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Cytogenetic Trends in Two Families of the Neotropical Catfishes: Heptapteridae and Pseudopimelodidae (Siluriformes)

Juceli Gonzalez Gouveia,[1] Vivian Patrícia Oliveira de Moraes-Manécolo,[1] Ana Claudia Swarça,[2] Alberto Sergio Fenocchio,[3] Lucia Giuliano-Caetano,[1] and Ana Lúcia Dias[1]

Abstract

Several neotropical Siluriformes groups suffered important taxonomic revisions based on the evaluation of morphological and molecular characteristics that allow the construction of new phylogenetic hypothesis. In the present study were cytogenetically analyzed six species belonging to Heptapteridae (*Cetopsorhamdia iheringi, Phenacorhamdia tenebrosa, Rhamdella eriarcha, Pimelodella meeki, Pimelodella australis, Heptapterus mustelinus*) and two to Pseudopimelodidae families (*Microglanis cottoides* and *Microglanis cibelae*) by means of differential staining techniques to describe more precisely cytogenetic similarities and differences. The diploid number of *R. eriarcha* with $2n = 58$ and *M. cibelae* with $2n = 56$ were reported for the first time. Also, the lowest chromosome number ($2n = 48$) for *P. tenebrosa* was described. The chromosome-banding techniques for to put in evidence nucleolar organizers impregnated by silver nitrate ([AgNORs], chromomycin A_3 [CMA_3], and rDNA 18S) showed for all studied species conserved patterns, characteristic for each family. The rDNA 5S showed high variability among species or populations of both families, these regions could be simple or multiple, syntenic, or not with rDNA18S. The chromosome markers showed that both families are related not only from a morphologic point of view but also by their karyotypic characteristics, however, some of the present cytogenetic results evidence the importance of new morphologic, molecular, and phylogenetic studies to improve the knowledge of these fish groups.

Keywords: Siluriformes, neotropical region, cytotaxonomy

Introduction

Historically Pimelodidae family was divided on morphological analyses in three branches: Pimelodinae, Pseudopimelodinae, and Heptapterinae, all of them having a wide geographical distribution on neotropical freshwaters.[1] These groups have upgraded to families Pimelodidae, Heptapteridae, and Pseudopimelodidae sharing several morphological characteristics that allow considering each of these as monophyletic entities.[2–5] Based on molecular trait, Sullivan *et al.*[6] joined these three families in a new clade, the superfamily Pimelodoidae, considering Pimelodidae as sister group of Pseudopimelodidae and Heptapteridae as sister group of *Conorhynchus*.

In a review and in several reports of cytogenetic data about these families, it has been stated that Heptapteridae shows diverse diploid numbers ranged from $2n = 42$ to $2n = 58$. However, species of the Pseudopimelodidae family are characterized by stability in chromosome numbers showing almost all $2n = 54$ chromosomes.[7–12]

Heptapteridae shows 218 species and 32 genera and Pseudopimelodidae 50 species and 9 genera[2]; however, the karyotypic data are relatively scarce, especially those related with ribosomal DNAs, and only available for some genera (Table 1).

Almost all Heptapteridae species show nucleolar organizers regions (NORs) on a single chromosome pair, situation confirmed by means of *in situ* hybridization with rDNA 18S probes in 24 species, only *Heptapterus mustelinus*[9,13] and *Pimelodella* sp.[14] show multiple NORs.

On the contrary, there was evidenced variation among the genera and species that belongs to Heptapteridae, having

Departments of [1]General Biology and [2]Histology, CCB (Centro de Ciências Biológicas), Universidade Estadual de Londrina, Londrina, Brazil.
[3]Department of Genetics, Universidad Nacional de Misiones, Posadas, Argentina.

Table 1. Cytogenetic Data (2*n*, Karyotype, and rDNA) in Heptapteridae and Pseudopimelodidae Species and Populations

Family/species	*Location*	2n	*Karyotype*	*18S rDNA*	*5S rDNA*	*Reference*
Heptapteridae						
Cetopsorhamdia iheringi	R. Capivara/SP	58	28m+24sm +6st	—	—	30
C. iheringi	R. Pardo/SP	58	28m+24sm +6st	—	—	30
C. iheringi	R. São Francisco/MG	58	22m+16sm +10st +10a	—	—	26
C. iheringi	R. das Marrecas/PR	58	22m+16sm +10st +10a	—	—	26
C. iheringi	Rb. Minhocas/MG	58	28m+26sm +4st	Simple, interstitial	Simple, interstitial	17
C. iheringi	Rb. Três Bocas/PR	58	28m+26sm +4st	Simple, interstitial	Simple, interstitial	Present study
Cetopsorhamdia sp.	Rb. Canta Galo/SP	58	22m+16sm +10st +10a	—	—	26
Heptapterus mustelinus	Arroyo Colombo/RS	54	32m+8sm +10st +4a	Multiple, terminal	Simple, interstitial	Present study
H. mustelinus	Rb. Pindorama/PR	54	26m +18sm +4st +6a	—	—	9
H. mustelinus	R. Forquetinha/RS	58	32m+12sm +4st +10a	Multiple, terminal	Simple, terminal	13
Imparfinis borodini (cited as *H. longicauda*)	Rb. Quinta/SP	52	22m+26sm +4st	—	—	30
I. borodini	R. Pereira/PR	50	24m+18sm +8st	Simple, terminal	Simple, interstitial	33
Imparfinis hollandi	Salto Osório, R. Iguaçu/PR	42	22m+10sm +10st	—	—	29
Imparfinis minutes	Rb. Jacuí-SP	58	42m+12sm +4st	Simple, interstitial	Multiple, interstitial	11
I. minutes	Cunha/SP	58	42m+12sm +4st	Simple, interstitial	Simple, interstitial	11
Imparfinis mirini	Rb. Jacutinga/PR	58	36m+22sm	Simple, interstitial	—	10
I. mirini	R. Paraitinga-SP	58	42m+12sm +4st	Simple, interstitial	Multiple, interstitial	11
I. mirini	R. Araras-SP	58	36m+18sm +4st	Simple, interstitial	Multiple, interstitial	11
I. mirini	R. Iperó-SP	58	42m+12sm +4st	Simple, interstitial	Multiple, interstitial	11
I. mirini	R. Passa Cinco-SP	58	42m+12sm +4st	Simple, interstitial	Multiple, interstitial	11
I. mirini	R. Pilar do Sul-SP	58	36m+18sm +4st	Simple, interstitial	Multiple, interstitial	11
I. mirini	Conchas-SP	58	42m+12sm +4st	Simple, interstitial	Multiple, interstitial	11
I. mirini	R. Capivara/SP	58	42m+12sm +4st	Simple, interstitial	Multiple, interstitial	11
Imparfinis cf. *piperatus*	R. Juquiá/SP	56	24m+12sm +20st	—	—	26
Imparfinis cf. *pipetarus*	R. Juquiá/SP	56	22m+26sm +4st +4a	—	—	37
Imparfinis piperatus	R. Araras/SP	58	32m+26sm	—	—	37
I. piperatus	R. Grande/SP	58	26m+22sm +8st +2a	—	—	37
Imparfinis schubarti	Piumhi/MG	58	18m+34sm +6st	Simple, interstitial	Simple, interstitial	16
I. schubarti	Rb. Três Bocas/PR	58	30m+28sm	Simple, interstitial	—	10
I. schubarti	R. Laranjinha/PR	58	30m+28sm	Simple, interstitial	—	10
I. schubarti	R. Iyaí/PR	58	28m+28sm +2st	Simple, interstitial	—	38
I. schubarti	R. Água das Araras/PR	58	30m+28sm	Simple, interstitial	Simple, interstitial	33
I. schubarti	R. Quexada/PR	58	30m+28sm	Simple, interstitial	Simple, interstitial	33
I. schubarti	R. Vermelho/PR	58	30m+28sm	Simple, interstitial	Simple, interstitial	33
I. schubarti	R. Taquari/PR	58	30m+28sm	Simple, interstitial	Simple, interstitial	33
Imparfinis aff. *schubarti*	Rb. Marrequinhas/PR	58	28m+28sm +2st	Simple, interstitial	—	39
Imparfinis aff. *schubarti*	Rb Três Bocas/PR	58	28m+28sm +2st	Simple, interstitial	—	39
Imparfinis aff. *schubarti*	Rb. Três Bocas/PR	58	22m+18sm +10st +8a	—	—	26
Imparfinis aff. *schubarti*	R. Água da Floresta/PR	58	28m+28sm +2st	Simple, interstitial	—	39
Imparfinis aff. *schubarti*	R. Vinícius/PR	58	28m+28sm +2st	Simple, interstitial	—	39
Imparfinis aff. *schubarti*	R. Taquari/PR	58	28m+28sm +2st	Simple, interstitial	—	39

(*continued*)

Table 1. (Continued)

Family/species	*Location*	2n	*Karyotype*	*18S rDNA*	*5S rDNA*	*Reference*
Imparfinis aff. *schubarti*	R. Jataizinho/PR	58	28m+28sm +2st	Simple, interstitial	—	39
Imparfinis aff. *schubarti*	Rb. Canta Galo/SP	58	22m+18sm +10st +8a	—	—	26
Imparfinis cf. *schubarti*	Rb. da Bacia do Alto Paraná	58	24m+22sm +12st	Simple, interstitial	—	8
Phenacorhamdia tenebrosa	Rb. da Bacia do Alto Paraná	58	30m+22sm +6st	Simple, interstitial	—	8
P. tenebrosa	R. Vermelho/PR	48	14m+8sm +20st +6a	Simple, terminal	Simple, interstitial	Present study
Pimelodella aff. *avanhandavae*	R. Tibagi/PR	52	30m+22sm	Simple, terminal	—	40
Pimelodella australis	Arroyo Ribeiro/RS	58	34m+10sm +8st +6a	Simple, terminal	—	13
P. australis	Lg. dos Quadros/RS	58	34m+10sm +8st +6a	Simple, terminal	Simple, interstitial	Present study
Pimelodella boschmai	Mogi-Guaçu Araras/SP	46	38m+8sm XY/XX	Simple, terminal	Simple, terminal	14
Pimelodella cf. *chagresi*	Alvarado River, Colombia	50	32m+14sm +4st XY/XX	Simple, terminal, sexual Y	Simple, interstitial, sexual Y	34
Pimelodella gracilis	Paraná Mariápolis/SP	46	34m+12sm	Simple, terminal	Multiple, terminal, interstitial	14
Pimelodella griffin	R. Miranda/MS	46	38m-sm +8st-a	—	—	41
Pimelodella lateristriga	Paraíba do Sul Angra/RJ	58	36m+22sm	Simple, terminal	Simple, interstitial	14
Pimelodella laurenti	Cordisburgo/MG	46	28m+14sm +4st	Simple, terminal	Simple, terminal	42
Pimelodella meeki	R. Limoeiro/PR	46	30m+12sm +4st	Simple, terminal	—	43
P. meeki	R. Couro de Boi/PR	46	30m+12sm +4st	Simple, terminal	—	43
P. meeki	R. Gabriel da Cunha/PR	46	30m+12sm +4st	Simple, terminal	—	43
P. meeki	R. São Miguel Arcanjo/SP	46	28m+12sm +6st	Simple, terminal	Simple, terminal	14
P. meeki	R. Pilar, do Sul/SP	46	28m+12sm +6st	Simple, terminal	Simple, terminal	14
P. meeki	Rb. Jacutinga/PR	46	26m+14sm +6st	Simple, terminal	—	10
P. meeki	Rb. da Bacia do Alto Paraná	46	26m+16sm +4st	Simple, terminal	—	8
P. meeki	R. Queixada/PR	46	26m+16sm +4st	Simple, terminal	Simple, terminal	Present study
Pimelodella sp.	Pardo Cardoso/SP	46	34m+12sm	Multiple, terminal	Multiple, terminal	14
Pimelodella sp.	Botucatu/SP	46	28m+12sm +6st	Simple, terminal	Multiple, terminal	42
Pimelodella sp.	Colina/SP	46	28m+12sm +6st	Simple, terminal	Simple, terminal	42
Pimelodella sp.	Guapiara/SP	46	26m+16sm +4st	Simple, terminal	Simple, terminal	42
Pimelodella sp.	Pirassununga/SP	46	28m+14sm +4st	Simple, terminal	Simple, terminal	42
Pimelodella sp.	R. Cuiabá/MT	46	26m+10sm +10st	Simple, terminal	—	43
Pimelodella spelaean	S. Domingos/GO	46	28m+14sm +4st	Simple, terminal	Multiple, terminal	42
Pimelodella taenioptera	R. Aricá Mirim/MT	52	26m+22sm +4st	Simple, terminal	—	43
Pimelodella vittata	Rb. Minhocas/MG	46	16m+22sm +8st	Simple, terminal	Simple, terminal	17
Rhamdella eriarcha	R. Forquetinha/RS	58	46m-sm +8st +4a	Simple, terminal	Simple, interstitial	Present study
Rhamdella microcephala	R. Machado/MG	56	18m+30sm +8st-a	—	—	44
Rhamdia branneri	Us. Salto Segredo—R. Iguaçu/PR	58	36m+14sm +4st +4a	—	—	31
Rhamdia hilarii	R. Onça/SP	58	36m+18sm +8a	—	—	45
R. hilarii	Monjolinho/SP	58	—	—	—	46
Rhamdia cf. *hilarii*	Rv. Jurumirim/SP; Rb. Quinta/SP; Rb. Jacutinga/SP; Rb. Hortelã/SP; R. Araquá/SP; R. Pardo/SP	58	30m+18sm +10st	—	—	47
R. hilarii	Rv. Lobo/SP	58	—	—	—	48

(*continued*)

TABLE 1. (CONTINUED)

Family/species	*Location*	*2n*	*Karyotype*	*18S rDNA*	*5S rDNA*	*Reference*
R. hilarii	Rv. "29"/SP	58	—	—	—	48
R. hilarii	R. Mogi-Guaçu/SP	58	—	—	—	48
R. hilarii	R. São Franscisco/MG	58	—	—	—	48
R. hilarii	R. Aguapey/Ar	58	—	—	—	48
R. hilarii	R. Mogi-Guaçu/SP	58	58m/sm	—	—	49
R. hilarii	R. Aguapey/Ar	58	26m+16sm +8st +8a	—	—	26
Rhamdia quelen	Lg. dos Quadros/RS	58	52m/sm/st +6a	Simples, simple, terminal	—	27
Rhamdia prope quelen	Rb. Minhocas/MG	58+3*n*=87	26m+16sm +14st +2a	Simple, terminal	Simple, interstitial	17
R. quelen	R. Guaíba/RS	58	52m/sm/st +6a	Simple, terminal	—	27
R. quelen	R. Mogi-Guaçu/SP	58	—	—	—	48
R. quelen	R. Iguaçu/SC	58	—	—	—	48
R. quelen	R. Paraná/Ar	58	—	—	—	48
R. quelen	R. Paraná/Ar	58	26m+16sm +8st +8a	—	—	26
R. quelen	R. Iguaçu/PR	58	32m+16sm +6st +4a	—	—	50
R. quelen	R. Iguaçu/PR	58	32m+16sm +6st +4a	—	—	51
R. quelen	R. Taquarussu/PR	58	26m+20sm +6st +6a	—	—	52
R. quelen	Rb. Maringá/PR	58	26m+22sm +6st +4a/ 26m+24sm +8st	—	—	52
R. quelen	Serra da Bodoquena/MS	58	36m+16sm +6st	—	—	53
R. quelen	Água dos Patos/SP	58	36m+16sm +6st	—	—	54
R. quelen	Água das Pedras/PR	58	36m+16sm +6st	—	—	54
R. quelen	Taquari/PR	58	36m+16sm +6st	—	—	54
R. quelen	R. Iguaçu/PR	58	32m+16sm +6st +4a	Simple, terminal	—	50
R. quelen	FUNPIVI/SC	58	36m+16sm +6st	Simple, terminal	—	54
R. quelen	Sangão—Cascavel/PR	58	32m+8sm +12st +6a	Simple, terminal	Simple, interstitial	15
R. quelen	R. Oeste—Cascavel/PR	58	40m+10sm +8st	Simple, terminal	Simple, interstitial	15
R. quelen	Angra dos Reis/RJ	58	36m+14sm +8st	Simple, terminal	Simple, interstitial	15
R. quelen	R. São José/RJ	58	36m+14sm +8st	Simple, terminal	Simple, interstitial	15
R. quelen	R. Paraíba, do Sul/RJ	58	40m+10sm +8st	Simple, terminal	Simple, interstitial	15
R. quelen	Araras/SP	58	44m+12sm +2st	Simple, terminal	Simple, interstitial	15
R. quelen	R. Capivra-Botucatu/SP	58	36m+10sm +12st	Simple, terminal	Simple, interstitial	15
R. quelen	Colina/SP	58	36m+10sm +12st	Simple, terminal	Simple, interstitial	15
R. quelen	Guapiara/SP	58	36m+10sm +12st	Simple, terminal	Simple, interstitial	15
R. quelen	Iguapé/SP	58	34m+16sm +8st	Simple, terminal	Simple, interstitial	15
R. quelen	R. Passacinco—Ipeúna/SP	58	40m+10sm +8st	Simple, terminal	Simple, interstitial	15
R. quelen	Fortuna—Mariápolis/SP	58	40m+10sm +8st	Simple, terminal	Multiple, interstitial	15
R. quelen	Piquete/SP	58	30m+14sm +12st +2a	Simple, terminal	Simple, interstitial	15
R. quelen	Sto. Antônio do Pinhal/SP	58	32m+8sm +12st +6a	Simple, terminal	Simple, interstitial	15
R. quelen	Rb. Lindóia/PR	3*n*=87	30m+14sm +10st +4a	Simple, terminal	Simple, interstitial	55
R. quelen	Mogi-Guaçu/SP	58	22m+18sm +12st +6a	Simple, terminal	Simple, interstitial	56
R. quelen	Araguaia	58	18m+18sm +14st +8a	Simple, terminal	Simple, interstitial	56
R. quelen	Lg. da Usina Elétrica Ney Braga/PR	58	38m/sm +14st +6a	Simple, terminal	Simple, terminal	57

(*continued*)

Table 1. (Continued)

Family/species	*Location*	2n	*Karyotype*	*18S rDNA*	*5S rDNA*	*Reference*
R. quelen	Lg. da Usina Elétrica Ney Braga/PR	$3n = 87$	57m/sm +21st +9a	Simple, terminal	Simple, terminal	[57]
R. quelen	Rb. da Bacia do Alto Paraná	58	32m+18sm +8st	Simple, terminal	Simple, terminal	[8]
Rhamdia sapo	Buenos Aires/Ar	58	44m/sm +14st/a	—	—	[58]
Rhamdia sp.	Rb. Grande	58	46m/sm +12st/a	Simple, terminal	Simple, interstitial	[59]
Rhamdia sp.	Rb. Grande/SP	$3n = 87$	69m/sm +18st/a	Simple, terminal	Simple, interstitial	[59]
Rhamdia sp.	Us. Salto Segredo—R. Iguaçu/PR	58	36m+14sm +4st +4a	—	—	[31]
Rhamdia voulezi	Us. Salto Segredo—R. Iguaçu/PR	58	36m+14sm +4st +4a			[31]
Rhamdiopsis prope microcephala	Rb. Minhocas/MG	56	12m+30sm +14st	Simple, terminal	Multiple, terminal	[17]
Taunaya bifasciata	Rb. Bacia do Alto Paraná	58	20m+18sm +20st	Simple terminal	—	[8]
Pseudopimelodidae						
Cephalosilurus apurensis	R. Orinoco/Venezuela	54	6m+28sm +14st +6a			[36]
Lophiosilurus alexandri	R. São Francisco	54	16m+18sm +10st +1a	Simple, terminal	Simple, terminal	[18]
Microglanis cibelae	R. Maquiné/RS	54	22m+16sm +12st +4a	Simple, terminal	Simple, interstitial	Present study
Microglanis aff. *cottoides*	Rb. Cavalo, R. Jaraguá do Sul/SC	54	10m+32sm +10st +2a			[36]
Microglanis cottoides	R. Forquetinha/RS	54 $3n = 81$	30m+14sm +6st +4a	Simple, terminal	Simple, interstitial	[12]
M. cottoides	R. Araquá e R. Capivara	54	22m+20sm +12st	—	—	[30]
M. cottoides	R. das Antas/PR	54	30m+14sm +6st +4a	Simple, terminal	Simple, terminal	Present study
M. cottoides	R. Batatal/PR	54	30m+14sm +6st +4a	Simple, terminal	Multiple, interstitial	Present study
M. cottoides	R. Iporanga/PR	54	30m+14sm +6st +4a	Simple, terminal	Multiple, interstitial	Present study
Pseudopimelodus bufonius	Aq. Trade/Amaznia	54	12m+30sm +12st	—	—	[36]
Pseudopimelodus mangurus	R. Mogi-Guaçu/SP	54	6m+26sm +12st +10a	—	—	[60]
Pseudopimelodus pulcher	R. Laranjinha/PR	54	20m+16sm +10st +8a	Multiple, terminal	—	[12]

2*n*, diploid number; a, acrocentric; GO, Goiás; Lg, lagoon; m, metacentric; MG, Minas Gerais; PR, Paraná; R, river; Rb, stream; rDNA, ribosomal DNA; RJ, Rio de Janeiro; RS, Rio Grande do Sul; Rv, reservoir; sm, submetacentric; SP, São Paulo; st, subtelocentric.

some cases with one or more chromosome pairs carrying rDNA 5S. *Rhamdia* shows the higher number of populations with these regions studied (Table 1), being predominant single pattern, except for *Rhamdia quelen* of Fortuna River (Sao Paulo State).[15] In this family, it was reported that rDNA 18S and 5S are located in the same chromosome pair in *Imparfinis mirini*, *Imparfinis minutus,*[11] *Imparfinis schubarti*,[16] and *Cetopsorhamdia iheringi.*[17]

Fluorescence *in situ* hybridization (FISH) cytogenetic analyses with rDNA probes in Pseudopimelodidae are restricted to three species, *Lophiosilurus alexandri* and *Microglanis cottoides* that show one pair carrying genes rDNA 18S and other different chromosomes with rDNA 5S.[12,18] In time, *Pseudopimelodus pulcher* has rDNA 18S located on two pairs.[12]

In view that species of these families are closely related and considered as sister groups from a phylogenetic[6] approach, they represent an interesting model for studies in cytotaxonomy and karyotypic evolution.

They were characterized with different cytogenetic markers in some species of fishes of Heptapteridae and Pseudopimelodidae families, comparing them with available data in the literature to suggest karyotypic trends in the group.

Materials and Methods

Collection sites and species analyzed

Thirty-three specimens of six heptapterid species were cytogenetically analyzed: *C. iheringi* and *Phenacorhamdia tenebrosa* from rivers of Paranapanema basin (Paraná state/Brazil), *Pimelodella meeki* from a tributary of the Ivaí river (Paraná state/Brazil), *H. mustelinus* and *Rhamdella eriarcha* from Laguna dos Patos basin (Rio Grande do Sul state/Brazil), and *Pimelodella australis* from Tramandaí basin (Rio Grande do Sul state/Brazil) (Table 2 and Fig. 1).

Twenty-five specimens of two species of Pseudopimelodidae family were cytogenetically analyzed: *M. cottoides* from Ribeira do Iguape river basin (São Paulo state/Brazil) and Tagaçaba river basin (Paraná state/Brazil) and *Microglanis cibelae* from Tramandaí basin (Rio Grande do Sul state/Brazil) (Table 2 and Fig. 1). All specimens were collected with the permission of Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), with license number 11399-1. The specimens were deposited in the Museu de Zoologia da Universidade Estadual de Londrina (MZUEL), Paraná, Brazil, with voucher numbers: *C. iheringi* (MZUEL14125), *H. mustelinus* (MZUEL5350), *P. meeki* (MZUEL14132); *P. tenebrosa* (MZUEL14126); *M. cottoides*: Batatal river (MZUEL8020); Iporanga river (MZUEL8021); Antas river (MZUEL7953), and *M. cibelae* (MZUEL7452).

Preparation of chromosomes, conventional staining, and chromosome banding

The metaphase chromosomes were obtained through the air-drying technique using kidney tissue in direct chromosome preparations.[19] After 30 metaphases counted 1 each specimen, the diploid number and karyotype were determined, and the chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a).[20] To determine the fundamental number (FN) metacentric, submetacentric, and subtelocentric chromosomes were considered biarmed, and acrocentric chromosomes were considered uniarmed. The nucleolar organizer regions (AgNORs) were detected by silver nitrate impregnation.[21] The guanine-cytosine (GC) bands were detected with chromomycin A_3 (CMA_3).[22]

Fluorescence in situ *hybridization*

FISH was performed according to Pinkel *et al.*[23] with modifications. The 18S rDNA probe was isolated from genome of

Table 2. Species Analyzed, Collection Sites, and Hydrographic Basins

Family/species	*Number of individuals*	*Collection sites*	*Basin*
Heptapteridae			
C. iheringi	3 females and 2 males	Três Bocas stream/PR (23°23′06.6″S 51°04′35.8″W)	Paranapanema
H. mustelinus	1 females and 2 unidentified sex	Arroyo Colombo/RS (30°06′02.0″S 51°41′42.0″W)	Laguna dos Patos
P. tenebrosa	5 females and 1 male	Vermelho river/PR (23°13′0.95″S 51°20′0.88″W)	Paranapanema
P. australis	1 female and 4 unidentified sex	Quadros Lagoon/RS (29°44′42.8″ S 50°06′54.3″W)	Tramandaí
P. meeki	4 females and 6 males	Quexada river/PR (23°56′9.65″S 51°39′26.08″W)	Ivaí
R. eriarcha	1 female, 2 males and 1 unidentified sex	Forquetinha river/RS (29°28′0.17″S 54°24′59.89″W)	Laguna dos Patos
Pseudopimelodidae			
M. cottoides	2 females and 4 males	Antas river/PR (25°13′35.2″S 48°34′03.9″W)	Tagaçaba
M. cottoides	7 females and 1 male	Iporanga river/SP (24°34′49″S 48°35″30.2″W)	Ribeira do Iguape
M. cottoides	5 females	Batatal river/SP (24°36′34.7″S 48°17′14.2″W)	Ribeira do Iguape
M. cibelae	6 females	Maquiné river/RS (29°39′10.4″S 50°12′31.8″W)	Tramandaí

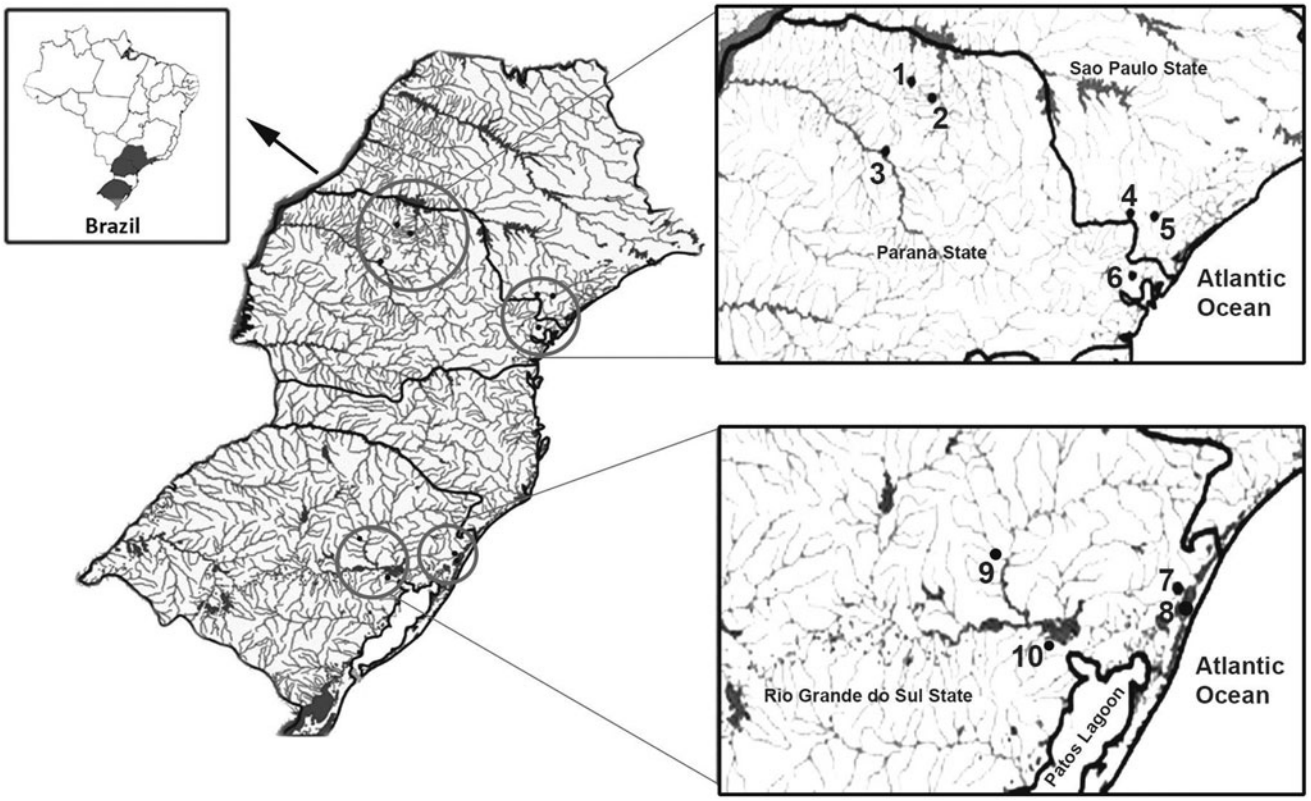

FIG. 1. Collection sites. Map of Brazil indicating, in the *right* side, Sao Paulo State, Parana State and Rio Grande do Sul State: Paranapanema basin: (1) Vermelho river; (2) Três Bocas stream; Ivaí basin: (3) Queixada river; Ribeira do Iguape basin: (4) Batatal river; (5) Iporanga river; Tagaçaba basin: (6) Antas river; Tramandai basin: (7) Maquine river; (8) Quadros lagoon; Laguna dos Patos basin: (9) Forquetinha river; and (10) Arroyo Colombo.

Prochilodus argenteus[24] and 5S rDNA of *I. schubarti* was isolated as described by Gouveia *et al.*[25] Probes were labeled with DIG-Nick Translation Kit (Roche Applied Science, Mannheim, Germany) cat number 11745816910 or BioNick™ Labeling System Kit (Invitrogen Life Technology, Carlsbad) cat number 18247015. Preparations were covered with 50 μL of hybridization mixture containing 100 ng of labelled probe (7.5 μL), 50% formamide (30 μL), dextran sulphate 50% (12 μL) and 20 × saline-sodium citrate (SSC) (10.5 μL). The preparations were denatured at 80°C for 10 min and hybridized overnight at 37°C in a humidified chamber. Posthybridization washes were carried out in 2 × SSC for 5 min., in 1 × phosphate-buffered saline, and 1 × (20 × SSC, Triton 100, nonfat milk, and distilled water, pH 7) all at 45°C. The probe was detected with 5 μL of avidin conjugated with fluorescein isothiocyanate (1:100) and anti-digoxigenin with rhodamine conjugate +45 μL of bovine serum albumin (5%) as appropriate. To amplify the signal, 40 μL of amplification solution (1 μL antiavidin-biotin conjugate and 39 μL of 1 × [20 × SSC, Triton 100, nonfat milk, and distilled water, pH 7]) was used. The slides were mounted with 25 μL of a medium composed of 23 μL of DABCO solution [1,4-diaza-bicyclo (2.2.2)-octane (2–3%), 20 mM Tris HCl, pH 8.0, and glycerol (100%), in distilled water], 1 μL of $MgCl_2$ 50 mM, and 1 μL of DAPI solution (20 μg/mL). Images were acquired with a Leica DM 4500 B microscope equipped with a DFC 300FX camera and Leica IM50 4.0 software of Nikon E800. Images were overlaid and processed in Adobe Photoshop using only cropping and functions affecting the whole image equally.

Results

Heptapteridae family

C. iheringi had $2n = 58$ and a karyotypic formula of 30m, 24sm, 4st, and FN = 116. The pair 16 submetacentric presented a secondary constriction located interstitially on the short arms (Fig. 2a), which coincided with the AgNORs, CMA_3 signals (Fig. 2a, box), and 18S rDNA (Fig. 4a, arrows). The 5S rDNA sites were also evidenced in the 16 pair in interstitial region, but in the long arm (Fig 4a, arrowhead).

H. mustelinus had $2n = 54$ and a karyotypic formula of 32m, 10sm, 10st, 2a, and FN = 106 (Fig. 2b). The AgNORs were evidenced in the short arm of the metacentric pair 5 and the long arm of the subtelocentric pair 23, both in the terminal region coincident with CMA_3 (Fig. 2b, box) and 18S rDNA sites (Fig. 4b, arrows). 5S rDNA sites were located on a pair of subtelocentric chromosomes in the short-arm terminal region, showing no synteny with 18S rDNA site (Fig. 4b, arrowhead).

P. tenebrosa had $2n = 48$ and a karyotypic formula of 14m, 10sm, 16st, 8a, and FN = 90 (Fig. 2c). The pair 12 presented a secondary constriction located terminally on the short arms, which coincided with the AgNORs and CMA_3 (Fig. 2c, box) and 18S rDNA (Fig. 4c, arrows). The 5S rDNA sites were also evidenced in the pair 12 in the interstitial region on the short arm, near the centromere. There was synteny between the 18S and 5S rDNAs (Fig. 4c, arrowheads).

P. australis had $2n = 58$ and a karyotypic formula of 34m, 10sm, 8st, 6a, and NF = 110 (Fig. 2d). The AgNORs were

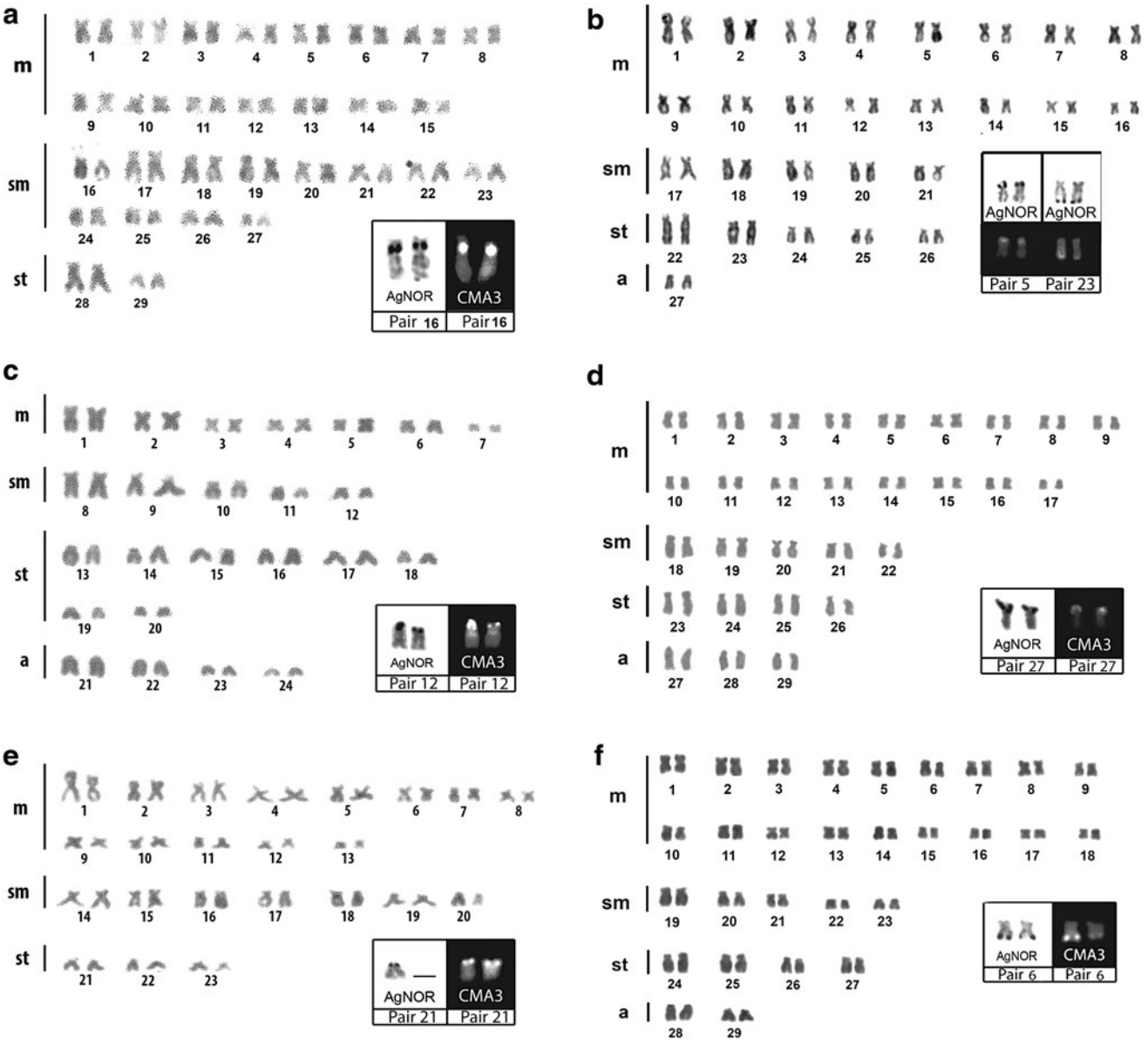

FIG. 2. Karyotype of Heptapteridae species: **(a)** *Cetopsorhamdia iheringi*; **(b)** *Heptapterus mustelinus*; **(c)** *Phenacorhamdia tenebrosa*; **(d)** *Pimelodella australis*; **(e)** *Pimelodella meeki*; and **(f)** *Rhamdella eriarcha. Inset box* shows location of AgNOR and CMA_3 staining. AgNOR, nucleolar organizer region with silver nitrate staining; CMA_3, chromomycin A_3.

observed terminally on the short arms of the pair 27 (acrocentric pair), which coincided with the CMA_3 signals (Fig. 2d, box) and 18S rDNA sites (Fig. 4d, arrows). 5S rDNA sites were located on a pair of acrocentric chromosomes in the interstitial region on the long arm, showing no synteny with 18S rDNA sites (Fig. 4d, arrowheads).

P. meeki had $2n = 46$ and a karyotypic formula of 26m, 14sm, 6st, and FN = 92 (Fig. 2e). The AgNORs were evidenced terminally on the short arm of the pair 21 (subtelocentric) (Fig. 2e, box), which coincided with the CMA_3 signals (Fig. 2e, box) and 18S rDNA sites (Fig. 4e, arrows). 5S rDNA sites were observed on a pair of subtelocentric chromosomes in the terminal region on the short arm, showing no synteny with 18S rDNA sites (Fig. 4e, arrowheads).

R. eriarcha had $2n = 58$ and a karyotypic formula of 36m, 10sm, 8st, 4a, and FN = 112 (Fig. 2f). The AgNORs were evidenced terminally on the long arm of the pair 6 (metacentric pair), which coincided with the CMA_3 signals (Fig. 2f, box) and 18S rDNA sites (Fig. 4f, arrows). 5S rDNA sites were observed in the interstitial region on the short arm of one metacentric pair, showing no synteny with 18S rDNA sites (Fig. 4f, arrowheads).

Pseudopimelodidae family

M. cibelae had $2n = 54$ and a karyotypic formula of 22m, 16sm, 12st, 4a, and FN = 104, with a secondary constriction located terminally on the short arm of the pair 26 (acrocentric pair) (Fig. 3a), which coincided with the AgNORs and CMA_3

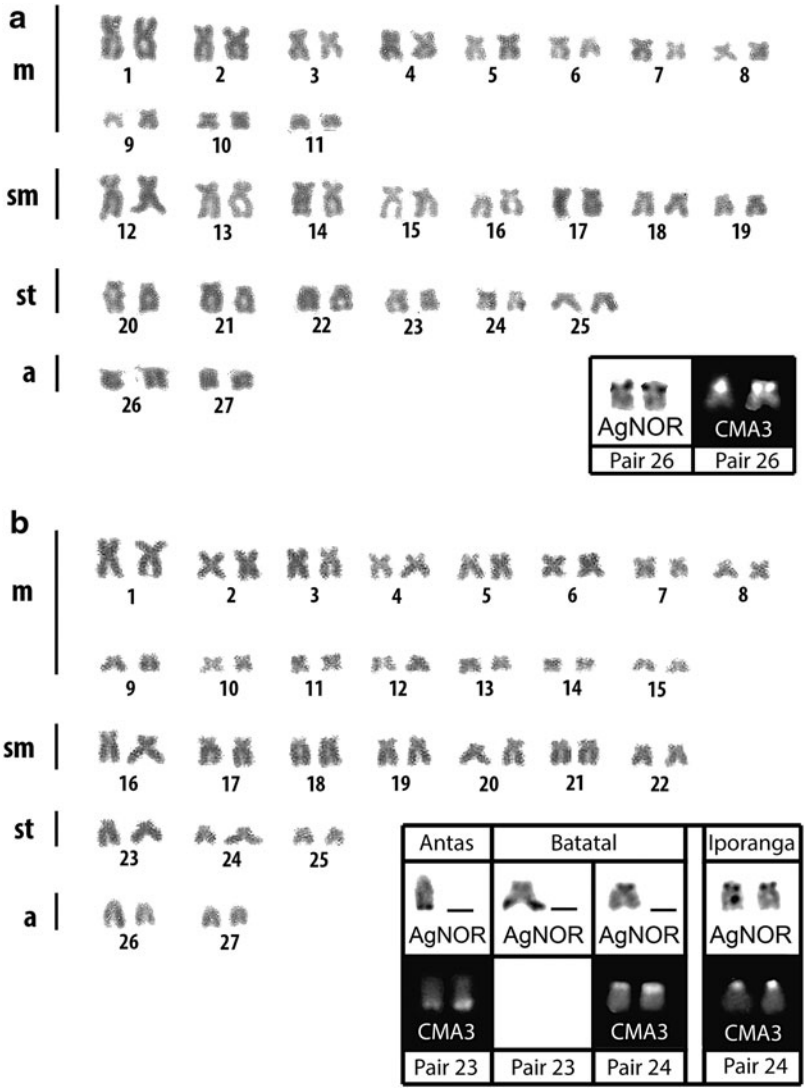

FIG. 3. Karyotype of Pseudopimelodidae species: **(a)** *Microglanis cibelae*; **(b)** *Microglanis cottoides. Inset box* shows location of AgNOR with silver nitrate staining and CMA_3 staining.

signals (Fig. 3a, box) and 18S rDNA sites (Fig. 4g, arrows). 5S rDNA sites were observed in the interstitial regions on the short arms of one subtelocentric pair, near the centromere (Fig. 4g, arrowheads).

All the population of *M. cottoides* had $2n = 54$ and a karyotypic formula of 30m, 14sm, 6st, 4a, and FN = 104 (Fig. 3b), however, each population showed variation in the location of AgNORs, CMA_3, and 18S rDNA.

In *M. cottoides* of the Antas River, the AgNORs are limited to the distal region of long arm of a single chromosome of the pair 23 (Fig. 3b, box). CMA_3 and 18S rDNA signals were evidenced at the terminal region on the long arm of the same pair (Figs. 3b, box and 4h, arrows). 5S rDNA sites were observed in the terminal regions of one subtelocentric pair, showing no synteny with 18S rDNA sites (Fig. 4h, arrowheads).

M. cottoides of the Batatal river presented AgNORs in the terminal region either, on the long arm of only one subtelocentric chromosome (pair 23) and in the terminal region of the short arm of one chromosome of the pair 24 (Fig. 3b, box). Coincidence among CMA_3 and 18S rDNA signals were observed in pair 24 (Figs. 3b, box and 4i, arrows). 5S rDNAs sites were evidenced in four chromosomes: a pair of acrocentric with interstitial bands on the long arm and a pair of subtelocentric chromosomes with interstitial bands on the short arm (Fig. 4i, arrowhead).

M. cottoides of the Iporanga river presented AgNORs in the terminal region on the short arm of one subtelocentric pair (pair 24) (Fig. 3b, box), which are coincident with CMA_3 signals (Fig. 3b, box) and 18S rDNA sites (Fig. 4j, arrows). 5S rDNA presented multiple sites, with two chromosome pairs in the interstitial region, being one acrocentric pair on the short arms and one subtelocentric pair on the long arms (Fig. 4j, arrowhead).

Discussion

Heptapteridae family

Present data constitute the first karyotypic description for *R. eriarcha* ($2n = 58$) and *P. tenebrosa* ($2n = 48$) confirming previous reports showing karyotypic variability within this family (Table 1).

Among Heptapteridae, most species show diploid numbers higher than 50[7,8] chromosomes being considered $2n = 58$ the basal one for the family[26] and the higher number of biarmed chromosomes (m, sm, st) reflects in a high FN. Meanwhile, new findings show interspecific variability and population

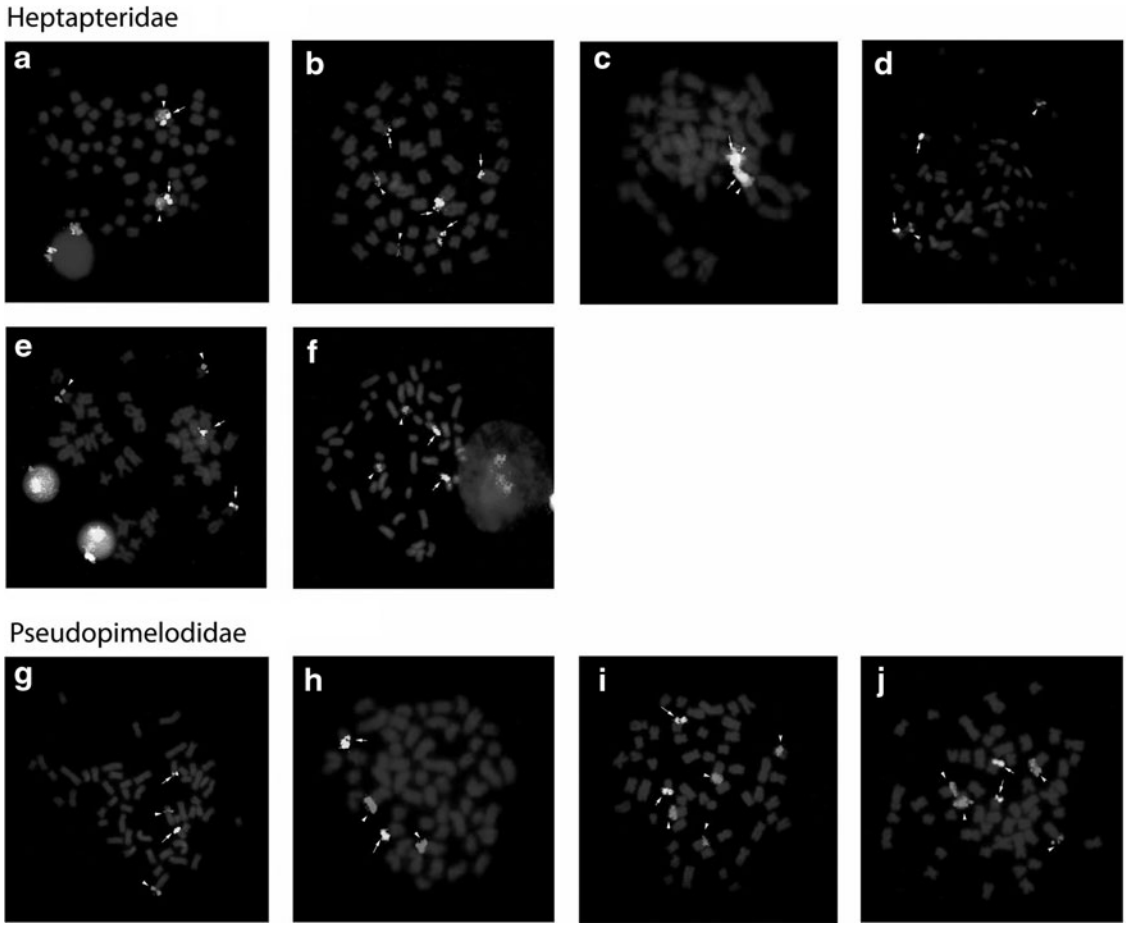

FIG. 4. Somatic metaphases of the Heptapteridae and Pseudopimelodidae species with 5S rDNA sites (*arrowheads*) and 18S rDNA sites (*arrows*): **(a)** *C. iheringi*; **(b)** *H. mustelinus*; **(c)** *P. tenebrosa*; **(d)** *P. australis*; **(e)** *P. meeki*; **(f)** *R. eriarcha*; **(g)** *M. cibelae*; **(h)** *M. cottoides* (Antas river); **(i)** *M. cottoides* (Batatal river); **(j)** *M. cottoides* (Iporanga river).

polymorphism (Table 1). The most heterogeneous genus is *Imparfinis* that shows 2*n* ranging from 42 to 58 chromosomes and *Pimelodella* with 2*n* ranging from 46 to 58 chromosomes (Table 1). On the contrary, *Rhamdia* and *Cetopsorhamdia* have a karyotype practically invariable with 58 chromosomes (Table 1). However, several species and populations of *Rhamdia* show numerical variation due to the presence of polymorphic B chromosomes.[15,27]

P. tenebrosa described in this article interestingly shows $2n = 48$, a chromosome number highly different ($2n = 58$), which was reported previously by Borba *et al.*[8] for the same species. Both populations belong to the higher Paraná River basin.[28] In this case, it is difficult to explain what chromosomal rearrangements may have occurred to lead to this difference; however, probably a mistake might have occurred in the species identification. Thus, chromosomal analysis could be important for cytotaxonomy and identification of cryptic species. *H. mustelinus* from Arroyo Colombo (Laguna dos Patos basin) have $2n = 54$ chromosomes (present article) such as *H. mustelinus* from Pindorama stream of the upper Paraná River basin (Brazil).[9] The same species from Laguna Patos hydrographic system, of the Forquetinha river, has been characterized by Moraes[13] showing 58 chromosomes. These numerical karyotypic differences could be explained by diverse chromosome rearrangements as fusions and translocations[10,17,29]; however, clear efforts in taxonomy and systematic classification are necessary.

As expected, CMA_3 staining and FISH with 18S rDNA exhibited fluorescent signals that correspond to the AgNOR sites. This correspondence has already been observed in almost species of Heptapteridae, frequently associated to terminal regions of one chromosome pair.[7,8] The exceptions were found in three genera *Cetopsorhamdia*, *Imparfinis*, and *Taunaya* with interstitial NORs (Table 1), feature considered synapomorphy.

Only *H. mustelinus* presented multiple sites with terminal NORs as observed in another population of *H. mustelinus*,[9] in *Imparfinis borodini*[30]; *Rhamdia* sp., and *R. quelen*,[31] but are not being confirmed by FISH.

The location and number of the rDNA 5S are relatively variable occupying preferentially interstitial regions on one chromosome pair or two chromosome pairs (Table 1), in some cases syntenic with regions rDNA 18S, as already described by other authors.[11,16,25,32–34]

Gouveia *et al.*[25,33] isolated 5S rDNA sequences and transposon (Tc1-mariner) from *I. schubarti* genome and found that transposable elements may be acting along these sequences, showing hybridization in some species of Heptapteridae and Pseudopimelodidae, and seem to be related to the movement of 5S rDNA genes in these fish genomes.

Pseudopimelodidae family

Present data represent the first karyotypic description for *M. cibelae*, showing a conservative diploid number $2n = 54$ in this family.

Recently, Souza-Shibatta *et al.*[35] reevaluated *M. cottoides* systematics, using morphological and molecular data, with sequence analysis of the COI (cytochrome oxidase I) gene. The authors analyzed different populations of *M. cottoides* from the South Atlantic and Uruguay basins and species that lived in sympatry, among them is *M. cibelae*. Phylogenetic analysis suggests that *M. cottoides* currently forms a non-monophyletic group, which includes endemic populations of the Uruguay River basin closer to *Microglanis malabarbai*, excluding *M. cibelae* that was grouped within the *M. cottoides* samples. Based on these results, the authors proposed *M. cibelae* as a junior synonym of *M. cottoides*.

Despite this, karyotypic data show differences among these two species being clear that *M. cottoides* presents a great number of metacentric chromosomes. Some of these cytogenetic differences were previously reported by other authors and could be attributed to inversions or translocations with consequences in the karyotypic formula.[12,30,36] The diverse karyotypic characterization could be related more to technical artifacts than real differences. However, these variations should also be considered to show the importance of a more detailed analysis of the characterization of these species by taxonomic studies, besides the fact that *M. cibelae* of the present work was collected in the type-locality of the description of this species.

Probably, the most likely NOR phenotype in *M. cottoides* is the short-arm (par 24) terminal region that is shared in the analyzed populations of this species.[12,36] Inversion and translocation events may explain NOR in the terminal region of the long arm in *M. cottoides* of the Antas river.

Among the Pseudopimelodidae also have been described species with multiple AgNORs as *Microglanis* aff. *cottoides*,[36] *Pseudopimelodus bufonius*,[36] *P. pulcher*,[12] and *M. cottoides* (present study). This condition of multiple NORs was confirmed by FISH only for *P. pulcher* (Table 1).

The location and number of the DNAr 5S are relatively variable occupying terminal or interstitial regions on one chromosome pair or multiple chromosome pairs, as observed in other species of this family (Table 1).

Final Remarks

Present and available data confirm some cytogenetic features shared and other exclusive for two families, that is, both families show a great number of biarmed chromosomes, nucleolar organizer regions frequently in one pair, staining always bright CMA_3 and 18S rDNAr and 5S rDNA not syntenic. As an exclusive trait Pseudopimelodidae has a conserved diploid number of 54 chromosomes in all analyzed species, however, Heptapteridae shows a highly variable chromosome number.

When 5S ribosomal genes are analyzed, it is not possible to establish an exclusive pattern to families, but these sites are located preferentially in interstitial regions. The difficulty in establishing karyotypic comparisons or cytossystematic relationships more accurate among the fish of these families is due to the scarcity of chromosome and non-homogeneous data, as well as to mistakes in the taxonomic identification.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Ana Lúcia Dias, Dra
Department of General Biology
CCB (Centro de Ciências Biológicas)
Universidade Estadual de Londrina
P.O. Box 6001
Londrina, Paraná CEP 86051-970
Brazil

E-mail: anadias@uel.br