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Short Communication

Cytogenetic studies in fishes of the genera *Hassar*, *Platydoras* and *Opsodoras* (Doradidae, Siluriformes) from Jarí and Xingú Rivers, Brazil

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Abstract

We studied the karyotypes of *Hassar* cf. *orestis* and an undescribed *Hassar* species from the Jarí River and *Opsodoras ternetzi*, *H. orestis* and *Platydoras* cf. *costatus* from the Xingú River, all with 2n = 58. Constitutive heterochromatin is located in the centromere in most metacentric pairs; in some chromosomes this banding is not present, or it is located on the whole chromosome arm or in the distal regions. The NOR is located on a single biarmed pair at a distal region of the short arm in *H.* cf. *orestis*, *H. orestis* and *P.* cf. *costatus* at a distal region of the long arm in *O. ternetzi* and at a proximal region of the long arm in the *Hassar* species. In all species (except for *Hassar* sp.) the CMA3 analysis revealed a rich G-C region coincident with the NOR. Probably inversions occurred in the NOR chromosome during the chromosomal differentiation of the Doradidae species here described.

Key words: chromosomes, Amazon, biodiversity, Siluriformes, banding, fluorochromes.

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The species of the Doradidae family are usually known as thorny catfishes. They are distributed throughout all the main river systems in the American continent (Higushi, 1992). In South America, these fishes can be found in a large range of freshwaters habitats, between the parallels 10° N and 35° S. Approximately 80 species are described from the Orinoco, Essequibo, Putumayo, Napo, Ucayali-Marañon, Amazonas and its tributaries (Negro, Madeira and Xingú), Tocantins-Araguaia and Paraguay-Paraná River systems. Some endemic species can be found in the São Francisco-Velhas River system in central eastern Brazil with two primitive forms limited to the Jequitinhonha and Paraguaçu River system (Higushi, 1992; de Pinna, 1998).

The fishes from this family have a size range from three centimeters to one meter. They are easily identified by the series of bony plates on each side of the body, where each plate has a curved torn of different size, depending on the species. Some have additional plates among the dorsal and adipose fins, or even covering most of the body. They are omnivorous fishes (Ferreira *et al.*, 1998) and are also called talking catfishes because they are able to make sounds by movements of the pectoral spine or as vibrations produced by the swim bladder (Nelson, 1994).

Based on the cladistic methodology applied for osteological traits, Higuchi (1992) divided this family of 70 species into 33 genera, where three were new. Nelson (1994) reports that the family Doradidae has around of 35 genera with 90 species. Sabaj and Ferraris Jr. (2003) recognized 72 valid species in 30 genera. Following this review, Moyer *et al.* (2004) provide a genus-level phylogeny for doradids based on mitochondrial (12S and 16S rRNA) and nuclear (elongation factor-1 alpha EF1α) gene sequences, as well as a re-evaluation of published morphological data. This phylogeny differs in some aspects from the one proposed by Higuchi (1992).

Few species have their karyotypes studied (Fenocchio *et al.*, 1993; Venere, 1998; Eler *et al.*, 2007). These data show that, even with few species cytogenetically studied in this family, a large variation among the karyotypes is evident, especially in chromosomes morphology (Table 1). In the present paper we describe the karyotypes of *Hassar* cf. *orestis*, *Hassar* sp., *Hassar orestis* Burgess, 1989, *Opsodoras ternetzi* Eigenmann, 1925 and *Platydoras* cf. *costatus* Linnaeus, 1758 aiming to contribute to their taxonomic classification and a better understanding of their chromosomal evolution.

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Table 1 - Chromosomal characterization of the family Doradidae. 2n = diploid number; FN = Fundamental Number; KF = Karyotypic formulae; NOR = Nucleolar Organizer Region; M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric; p = short arm; q = long arm. M = male; F = female.

Species	2n	FN	KF	NOR	Reference
Hassar cf. *orestis*	58	116	32M+18SM+8ST	20° SM, p, distal	Present paper
Hassar orestis	58	114	42M+14SM+2A	SM, q, distal	Venere (1992)
Hassar orestis	58	116	32M+20SM+6ST	22° SM, p, distal	Present paper
Hassar sp.	58	116	32M+18SM+8ST	7° M, q, proximal	Present paper
Hassar wilderi	58	116	32M+16SM+10ST	SM, p, distal	Venere (1992)
Leptodoras acipenserinus	58	112	24M+16SM+14ST+4A	ST, p, distal	Venere (19992)
Opsodoras sp.	58	109 F 108 M	21M+18SM+12ST+7A 20M+18SM+12ST+8A	SM, p, distal	Venere (1992)
Opsodoras ternetzi	58	114	44M+12SM+2A	24° SM, q, distal	Present paper
Platydoras cf. *costatus*	58	104	26M+16SM+4ST+12A	20° SM, p, distal	Present paper
Pseudodoras niger	58	102	20M+16SM+8ST+14A	15° SM, p, distal	Fenocchio *et al.* (1993)
Rhynodoras d'orbignyi	58	102	20M+20SM+4 ST+14A	16° SM, p, distal	Fenocchio *et al.* (1993)
Rhynodoras sp.	58	104	18M+16SM+12ST+12 A	SM, p, distal	Venere (1992)
Trachydoras paraguaiensis	56	112	32M+20SM+4ST	SM, q, interstitial	Fenocchio *et al.* (1993)
Wertheimeria maculata	58	104	24M+14SM+8ST+12A	M, p, distal	Eler *et al.* (2007)
Hassar wilderi	58	116	32M+16SM+10ST	ST, p, distal	Eler *et al.* (2007)

We analyzed fishes of the family Doradidae, from Rivers Jarí (PA) and Xingú (PA). From Jarí River (S = 03° 18' 14,9" and W = 52° 03' 29,3") three females and two males of the *Hassar* cf. *orestis* species were analyzed as well as three females of the species *Hassar* sp. From Xingú River (S = 02° 37' 44,3" and W = 51° 57' 06,4"), three females and two males of the *Opsodoras ternetzi* species were analyzed as well as one male and one female of the species *Hassar orestis*. Also from this River (S = 03° 50' 46,6" and W = 52° 29' 22,3") four females of the species *Platydoras* cf. *costatus* were analyzed. Voucher specimens were deposited in the fish collection of the Museu Paraense Emilio Goeldi in Belém, Pará, Brazil: *Hassar orestis* (MPEG 12463) and *Opsodoras ternetzi* (MPEG 12464), both from Xingú River. The other specimens here described remain uncatalogued.

Mitotic chromosome preparations were obtained from kidney cells using the air-drying technique of Bertollo *et al.* (1978). C-banding (Sumner, 1972), Ag-NOR staining (Howell and Black, 1980) and Chromomycin A3 (CMA_3) banding (Schweizer, 1980) were applied. Chromosome morphology was determined on the basis of arm ratio as proposed by Guerra (1986), and chromosomes were classified as metacentrics (M), submetacentrics (SM), subtelocentrics (ST) and acrocentrics (A).

The five species here studied have 2n = 58 chromosomes. *Hassar* cf. *orestis* (Figure 1a) and *Hassar* sp. (Figure 1b) have 32M+18SM+8ST and FN = 116 *Opsodoras ternetzi* (Figure 1c) has 44M+12SM+2A and FN = 114, *Hassar orestis* (Figure 1d) has 32M+20SM+6ST and FN = 116, and *Platydoras* cf. *costatus* (Figure 1e) has 26M+16SM+4ST+12A and FN = 104. None of these species has shown any sex chromosome heteromorphism.

Single Ag-NORs were observed in all species. In *Hassar* cf. *orestis* (Figure 1a) from Jarí River, and in *Hassar orestis* (Figure 1d) and *Platydoras* cf. *costatus* (Figure 1e) from Xingú River the NOR is located at a distal position of the short arm, while in *Hassar* sp. (Figure 1b) it is at interstitial position of the long arm and in *Opsodoras ternetzi* (Figure 1c) it locates to a distal position of the long arm on submetacentric chromosomes.

Constitutive heterochromatin could be found in the centromeric region of most of metacentric chromosomes, showing a faint banding pattern in all the species here studied. It was possible also to find heterochromatic blocks in the short arms of *Hassar orestis* (Figure 2d) and O*psodoras ternetzi* (Figure 2c). CMA_3 banding in the species *Hassar* cf. *orestis* (Figure 2a), *Opsodoras ternetzi* (Figure 2c), *Hassar orestis* (Figure 2d) and *Platydoras* cf. *costatus* (Figure 2e) showed regions rich in G-C base pairs coincident with the NOR location. Unfortunately, it was not possible to do this banding in *Hassar* sp.

Table 1 summarizes the karyotype data obtained in the present study and those available in the literature. When we compare the results here obtained with the ones described by Venere (1998) we can note that there is good agreement on the diploid number in the genus *Hassar,* except for a small difference in the fundamental number of *Hassar oresti*, because the karyotype described by that author has one acrocentric pair which is not present in the

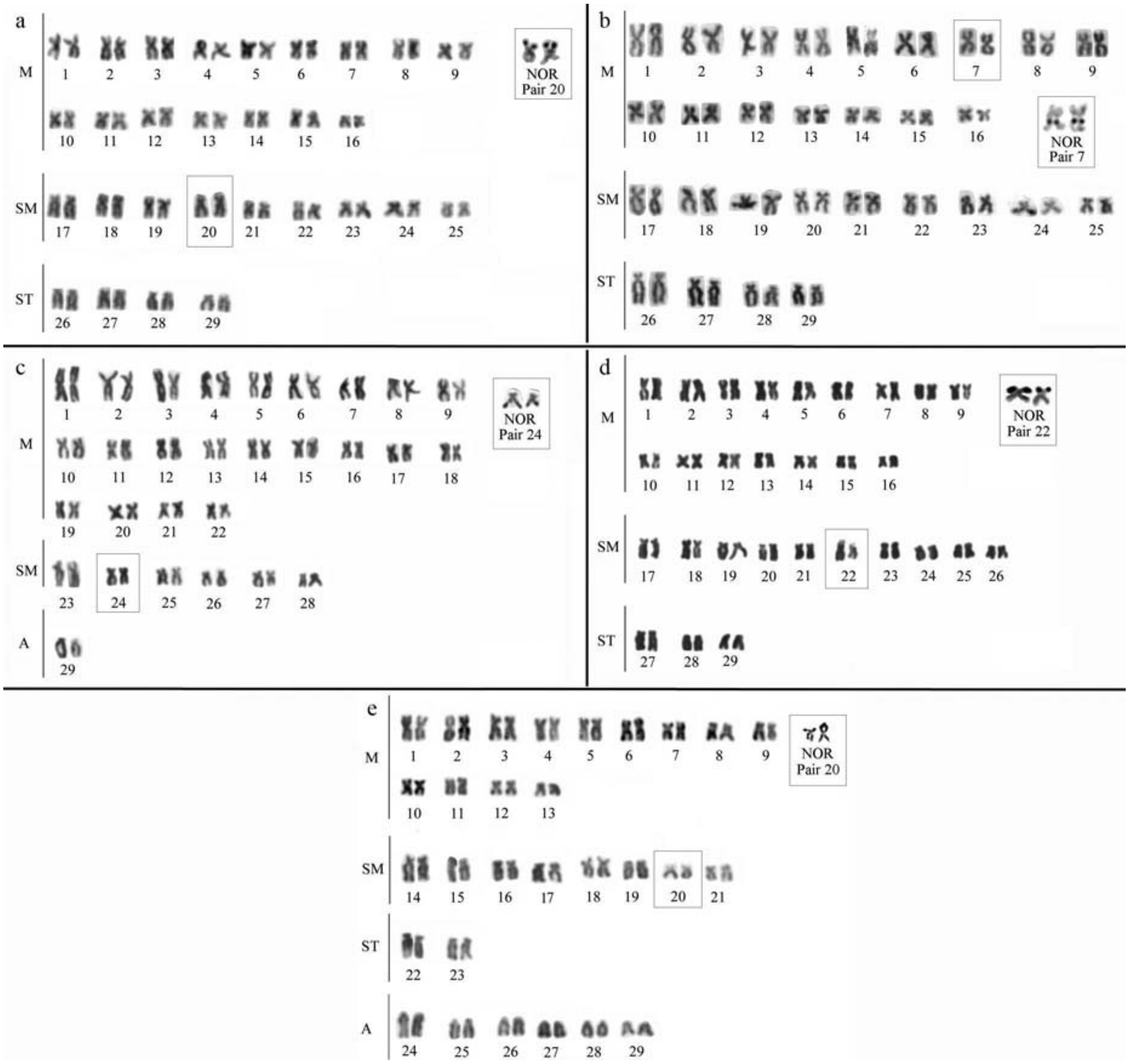

Figure 1 - Giemsa stained karyotypes of the species: a) *Hassar* cf. *orestis*; b) *Hassar* sp.; c) *Opsodoras ternetzi*; d) *Hassar orestis*; e) *Platydoras* cf. *costatus*. Within the boxes are the NOR-stained chromosomes of each species.

karyotype here described. The species *Opsodoras ternetzi* here described also has the same diploid number, however, the karyotype is different from the species *Opsodoras* sp. (Venere, 1998) because it does not have sex chromosome heteromorphism or acrocentric pairs.

The family Doradidae has around of 80 species (de Pinna, 1998), from which 14 were already karyotyped and most have 2n = 58. Probably this is the modal diploid number of this family, the same diploid number which is also considered ancestral for Siluriformes (Oliveira *et al.*, 1988). Eler *et al.* (2007) described the karyotype of *Wertheimeria maculata,* which according to Higushi (1992) is a sister taxon of all Doradidae. This species has 2n = 58 (24M+14SM+8ST+12A), supportig the hypothesis that this diploid number is plesiomorphic for the Doradidae.

The C-banding pattern was similar for the entire group, with a heterochromatic faint pattern. Fenocchio and Bertollo (1992) noted that it is very difficult to obtain good C-banding in Pimelodidae, a family phylogenetically related to Doradidae. This can best be explained by the peculiar traits of the chromatin of these fishes rather than by the absence of heterochromatic regions.

When looking at *Hassar wilderi* and *Opsodoras* sp. which were already analyzed using CMA$_3$ banding (Venere, 1998), we can conclude that this G-C base pairs specific fluorochrome is useful for visualizing the NOR in fishes. According to Pendás *et al.* (1993), the positive correlation between NOR and CMA$_3$ banding occurs because the rRNA genes from NOR region are interspersed by DNA sequences which are rich in G-C base pairs.

The single NOR in the distal position of a short arm in the species *Hassar* cf. *orestis* from Jarí River, *Hassar orestis* and *Platydoras* cf. *costatus* from Xingú River is similar to the one described for *Hassar wilderi*, *Leptodoras*

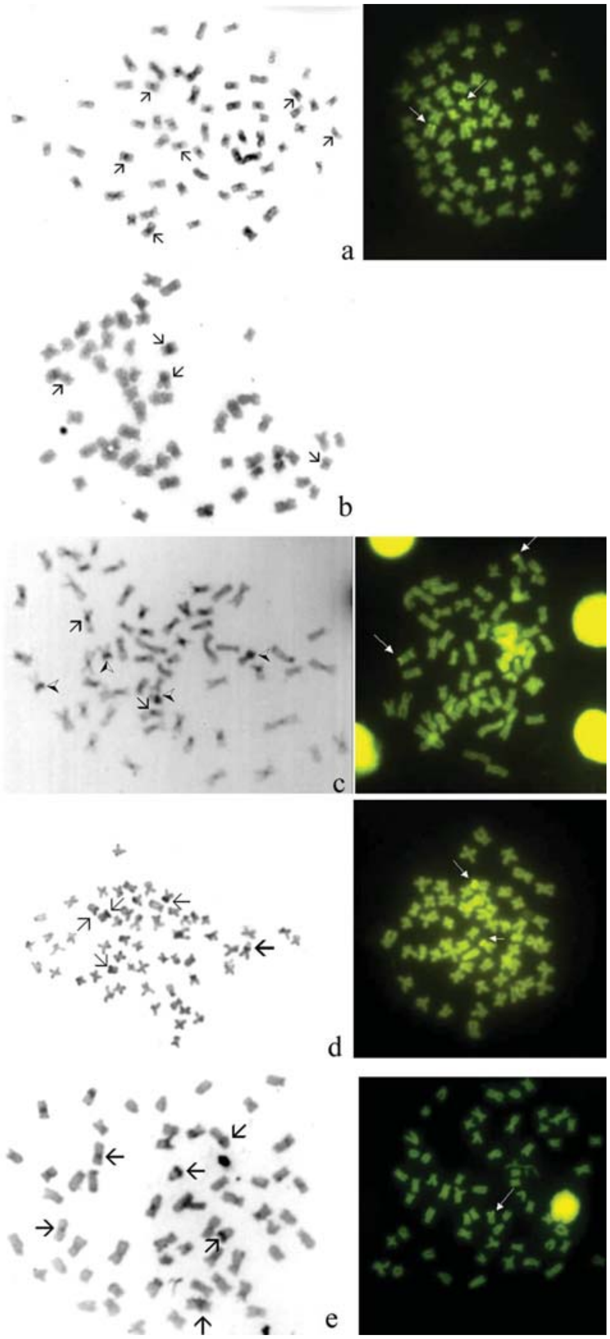

Figure 2 - C-banded (left) and CMA$_3$ banded (right) karyotypes of the species: a) *Hassar* cf. *orestis*; b) *Hassar* sp.; c) *Opsodoras ternetzi*; d) *Hassar orestis*; e) *Platydoras* cf. *costatus*. In the C-banded karyotype shown in 2c, the arrows point to centromeric heterochromatin, while the arrow heads show heterochromatic blocks in the short arms.

acipenserinus, Opsodoras sp. and *Rhynodoras* sp. (Venere, 1998), and also for *Pseudodoras niger* and *Rhynodoras d'orbignyi* (Fenocchio *et al.* 1993). In *Hassar* sp. from Jarí, the NOR is similar to the one in *Trachydoras paraguaiensis* (Fenocchio *et al.* 1993) since both are located in an interstitial region in the long arm of a submetacentric pair. In *Opsodoras ternetzi* from Xingú the NOR is located at the distal region of the long arm of a submetacentric chromosome pair same as in *Hassar orestis* (Venere, 1998).

The results here obtained for the species *Hassar orestis* from Jarí and Xingú Rivers show that the NOR is located in the distal region of the short arm. One chromosome pair with heterochromatic blocks in the short arms in *Hassar orestis* (Figure 2d) from Xingú River is probably the NOR bearer chromosome, showing a more evident secondary constriction in one copy of the pair which seems to be a species-specific marker. Venere (1998) studied the same species from Araguaia River and found the NOR in the distal region of the long arm. These results are important since they show the possibility of using the NOR as a population marker.

If one accepts that the analyzed NOR bearer chromosomes in the genus *Hassar* are homologous, potential paracentric and/or pericentric inversions could have played a role in the karyotypic evolution of these species, moving the NOR to different places of the chromosomes. According to Almeida-Toledo (1998), NOR position changes can be an important marker for karyotypic differences among populations or species of fishes. The NOR size polymorphisms are very frequent in fishes and can be found also in mammals and other vertebrates and seems to be a general trait of NORs. This size polymorphism was observed in all the species here studied. Concerning the *Opsodoras ternetzi* we observed a difference in NOR position when compared with the *Opsodoras* sp. (Venere, 1998). In the first one the staining occurred in the distal region of the long arm of a metacentric pair, while in the latter the staining was in the distal region of the short arm of a subtelocentric pair. Apart from this, the modal number is conserved as in other species of this group, but the chromosome formulae and the fundamental numbers change with respect to M+SM+ST+A, suggesting that inversions can be involved not only in the NOR bearers but also in the differentiation of other chromosomal pairs.

According to Lundberg and Friel (2004), Auchenipteridae is the family that is most closely related to Doradidae. Souza *et al.* (2001) described the karyotypes of four species of this family, where three species have a single NOR located in a distal portion of a short arm, like *Hassar* cf. *orestis* from Jarí River, *Hassar orestis* and *Platydoras* cf. *costatus* from Xingú River here presented, as well as *Hassar wilderi*, *Leptodoras acipenserinus*, *Opsodoras* sp. and *Rhynodoras* sp. (Venere, 1998) and *Pseudodoras niger* and *Rhynodoras d'orbignyi* (Fenocchio *et al.*, 1993). The ancestral location of NORs may be in the distal portion of a short arm, since it is found in species from both Doradidae and Auchenipteridae. Cytogenetic research on other species of this family should help to define the phylogenetic relationships within this group, as well as the understanding of the chromosomal evolutionary mechanisms that acted in the chromosomal differentiation of these species.

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References

Almeida-Toledo LF (1998) Cytogenetic markers in Neotropical freshwater fishes. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS and Lucena CAS (eds) Phylogeny and Classification of Neotropical Fishes. Edipucrs, Porto Alegre, pp 583-588.

Bertollo LAC, Takahashi CS and Moreira-Filho O (1978) Citotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Rev Bras Genet 2:103-120.

Eler ES, Dergam JA, Vênere PC, Paiva LC, Miranda GA and Oliveira AA (2007) The karyotypes of the thorny catfishes *Wertheimeria maculata* Steindachner, 1877 and *Hassar wilderi* Kindle, 1895 (Siluriformes, Doradidae) and their relevance in doradids chromosomal evolution. Genetica 130:99-103.

Fenocchio AS and Bertollo LAC (1992) Karyotype similarities among Pimelodidae (Pisces, Siluriformes) from the Brazilian Amazon region. Cytobios 69:41-46.

Fenocchio AS, Jorge LC, Venere PC and Bertollo LAC (1993) Karyotypic characterization and nucleolus organizer regions in three species of Doradidae (Pisces, Siluriformes). Rev Bras Genet 4:1097-1101.

Ferreira EJG, Zuanon JAS and Santos GM (1998) Peixes Comerciais do Médio Amazonas: Região de Santarém, Pará. IBAMA, Brasília, 214 pp.

Guerra MS (1986) Reviewing the chromosome nomenclature of Levan *et al.* Rev Bras Genet 4:741-743.

Higuchi H (1992) A phylogeny of the South American thorny catfishes (Osteichthyes, Siluriformes, Doradidae). PhD Thesis, Harvard University, Cambridge.

Howell WM and Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: A 1-step method. Experientia 36:1014-1015.

Lundberg JG and Friel JP (2003). Siluriformes. Catfishes, v. 20 January 2003 (under construction). http://tolweb.org/Siluriformes/15065/2003.01.20. In: Tree of Life Web Project, http://tolweb.org/.

Moyer GR, Burr BM and Krajewski C (2004) Phylogenetic relationships of thorny catfishes (Siluriformes, Doradidae) inferred from molecular and morphological data. Zool J Linn Soc 140:551-575.

Nelson JS (1994) Fishes of the World. John Wiley and Sons, New York, 164 pp.

Oliveira C, Almeida-Toledo LF, Foresti F, Britski HA and Toledo Filho SA (1988) Chromosome formulae of Neotropical freshwater fishes. Rev Bras Genet 3:577-624.

Pendás AM, Morán P and García-Vázquez E (1993) Ribosomal RNA genes are interspersed throughout a heterochromatic chromosome arm in Atlantic Salmon. Cytogenet Cell Genet 63:28-130.

de Pinna MCC (1998) Phylogenetic relationships of Neotropical Siluriformes (Teleostei, Ostariophysi): Historical overview and synthesis of hypothesis. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS and Lucena CAS (eds). Phylogeny and Classification of Neotropical Fishes. Edipucrs, Porto Alegre, pp 279-330.

Sabaj MH and Ferraris Jr CJ (2003) Family Doradidae. In: Reis RE, Kullander SO and Ferraris Jr CJ (eds). Check list of the freshwater fishes of South and Central America. Edipucrs, Porto Alegre, pp 456-469.

Schweizer D (1980) Simultaneous fluorescent staining of R bands and specific heterochromatic regions (DA/DAPI bands) in human chromosomes. Cytogenet Cell Genet 27:190-193.

Souza EL, Feldberg E and Nakayama CM (2001) Estudos cromossômicos na família Auchenipteridae (Siluriformes) na área do Catalão, AM. In: Anais da X Jornada de Iniciação Científica de PIBIC/INPA, Manaus, pp 150-153.

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75:304-306.

Venere PC (1998) Diversificação cariotípica em peixes do médio rio Araguaia, com ênfase em Characiformes e Siluriformes (Teleostei, Ostariophysi). Ph.D. Thesis, Universidade Federal de São Carlos, São Carlos.

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