeric region on the short arm (Swarça *et al*., 2005a; Matoso *et al*., 2011; Moraes-Neto *et al*., 2011; Swarça *et al*., 2017). Cytogenetic studies in Pimelodidae indicate the features described above for *Steindachneridion* and *Phractocephalus* are observed in most analyzed species, and possibly represent the ancestral condition for the family. Different diploid number found in the species of the *Calophysus*-*Pimelodus* clade reflect a process of karyotype evolution divergent from the other Pimelodidae, and are considered derived condition in the family.

Our results increase cytogenetic information for Pimelodidae providing the first report of synteny between 18S and 5S DNAr genes in *Pimelodus maculatus*, and cytogenetic differences found reinforce the hypothesis of the existence of more than one species in which is currently attributed to the widespread *P. maculatus*. Associating cytogenetic data with available phylogeny enable us identifying features that distinguish subgroups within Pimelodidae, such as NORs location and position of the 5S rDNA sites. Such data facilitate establishing relationships between groups and help understanding the chromosomal evolution of this family.

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