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A cytogenetic study of *Diplotnystes mesembrinus* (Teleostei, Siluriformes, Diplomystidae) with a discussion of chromosome evolution in siluriforms

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Abstract — The mitotic chromosomes, nucleolus organizer regions (NORs), C-banding pattern and nuclear DNA content of *Diplomystes mesembrinus* were studied. The karyotype, with *2n=56* chromosomes (22m+24sm+6st+4a), has a high chromosome arm number (NF = 102), one chromosome pair with NORs, and a very small amount of heterochromatln. The NOR-bearing arm is entirely heterochromatic and exhibits a marked size polymorphism. The diploid DNA content detected in erythrocyte nuclei of D. *mesembrinus* was 2.57 ± 0.15 pg/nucleus. The chromosome evolution in Siluriformes is discussed on the basis of available cytogenetic data and it is proposed that 2n=56 is synapomorphic for the order.

Key words: C-band; cytogenetics; Diplomystidae; *Diplomystes mesembrinus;* DNA content; NOR; Siluriformes.

INTRODUCTION

The catfish family Diplomystidae includes six species: *Diplomystes chilensis, D. nabuelbutaensis* and *D. camposensis* from Chile, D. *viedmensis*, *D. cuyanus* and *D. mesembrinus* from Argentina (AZPELICUETA 1994). Fishes of this family are only found in the Southern Neotropical region, and represent one of the most meridional records of freshwater Siluriformes (AZPELICUETA 1994).

The family Diplomystidae is the most basal siluriform lineage (FINK and FINK 1981; ARRA-TIA 1987; DE PINNA 1998) and information on the group is fundamental for understanding the evolution of other siluriforms. Although several morphological analyses have been performed on fishes of this group (FINK and FINK 1981, 1996; ARRATIA 1987; AZPELICUETA 1994; DE PINNA 1998) there are few data about other categories of characters. Preliminary cytogenetic studies with *D. camposensis* and *D. nabuelbutaensis* showed that they have *2n=56* chromosomes (ARRATIA and CAMPOS 1997).

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Until recently, only a few specimens of *D. mesembrinus* were known (AZPELICUETA 1994; AZPELICUETA and GOSZTONYI 1998). The objective of the present paper is to describe the karyotype, the location of the nucleolus organizer regions, the pattern of heterochromatin distribution, and the nuclear DNA content of *D. mesembrinus* and to compare these results with the cytogenetic information available for other members of the order Siluriformes.

MATERIALS AND METHODS

Thirteen specimens (11 males and 2 females) of D. *mesembrinus* collected in the rio Chubut near Los Altares, Chubut, Argentina were analysed. Specimens were deposited in the fish collection of the Laboratorio de Biologia de Peixes, UNESP, Botucatu, SP, Brazil (LBP 449).

Chromosome spreads and staining techniques were performed by the method of FORESTI *et al.* (1993). Chromosome morphology was determined on the basis of arm ratio as proposed by LEVAN *et al.* (1964) and the chromosomes were classified as metacentrics (M), submetacentrics (SM), subtelocentrics (ST) and acrocentrics (A). NF (chromosome arm number) was determined considering M/SM chromosomes to have two arms and ST/A chromosomes to have one arm. For DNA content determination, blood was collected by caudal vein puncture, spread over slides and air dried. The DNA content of eight fishes was determined according to the technique described by CARVALHO *et al.* (1998). The absorb-ance of fish nuclei from each slide was standardized as a percentage of the mean absorbance of three controls: chicken erythrocytes, common carp erythrocytes, and rainbow trout erythrocytes. Microdensit-ometry analysis was performed under a Zeiss microscope using a 100x oil-immersion objective. Analyses were done using the OPTIMAS software, version 4.1. For each fish, 15 nuclei were measured from each of two slides (30 nuclei per individual).

RESULTS AND DISCUSSION

The diploid number found for males and females of *D. mesembrinus, 2n=56* (Fig. 1) was the same as described for *D. camposensis* and D. *nahuelbutaensis* (ARRATIA and CAMPOS 1997), suggesting a conservative diploid number for Diplomystidae.

Preliminary studies based on analyses of the most frequent diploid number in Siluriformes (LEGRANDE 1981; OLIVEIRA *et al.* 1988) suggested that *2n=58* could be the ancestor diploid number for the order. However, considering that the diploid number found in all species of Diplomystidae studied is *2n=56,* and that this family is the most primitive family of Siluriformes (FiNK and FINK 1981; ARRATIA 1987; DE PINNA 1998), this hypothesis needs to be revised.

In the order Characiformes, a sister group of Siluriformes and Gymnotiformes (FINK and FINK 1981, 1996), the most common diploid number is *2n=54* and of about 450 species karyotyped so far (KLINKHARDT *et al.* 1995; CLAUDIO OLIVEIRA unpublished database of cy-togenetic data for Neotropical fish) only two, *Curimata ocellata* (FELDBERG *et al.* 1992) and *Potamorhina latior* (FELDBERG *et al.* 1993), of the Curimatidae family, have 2n=56 chromosomes. Among the Gymnotiformes, the sister group of Siluriformes (FiNK and FINK 1981, 1996), the diploid number ranges from *2n=22* for *Apteronotus albifrons* (HINEGARDNER and ROSEN 1972) to 2n=52 for *Rhamphichthys* cf. *pantherinus* (ALMEIDA-TOLEDO 1978). Among Siluriformes, in addition to Diplomystidae, 272=56 chromosomes are found in species of the families Ageneiosidae, Ariidae, Bagridae, Calli-chthyidae, Clariidae, Doradidae, Heterop-neustidae, Ictaluridae, Loricariidae, Mochoki-dae, Pimelodidae, Sisoridae, and Trichomyc-teridae (Table 1) (FITZSIMONS *et al.* 1988;

TABLE 1 — Summary of cytogenetic data available for the order Siluriformes. For the 321 species karyotyped 352 different diploid number were described.

Family	Diploid number distribution														Number of species karyo-typed	Diploid number range
	≤44	46	48	50	52	54	56	58	60	62	64	66	68	≥70		
Ageneiosidae*	—	—	—	—	—	—	2	—	—	—	—	—	—	—	2	56
Amblyciptidae	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2	24-34
Ariidae*	—	—	—	—	—	4	3	—	—	—	—	—	—	—	7	54-56
Aspredinidae	—	—	—	3	—	—	—	—	—	—	—	—	—	—	3	50
Auchenipteridae	—	—	—	—	—	—	—	6	—	—	—	—	—	—	6	58
Bagridae*	4	—	3	5	10	7	4	4	2	—	—	—	—	—	35	28-60
Callichthyidae*	6	9	1	2	4	1	4	4	7	7	2	2	1	16	53	40-134
Clariidae*	—	—	—	1	1	4	4	1	—	—	—	—	—	—	10	50-58
Cranoglanididae	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	74
Diplomystidae*	—	—	—	—	—	—	3	—	—	—	—	—	—	—	3	56
Doradidae*	—	—	—	—	—	—	1	9	—	—	—	1	—	—	11	56-66
Heteropneustidae*	—	—	—	—	—	—	1	1	—	—	—	—	—	—	2	56-58
Ictaluridae*	9	3	3	3	2	4	2	2	4	2	—	1	—	—	31	40-66
Loricariidae*	1	—	2	—	9	16	2	1	—	1	2	2	4	16	56	36-80
Mochokidae*	—	—	—	—	—	8	1	—	—	—	—	—	—	—	9	54-56
Nematogeniidae	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	94
Pimelodidae*	—	8	—	2	3	2	23	18	2	1	—	—	—	—	54	46-62
Plotosidae	2	—	1	—	—	—	—	—	—	—	—	—	—	—	2	36-48
Schilbeidae	—	—	—	—	—	—	—	3	—	1	—	1	—	—	4	58-66
Siluridae	3	—	2	—	—	1	—	6	3	—	—	—	—	1	14	28-86
Sisoridae*	1	—	—	1	—	—	1	—	—	—	—	—	—	—	3	36-56
Trichomycteridae*	—	—	—	—	1	7	1	—	1	1	1	—	—	—	12	52-64
Total	28	20	12	17	30	54	52	55	19	13	5	7	5	35	321	28-134

* Families with species with $2n$=56 chromosomes.

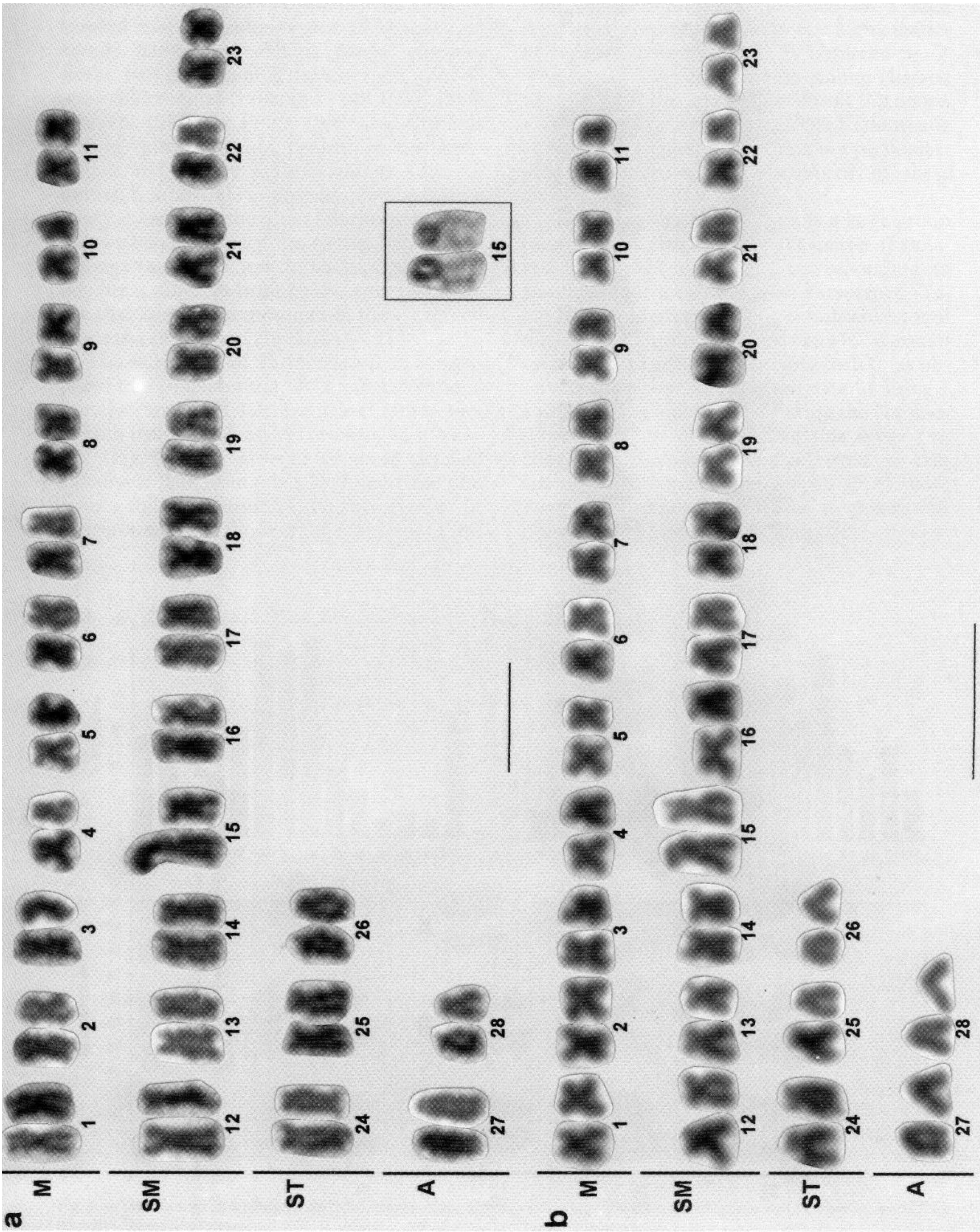

Fig. 1 — Karyotypes of a male (a) and a female (b) *Diplomystes mesembrinus* with $2n = 56$, and, in the inset, the chromosome pair with NORs. Bars = 10 µm.

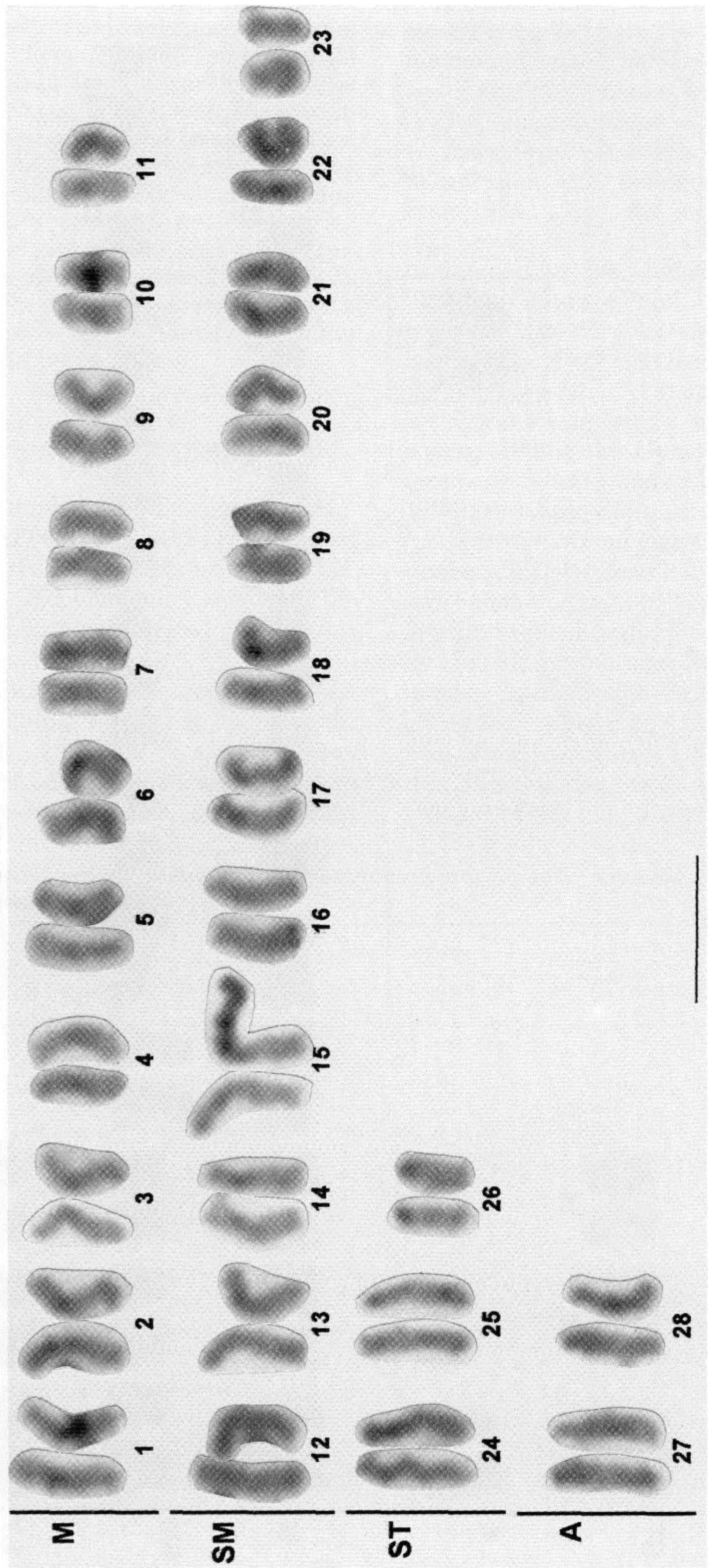

Fig. 2 — Karyotype of a male *Diplomystes mesembrinus* stained by the C-banding technique. Note the reduced amount of heterochromatin and the conspicuous size polymorphism of the heterochromatic NOR-bearing chromosome arms (pair 15). Bar = 10 µm.

KLINKHARDT *et al.* 1995; CLAUDIO OLIVEIRA unpublished database of cytogenetic data for Neotropical fish). Considering that all *Diplomystes* species karyotyped exhibit 2n=56 chromosomes, that this group is the sister group of all other Siluriformes (FINK and FINK 1981, 1996; ARRATIA 1987; DE PINNA 1998) and that this diploid number is not present in Gymnotiformes and is very unusual among Characiformes, it is possible to propose that 2n=56 is a synapomorphy for the order Siluriformes. Since the two known characiform species with 2n=56 belong to a group where the widespread diploid number is different from 2n=56, we may postulate that 2n=56 chromosomes in these characi-forms is a convergent condition.

The karyotype of *D. mesembrinus* is composed of 22 metacentric, 24 submetacentric, 6 subtelocentric, and 4 acrocentric chromosomes in both males and females, resulting in a NF = 102 (Fig. 1). The absence of more detailed karyotypic data from other Diplomystidae prevents a full comparison of the karyotype of *D. mesembrinus* with those of other species of this family. The occurrence of a high fundamental number, reflecting the presence of a high number of chromosomes and/or a high number of biarmed chromosomes, as observed in D. *mesembrinus*, is very common among characiforms, siluriforms and gymnotiforms (OLIVEIRA *et al.* 1988; FITZSIMONS *et al.* 1988; KLINKHARDT *et al.* 1995), suggesting that this is a plesiomor-phic condition widely distributed in these groups.

The fish order Siluriformes is a large group of organisms with about 2,400 species (FERRA-RIS 1995), 321 of which (corresponding to about 13 % of the order) were karyotyped (Table 1). Considering that the possible ancestor of the order Siluriformes had 2n=56 chromosomes and an arm number close to 100 (as observed in *D. mesembrinus)* the data in Table 1 and Figure 3 a indicate that during the chromosome evolution of species of this order few changes in diploid number occurred, so that among the 321 species karyotyped 45.7% have 2n=56±2 chromosomes. To try to answer the question of why the diploid number is so con-

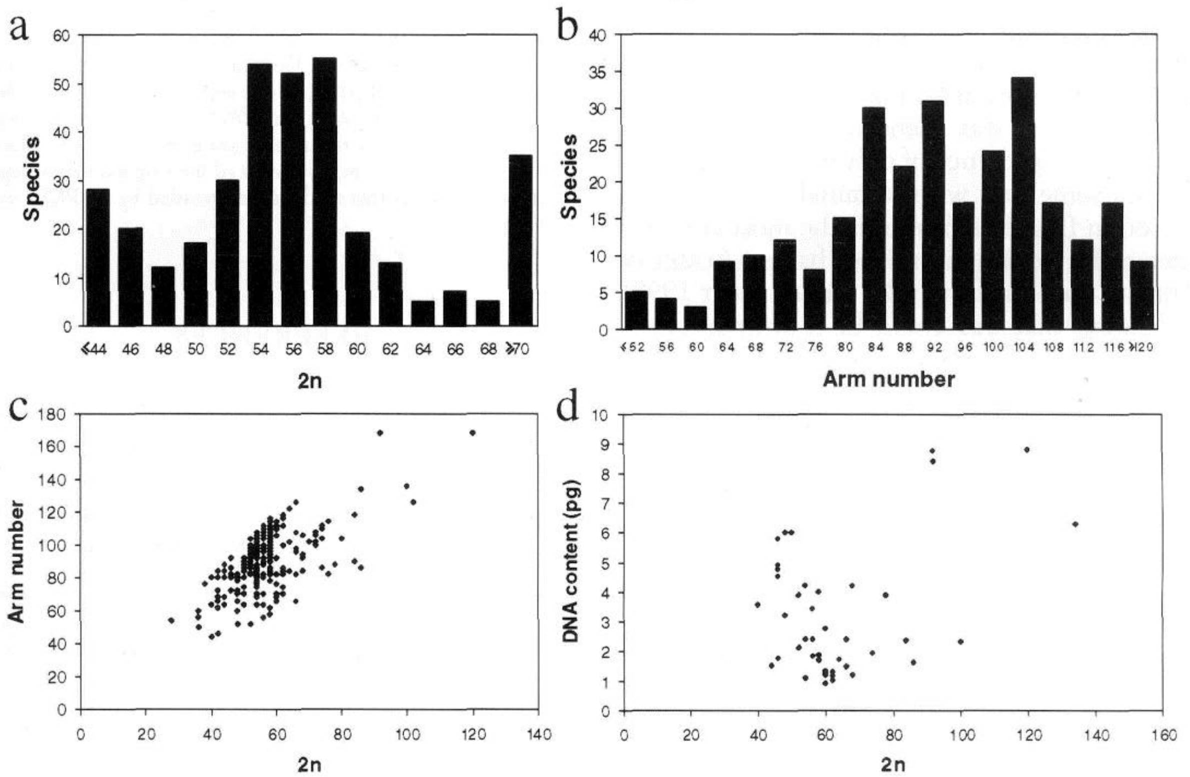

Fig. 3 — Distribution of diploid numbers (a), chromosome arm numbers (b), diploid vs. chromosome arm number (c), and DNA content (d) for Siluriformes.

served in a group with a very large number of species, and also with a variety of population size and structure, it is possible to hypothesize that this number of chromosomes has been conserved since, in some way, it is the best one for cell maintenance in these organisms. On the other hand, in some groups of siluriforms, such as the families Callichthyidae, Ictaluridae and Loricariidae, we found many species with different diploid numbers (Table 1) suggesting that the evolutionary history of these groups was different from that of the other siluriforms.

Among the karyotyped siluriform species, 45.7% have a diploid number smaller than *2n=56* and 39.5% have a diploid number higher than 2n=56 (Table 1), suggesting that a reduction of diploid number was better withstood than an increase in diploid number. Additionally, Figures 3b and 3c show that changes in diploid number were not directly produced by changes in chromosome arm number, suggesting that chromosome rearrangements that do not change the diploid number seem to be fixed more frequently than chromosome rearrangements like Robertsonian translocations that change the diploid number.

In *D. mesembrinus* the NORs were found in a large submetacentric pair in the terminal region of the short arm (Fig. 1), and a wide size polymorphism was identified in several individuals. The presence of only one NOR-bearing chromosome pair with terminal NORs, as observed in *D. mesembrinus*, is the most common feature found among teleost fishes (ALMEIDA-TOLEDO and FOREST: 1985; KLINKHARDT 1998), indicating that this could be a symplesiomorphic character for the group. The occurrence in several siluriforms of more than one chromosome pair with NORs and/or NORs located in interstitial positions on the chromosomes could be apomorphies for the species that have them.

C-banding showed the occurrence of a very small amount of heterochromatin near the centromeric region of almost all chromosomes of *D. mesembrinus* (Fig. 2). The only exception was a large heterochromatic segment observed in the short arm of the NOR-bearing chromosomes (Fig. 2). The existence of a small amount of heterochromatin in the chromosomes of D. *mesembrinus* resembles the data obtained for many other teleost species, including Siluriformes (GOLD *et al.* 1990). Considering its wide distribution, this character may represent a symplesiomorphic condition for teleosts.

The mean diploid DNA content observed in erythrocyte nuclei of *D. mesembrinus* was 2.57 + 0.15 pg. This value is higher than the mean value of 2.0 pg/diploid nuclei observed for teleosts (HINEGARDNER and ROSEN 1972). However, it is lower than the mean values observed for Characiformes (3.0 pg/diploid nuclei) (CARVALHO *et al.* 1998) and Siluriformes (3.2 pg/diploid nuclei). Since the available data about the DNA content of Siluriformes are limited to 43 species and most of these data are from the fam ily Callichthyidae (23 species), in which some species apparently evolved by polyploidy (OLIVEIRA *et al.* 1993), any conclusions about changes in this parameter are tentative. Consid ering that the DNA content of Siluriformes ranges from 0.91 to 8.8 pg/diploid nuclei (Fig. 3d), it is possible to suggest that large changes in DNA content occurred in the evolution of this group. On the other hand, many changes in DNA content were not produced by changes in diploid number (Fig. 3d), in agreement with the above hypothesis that a diploid number better adapted for this group of organisms may exist.

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