

Cytogenetic Studies in Hemiodidae (Ostariophysi, Characiformes) Fishes from the Central Amazon

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The Amazon basin offers an excellent region for ichthyogenetic studies, especially if we consider the richness of fish species, about 3,000 species, and their distribution in the different aquatic habitats. Porto *et al.* (in press), reported cytogenetic data for 211 nominal amazonian fish species. These data consist of chromosomal characterizations of fish species, detection of inter- and intra-specific polymorphisms, information on sex chromosome systems and records of supernumerary chromosomes.

The hemiodid fishes are endemic in South America and have been reported in the Amazon, Orinoco, Guiana, Paraná-Paraguai and Plata basins. They are fusiform, slender, pelagic and good jumpers, and inhabit in large lakes, fast moving water, and floodplains (Roberts 1974, Fink and Fink 1978, Santos *et al.* 1984). The systematics and taxonomy of the hemiodids is still unclear. Taxonomists are in disagreement, not only about the family name, but also the genus and species taxonomy (Roberts 1974, Géry 1977). The phylogenetic relationships of Hemiodidae is not available and its affinity with others families is uncertain. In the past, the Hemiodidae or part of the family were considered related with Parodontidae and Curimatidae (Roberts 1974). However, recently Uj (1990) and Buckup (1991) tentatively produced different hypotheses for the relationships to Characiformes and both considered the Characidiidae (or Characidiinae) to be the sister-group of the Hemiodidae.

The aim of this work is to describe the chromosomes of hemiodid species whose taxa have not been karyotyped previously, using the conventional Giemsa staining and Ag-banding, and to compare with cytogenetic data of other characiform families contributing to the understanding of the chromosome evolution of the order.

Material and methods

In this study we examined seven hemiodid species belonging to two subfamilies and two genera. The last systematic revision in the family was performed by Roberts (1974) and his paper was considered the basis for the characterization and diagnosis of the family, subfamilies and genera. However, family and subfamily terminology of this group and other characiform groups followed Géry (1977). The subfamilies, species and collection localities at Central Amazon are as follows: 1) Anodinae-*Anodus elongatus* (lago Camaleão-Marchantaria island), *A. melanopogon* (lago Camaleão, lago Catalão and lago do Rei), *Anodus sp* (lago Camaleão and lago Catalão) and, 2) Hemiodinae-*Hemiodus immaculatus* (lago do Rei), *H. cf. microlepis* (lago Camaleão and lago Catalão), *H. ocellatus* (Uatumã river) and, *H. unimaculatus* (Uatumã river). Collecting sites are shown in Fig. 1.

Metaphase chromosomes were obtained directly from kidney cell suspension (Bertollo *et*

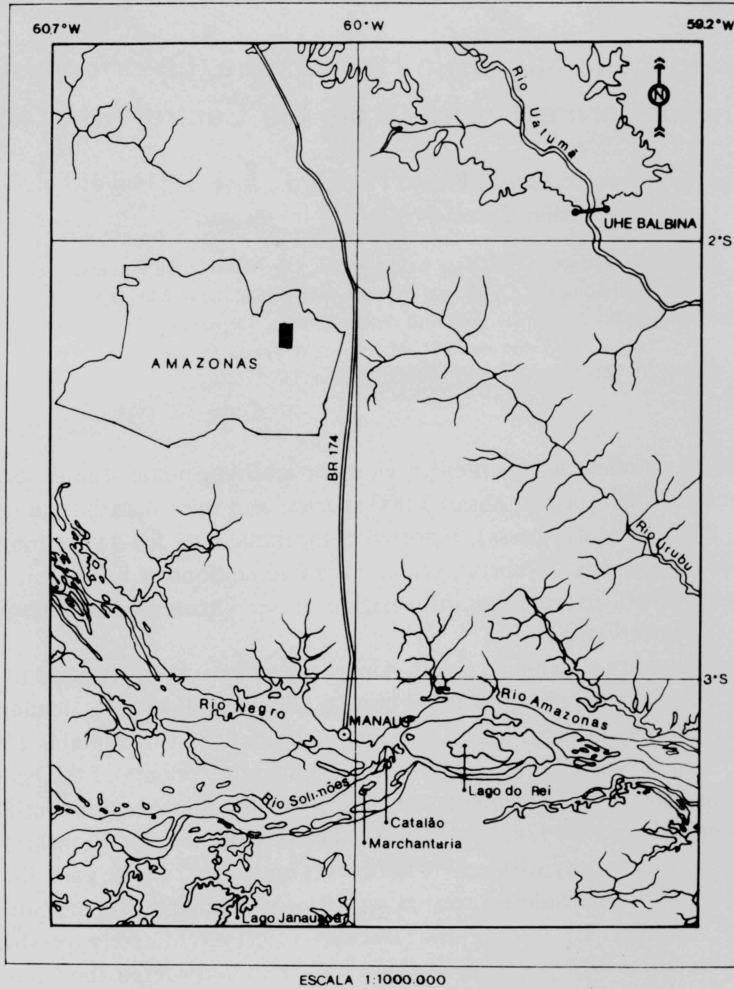


Fig. 1. Collection sites of the hemiodid species studied in the Central Amazon.

al. 1978) and silver staining of Nucleolus Organizer Regions (NORs) following Howell and Black (1980). The chromosomes were analysed measuring short arm length, long arm length and total length, with the help of a dry-tip compass and a pachymeter. Mean values were calculated for each chromosome pair. Chromosome morphology was determined on the basis of arm ratios as proposed by Levan *et al.* (1964).

Results and discussion

The same diploid number ($2n=54$) was found for all analysed species, with a high incidence of meta-submetacentric chromosomes. Up to six subtelocentric chromosomes were found in the species. *Anodus elongatus* has 26 metacentrics, 24 submetacentrics and 4 subtelocentrics, *A. melanopogon* has 20 metacentrics, 28 submetacentrics and 6 subtelocentrics, *Anodus* sp. has 24 metacentrics, 24 submetacentrics and 6 subtelocentrics, *Hemiodus immaculatus* has 22 metacentrics, 26 submetacentrics and 6 subtelocentrics, *H. cf. microlepis* has 20 metacentrics, 30 submetacentrics and 4 subtelocentrics, *H. ocellatus* has 26 metacentrics, 24 submetacentrics and 4 subtelocentrics and *H. unimaculatus* has 26 metacentrics, 24 submetacentrics and 4 subtelocentrics (Fig. 2, Table 1). For all the species the largest chromosome

Table 1. Karyotypic characters of the hemiodids studied at Central Amazon ($2n$ = diploid number, M = metacentric, SM = submetacentric, ST = subtelocentric, NORs = nucleolar organizer regions, q = long arm)

Species	N. specimens		N. cells		Chromosome types					NORs localization		
	Males	Females	Males	Females	2n	M	SM	ST	A	Pair	Position	Arm
<i>Anodus elongatus</i>	01	0	51	0	54	24	26	04	—	26 ST	terminal	q
<i>Anodus melanopogon</i>	06	16	117	430	54	20	28	06	—	25 ST	terminal	q
<i>Anodus</i> sp.	04	04	182	156	54	24	24	06	—	25 ST	terminal	q
<i>Hemiodus immaculatus</i>	05	03	39	64	54	22	26	06	—	25 ST	terminal	q
<i>Hemiodus</i> cf. <i>microlepis</i>	01	01	44	51	54	20	30	04	—	? SM	terminal	q
<i>Hemiodus ocellatus</i>	01	02	22	41	54	26	24	04	—	15 SM	subterm.	q
<i>Hemiodus unimaculatus</i>	01	06	26	224	54	26	24	04	—	17 SM	terminal	q

pair was of the metacentric type, except for *A. melanopogon* which was submetacentric.

The location/position of the active NORs (Nucleolar Organizer Regions) is present on the terminal region of the long arm of a large subtelocentric chromosome pair in *A. elongatus* (26th pair), *A. melanopogon* (25th pair), *Anodus* sp. (25th pair) and *H. immaculatus* (25th pair), and in the terminal region of the long arm of a submetacentric chromosome pair in *H. cf. microlepis* (in this species it was not possible to obtain photomicrography) and *H. unimaculatus* (17th pair). *H. ocellatus* presented the NOR located on the subterminal region of a submetacentric chromosome pair (15th) (Fig. 2).

Comparing the gross karyotypes of Hemiodidae and Characidiidae (considered phylogenetically related) they are not similar, the characidiids present $2n = 50$ with M-SM chromosomes and in some cases present multiple NORs (Miyazawa 1991). By other hand, the hemiodids share some karyotypic features with some Characiformes such as Prochilodidae, Curimatidae, Anostomidae, Chilodidae, which are considered a monophyletic assemblage (Vari 1983), and Parodontidae in respect to the diploid number ($2n = 54$), karyotypic macrostructures (principally M-SM chromosomes) and number of NOR-bearing chromosomes (single pair) (Scheel 1973, Galetti Jr. *et al.* 1981, 1984, Moreira Filho *et al.* 1984, 1985, Venere and Galetti Jr. 1989, Pauls and Bertollo 1990, Cestari *et al.* 1990, Feldberg *et al.* 1992).

Within these characiform families the karyotypes of parodontids (Moreira-Filho *et al.* 1984, 1985) are most similar to hemiodids. The main karyotypic similarities are the sharing of 2 to 3 ST chromosome pairs and the NOR located in the largest one in almost all species of both families. Also, the gross chromosomal similarities between the species of Anodinae and Hemiodinae and subsequently the chromosomal similarities between the species of Hemiodidae and Parodontidae are highly evident.

Amemiya and Gold (1990) has shown that the NOR chromosomes and the phenetic similarities in NOR chromosomes among Cyprinidae species are potentially informative data in the study relative to relationships. Recently, G-banding has complemented his work (Li and Gold 1991, Gold and Li 1991).

Considering the NOR sites of hemiodids as phenetic similarities, one can putatively consider that the large ST NOR chromosome is primitive for hemiodids, then *A. elongatus*, *A. melanopogon*, *Anodus* sp. and *H. immaculatus* share the same character. Preliminary data on *Argonectes scapularis*, a Bivibranchinae hemiodid, indicate that their NOR chromosomes is a large ST, also (unpublished data). Since these hemiodid species and the parodontids present the NOR sites on the terminal region of the long arm of the largest subtelocentric chromosome pair it would be of interest to check with longitudinal chromosome banding if the NOR chromosomes are or not homoeologous. In other clade one can include *H. cf. microlepis*, *H. ocellatus* and *H. unimaculatus* whose NOR chromosomes are SM.

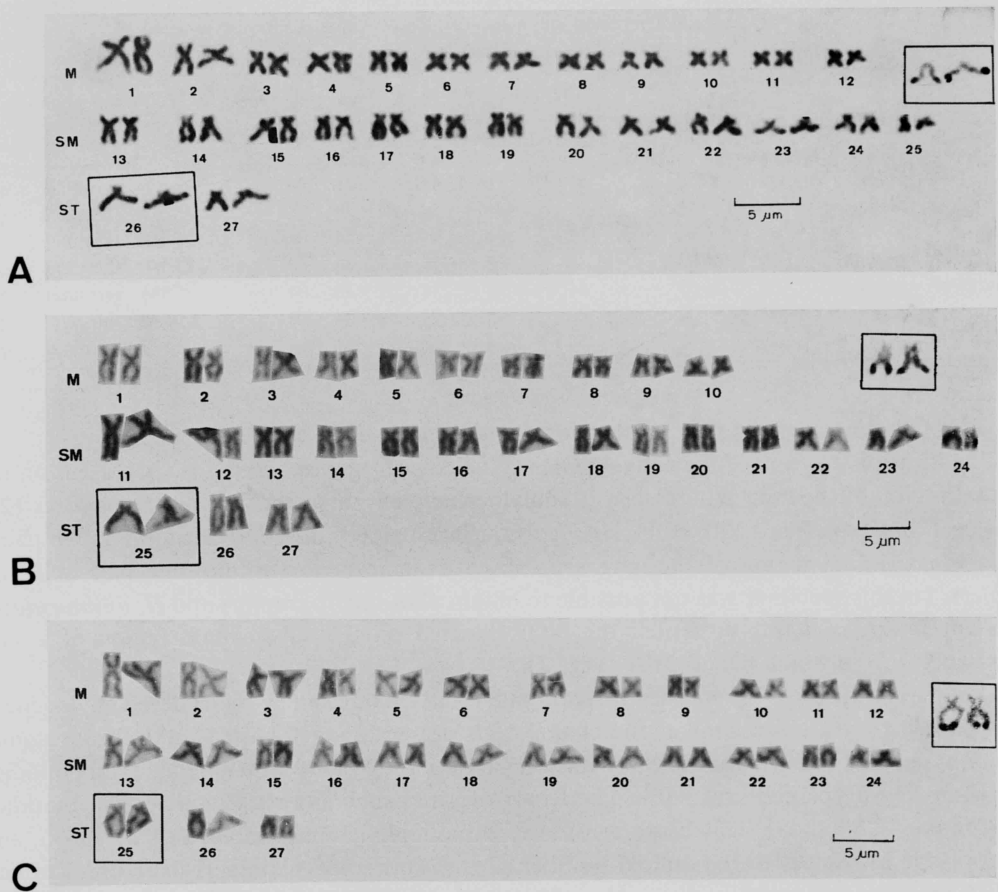
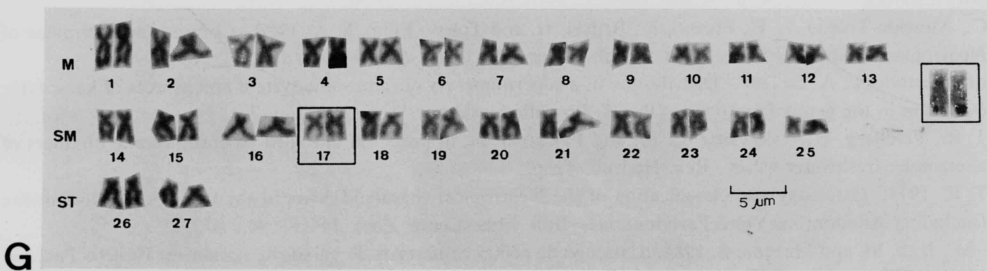
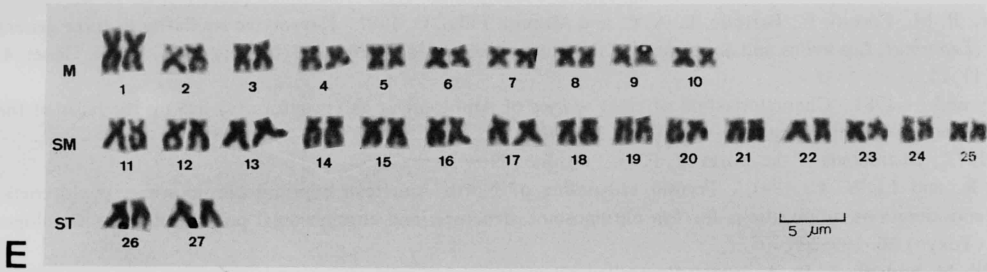
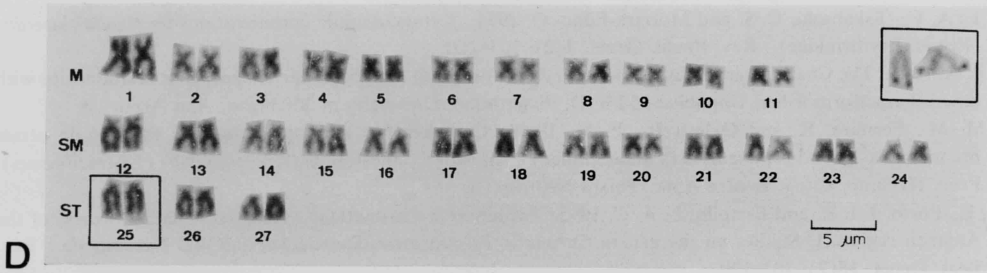


Fig. 2. Karyotypes and nucleolar chromosome of Hemiodidae: A) *Anodus elongatus* (26M + 24SM + 4ST), B) *Anodus* sp. (24M + 24SM + 6ST), C) *A. melanopogon* (20M + 28SM + 6ST), D) *Hemiodus immaculatus* (22M + 26SM + 6ST), E) *H. cf. microlepis* (20M + 30SM + 4ST), F) *H. ocellatus* (26M + 24SM + 4ST) and G) *H. unimaculatus* (26M + 24SM + 4ST).

The cytogenetic data available thus far shows that the Hemiodidae is an additional family of Characiformes to present $2n = 54$ and single NORs. As reviewed by Oliveira *et al.* (1988), both features have been considered important trends in the chromosomal evolution of Characiformes.

Summary

Karyotypes of seven hemiodid fish species have a similar macrostructure, with $2n = 54$ and $FN = 108$. The chromosome types range from 24 to 25 M-SM chromosome pairs and from 2 to 3 ST chromosome pairs. The species differ in the karyotypic formulae and the NORs are located on a single pair of ST or SM chromosomes. The results are compared with other characiforms, especially those with a similar karyotypic macrostructures, in order to evaluate the possible derivations of the chromosomal patterns of the group.



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Literature cited

Amemiya, C. T. and Gold, J. R. 1990. Chromosomal NOR phenotypes of seven species of North American Cyprinidae, with comments on cytosystematic relationships of the *Notropis volucellus* species group, *Opsopoe-*

- odus emiliae*, and the genus *Pteronotropis*. *Copeia*, 1990(1): 68–78.
- Bertollo, L. A. C., Takahashi, C. S. and Moreira-Filho, O. 1978. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). *Rev. Brasil. Genet.* **1**(2): 103–120.
- Buckup, P. A. 1991. The Characiiinae: a phylogenetic study of the South American darters and their relationships with other characiform fishes. Unpublished Ph. D. dissertation, University of Michigan, Ann Arbor.
- Cestari, M. M., Ferreira, R. and Galetti Jr., P. M. 1990. Complemento cariotípico de duas espécies de peixes ornamentais: *Chilodus punctatus* (Chilodontidae) e *Anostomus anostomus* (Anostomidae) (Characiformes). *Proc. III Simp. Citog. Evol. e Aplic. Peixes Neotrop.*: 3.
- Feldberg, E., Porto, J. I. R. and Bertollo, L. A. C. 1992. Evolution in Curimatidae (Teleostei, Characiformes) of the Amazon region. I. Studies on the genera *Curimata*, *Psectrogaster*, *Steindachnerina* and *Curimatella*. *Rev. Bras. Genet.* **15**(2): 103–120.
- Fink, W. L. and Fink, S. V. 1978. A Amazônia central e seus peixes. *Acta Amazonica* (Supl.) **8**(4): 19–42.
- Galetti Jr., P. M., Foresti, F., Bertollo, L. A. C. and Moreira Filho, O. 1981. Karyotypic similarity in three genera (*Leporinus*, *Leporellus* and *Schizodon*) of the family Anostomidae (Pisces, Teleostei). *Rev. Brasil. Genet.* **4**: 11–15.
- , —, — and — 1981. Characterization of eight species of Anostomidae (Cypriniformes) fish on the basis of the nucleolus organizer regions. *Caryologia* **37**: 401–406.
- Géry, J. 1977. *Characoids of the world*. T. F. H. Pub. Inc., New Jersey, USA. 672 p.
- Gold, J. R. and Li, Y. C. 1991. Trypsin G-banding of North American cyprinid chromosome: phylogenetic considerations, implications for fish chromosome structure, and chromosomal polymorphisms. *Cytologia* (Tokyo) **56**: 199–208.
- Howell, W. M. and Black, D. A. 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* **36**: 1014–1015.
- Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* **52**: 201–220.
- Li, Y. and Gold, J. R. 1991. Cytogenetic studies in North American minnows (Cyprinidae). XXII. Chromosomal nucleolar organizer regions in the genus *Pimephales*. *Can. J. Zool.* **69**: 2826–2830.
- Miyazawa, C. S. 1991. Estudo cariotípico de espécies e populações distintas do gênero *Characidium* (Characidiinae, Characidae) considerações citotaxonômicas e evolutivas. Unpublished M. S. thesis. Universidade Federal de São Carlos, São Paulo, Brasil. 82 p.
- Moreira-Filho, O., Bertollo, L. A. C. and Galetti, Jr., P. M. 1984. Structure and variability of nucleolar organizer regions in Parodontidae fish. *Can. J. Genet. Cytol.* **26**: 564–568.
- , — and — 1985. Karyotypic study of some species of family Parodontidae (Pisces, Cypriniformes). *Caryologia* **38**(1): 47–55.
- Oliveira, C., Almeida-Toledo, L. F., Foresti, F., Britski, H. and Toledo-Filho, S. A. 1988. Chromosome formulae of Neotropical freshwater fishes. *Rev. Brasil. Genet.* **11**(3): 577–624.
- Pauls, E. and Bertollo, L. A. C. 1990. Distribution of a supernumerary chromosome system and aspects of karyotypic evolution in the genus *Prochilodus* (Pisces, Prochilodontidae). *Genetica* **81**: 117–123.
- Porto, J. I. R., Feldberg, E., Nakayama, C. M. and Falcaõ, J. N. in press. A checklist of chromosome numbers of amazonian freshwater fishes. *Rev. Hydrob. Trop.*
- Roberts, T. R. 1974. Osteology and classification of the Neotropical characoid fishes of the families Hemiodontidae (including Anodontinae) and Parodontidae. *Bull. Mus. Comp. Zool.* **146**(9): 411–472.
- Santos, G. M., Jégu, M. and Merona, B. 1984. Catálogo de peixes comerciais do baixo rio Tocantins: Projeto Tucuruí. Manaus, Eletronorte/CNPq/INPA. 84 p.
- Scheel, J. J. 1973. Fish chromosome and their evolution. Internal reports of Danmarks Akvarium, Charlottenlund, Danmark, 22 p.
- Uj, A. 1990. Étude comparative de l'osteologie cranienne des poissons de la famille des Characidae et son importance phylogenetique. Docteur these. Faculté des Sciences, Departement de Zoologie et de Biologie Animale. Univesité de Geneve. 241 p.
- Vari, R. P. 1983. Phylogenetic relationships of the families Curimatidae, Prochilodontidae, Anostomidae and Chilodontidae (Pisces, Characiformes). *Smiths. Cont. Zool.* **11**(4): 27–43.
- Venere, P. C. 1991. Citogenética comparativa de peixes da familia Curimatidae. Masters dissertation. Universidade Federal de São Carlos, São Paulo, Brasil. 134 p.
- and Galetti Jr., P. M. 1989. Chromosome relationships of some Neotropical Characiformes of the family Curimatidae. *Rev. Brasil. Genet.* **12**(1): 17–25.