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Karyotypes of three «small» *Barbus* species (Cyprinidae) from Republic of Guinea (Western Africa) with a review on karyology of African small *Barbus*

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SUMMARY — Karyotypes of three barbels belonging to the group of «small» African species of *Barbus* sensu lato, *B. bigornei*, *B. ablabes* and *B. macrops* from the Republic of Guinea (Western Africa), were investigated. Diploid chromosome ($2n$) and chromosome arm (NF) numbers were for *B. bigornei* $2n$ = 48 and NF = 96, for *B. ablabes* $2n$ = 50 and NF = 98, and for *B. macrops* $2n$ = 50 and NF = 92, respectively. The first pair of metacentric chromosomes in all karyotypes was remarkably larger, and it can be considered as a «marker» element for these 3 species. The karyotype characteristics of *Barbus* species under study demonstrate that they belong to the diploid group of African barbels and they are, in fact, not related to the genus *Barbus* sensu stricto which is of a distinct evolutionary polyploid origin. Karyology of this poorly studied African cyprinid group is reviewed and discussed.

INTRODUCTION

Numerous African species assigned to the genus *Barbus* involve in fact two distinct groups: «small» (about 230 species) and «large» (about 70 species) barbels (SKELTON *et al.* 1991). These two groups differ especially in adult size (i.e. about 150 mm SL and 700-900 mm SL, respectively) and type of scale striation (radiating vs. longitudinal) (BANISTER 1987; LÉVÊQUE *et al.* 1990; SKELTON *et al.* 1991). However, the cyprinid genus *Barbus* sensu lato is a paraphyletic taxon to which a number of unrelated species and/or groups from Africa, Europe and Asia have been included (HOWES 1987).

Biochemical (AGNÈSE *et al.* 1990; BERREBI *et al.* 1990) as well as karyological (e.g. VERVOORT 1980; RAB 1981; VASIL'EV 1985; YU *et al.* 1987, 1989; COLLARES-PEREIRA and MADEIRA 1990; OELLERMAN and SKELTON 1990; GOLUBTSOV and KRYSANOV 1993; RAB *et al.* 1993; GUEGAN *et al.* 1995) investiga-

tions demonstrated that only those species with evolutionary tetraploid ($2n$ = 100) and hexaploid ($2n$ = 148-150) levels are assignable to the genus *Barbus* sensu stricto (and/or to the broader category of barbin lineage), while those possessing a diploid chromosome number ($2n$ = ± 50) belong to distinct lineages of cyprinine cyprinids (sensu HOWES 1991) such as *Puntius* and related genera (MAGTOON and ARAI 1989, 1990; ARAI and MAGTOON 1991; YU *et al.* 1987, 1989).

For African *Barbus* species, the existence of a polyphyletic assemblage was stressed and discussed by RAB (1981) and put in the limelight by GOLUBTSOV and KRYSANOV (1993) again. From a karyological view point, this African group of «small» barbels, similarly as nearly all other African ichthyofauna, it remains practically unknown. Tab. 1 shows that there are 4 reports on the chromosome numbers and/or karyotypes for 10 African «small» *Barbus* species only.

This present report deals with the description of karyotypes of three species of «small» barbels, namely *Barbus bigornei* (Lévêque, Teugels and Thys van den Audenaerde, 1988), *B. ablabes* (Blekeer, 1863) and *B. macrops* Boulenger, 1911, all of them caught in Republic of Guinea (Western Africa). The karyology of this poorly studied African cyprinid group is finally reviewed and discussed.

MATERIALS AND METHODS

The specimens used in this study represent a part of large sample during a joint French-Spaniard expedition in Republic of Guinea in 1993. All specimens karyotyped were preserved and are deposited as vouchers in the Museo Nacional de Ciencias Naturales (MNCN) at Madrid (Spain). The analyzed material consisted of 3 specimens (1 male, 2 females) of *Barbus bigornei* (No. 83838-40 MNCN) from the Mongo river (Upper Little Scarcies basin) at Marela, 1 specimen (female) of *Barbus ablabes* (No. 83857) from the Kaba River (Upper Little Scarcies basin) at Kouloundala, and 1 specimen (sex unknown) of *Barbus macrops* (No. 83960) from the Samou river (Konkouré basin) at Débélé.

Chromosome preparations were made directly in field conditions according to the method described in DOUSSAU DE BAZIGNAN and OZOUF-COSTAZ (1985). Fixed cell suspensions were kept in deep freezer until their analysis in the laboratory. Because cell suspensions were fixed with ethanol (instead of methanol) acetic acid fixative and such suspensions did not provide suitable metaphase plates, the protocol was modified as follows. The suspensions were refixed in cold, freshly made methanol-acetic acid fixative at least five times. The chromosome preparations were made by dropping of cell suspension either onto dry slides or, if unsuccessful, onto slides wetted with chloroform. After drying, the slides were stained with 5% of Giemsa stain and, if necessary and to get a better contrast, they were slightly counter-stained with 50% of silver nitrate. Selected and photographed metaphases were destained and nucleolar

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TABLE 1 — Comparison of current and previously published data on the chromosome number (2*n*), haploid karyotype characteristics and chromosome arm number (N.F.) of species of «small» African *Barbus* and related genera.

Species	2*n*	Haploid karyotype	N.F.	No. and sex of material examined	Locality	Reference
Barbus viviparus Weber, 1897	24*	not reported	—	not	Natal, aquarium stock	POST 1965
Barbus barilioides Boulenger, 1914 **	48	16m + 8sm	96	3 juv.	Angola, aquarium stock	RAB 1981
Barbus holotaenia Boulenger, 1904	50	12m + 13sm	100	1♂ 2♀	Zaire, aquarium stock	RAB 1981
Barbus anema Boulenger, 1903	50	21m, sm + 4a	92	3 ?	Alvero R., Ethiopia	KRYSANOV & GOLUBTSOV 1993
Barbus kerstenii Peter, 1868	50	17m, sm + 8a	84	17 ?	L. Abaya, Hare R., Sile R., Ethiopia	KRYSANOV & GOLUBTSOV 1993
Barbus paludinosus Peters, 1852	50	23m, sm + 2a	96	15 ?	Bulbula R., Ethiopia	KRYSANOV & GOLUBTSOV 1993
Barbus sp. 1	50	22m, sm + 3a	94	1 ?	Alvero R., Ethiopia	KRYSANOV & GOLUBTSOV 1993
Barbus sp. 2	50	22m, sm + 3a	94	1♂, 1♀, 11 ?	L. Abaya, Ethiopia	KRYSANOV & GOLUBTSOV 1993
Barbus sp. 3	50	24m, sm + 1a	98	2♂, 7♀ 9 ?	Hare R., Kulfo R., Ethiopia	KRYSANOV & GOLUBTSOV 1993
Barbus ablabes Bleeker, 1863	50	9m, + 15sm + 1st-a	96	1♀	Mongo R., Guinea	this report
Barbus bigornei Leveque, Teugels and Thys van den Audenarde, 1988	48	9m + 15sm	98	1♂, 2♀	Kaba R., Guinea	this report
Barbus macrops Boulenger, 1911	50	7m, + 14sm + 4st-a	92	1 ?	Samour R., Guinea	this report
Caecobarbus geertsii Boulenger, 1921	50	6m, + 14sm + 5st	100	2♂ 3♀	cave near Thysville, Zaire	VERVOORT 1980

Explanations: m - metacentric, sm - submetacentric, a - acrocentric chromosomes; * only haploid chromosome number reported; ** the name is a synonyme of *Barbus fasciolatus* Gunther, 1868.

organizer regions (NORs) were analyzed by the colloidal silver nitrate method of HOWELL and BLACK (1980). Chromosomes were classified according to LEVAN *et al.* (1964).

RESULTS

Barbus bigornei. - Diploid chromosome number $2n = 48$. The karyotype consists of 9 pairs of metacentric and 15 pairs of submetacentric chromosomes, NF = 96 (Fig. 1A). NORs are located telomerically in one middle-sized submetacentric pair (Figs. 2A, B).

Barbus ablabes. - Diploid chromosome number $2n = 50$. The karyotype consists of 9 pairs of metacentric, 15 pairs of submetacentric and 1 pair of subtelocentric to acrocentric chromosomes, NF = 98 (Fig. 1B). NORs are located telomerically in one middle- sized submetacentric pair (Figs. 2C, D).

Barbus macrops. - Diploid chromosome number $2n = 50$. The karyotype consists of 7 pairs of metacentric, 14 pairs of submetacentric and 4 pairs of subtelocentric to acrocentric chromosomes, NF = 92 (Fig. 1C). The location of NORs could not be precisely located but interphase nuclei displayed 2 positive signals (Fig. 2E).

Results for all three species are summarized in Tab. 1.

DISCUSSION

Karyotypes of cyprinids, both evolutionary diploid and polyploid, are generally characterized by the presence of small elements with their centromere position placed gradually from a median to a nearly terminal position. This previous morphological characteristic plus the effect of chromosome arm contraction during mitosis due to temporal and dose colchicine treatment make difficult precise assignment of a number of chromosome pairs to particular categories (RAB and ROTH 1989). Moreover and in spite of difficulties in preparing chromosome suspensions in the field leading to their relative poor quality, the karyotype features of these three barbels may however permit to discuss about the composition of their chromosomal sets. This is more especially the case of *B. macrops* for which we had chance to analyze a limited number of metaphases (Fig. 1C). Interestingly, the first pair of metacentric chromosomes in the karyotypes of all three species was distinctly larger, and it can be

Fig. 1. — Karyotypes of *Barbus bigornei* (A), *B. ablabes* (B) and *B. macrops* (C); karyogram of *B. macrops* is a camera lucida interpretation of metaphase plate displayed in the inset. m - metacentric, sm - submetacentric, st - sub- telocentric and a - acrocentric chromosomes. Scale bars equal 5 µm.

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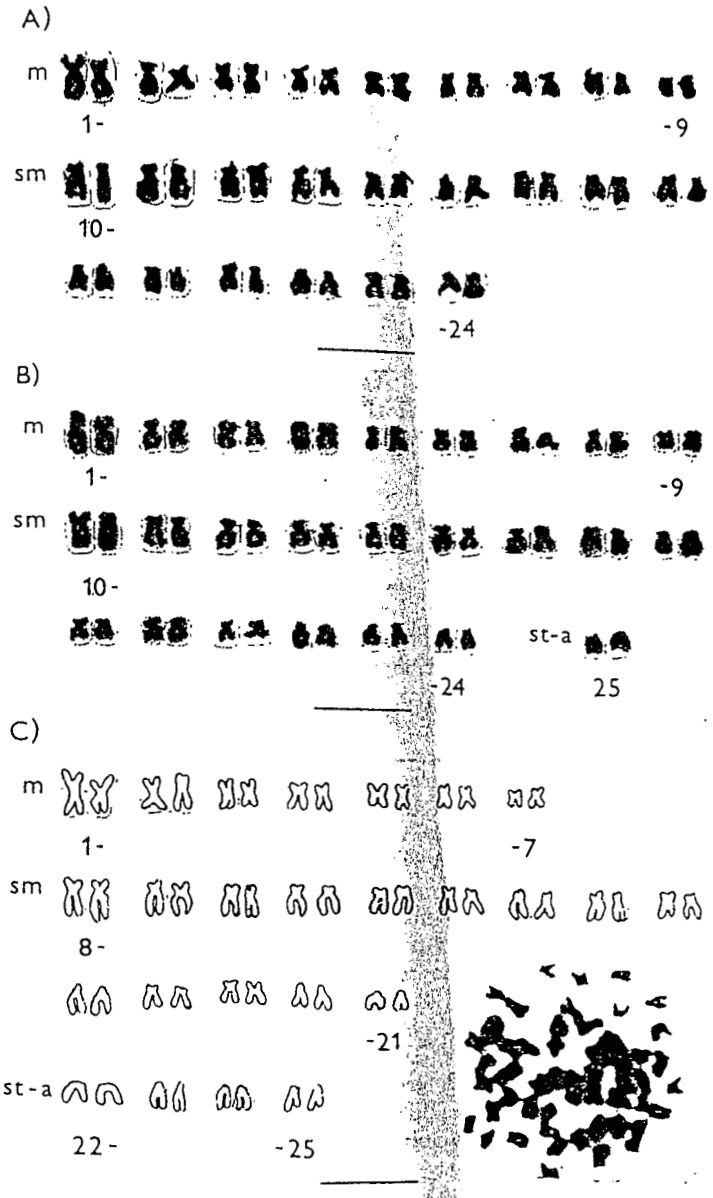


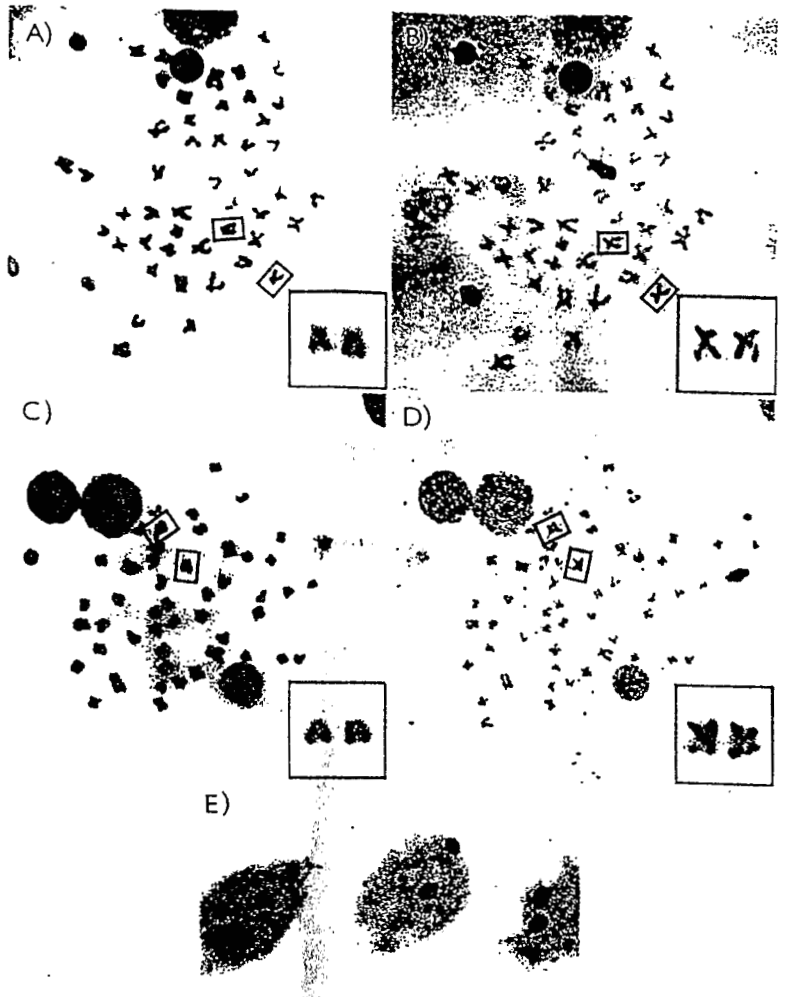
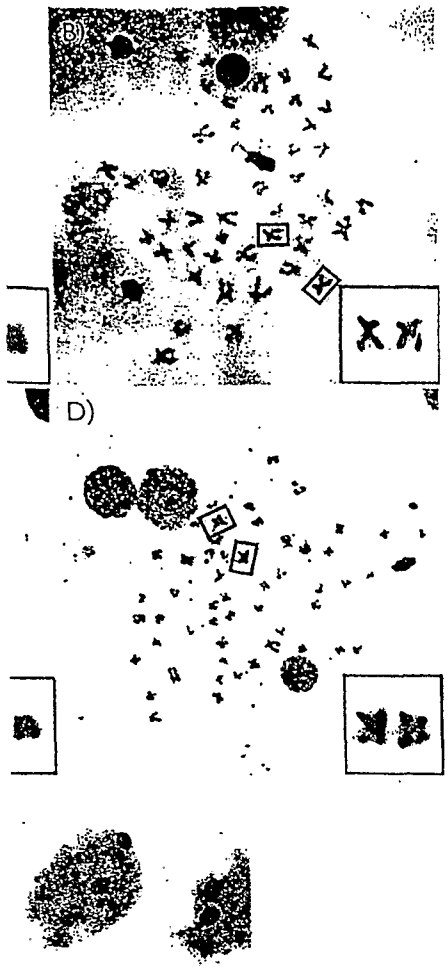


Fig. 2 — The metaphase plates (A - G) and interphase nuclei (E) of *Barbus bigornei* (A, B), *B. ablabes* (C, D) and *B. macrops* (E) stained sequentially with Giemsa (A, C) and silver (B, D) or nonsequentially with silver (E). The NOR bearing chromosome pairs in *Barbus bigornei* (A, B) and *B. ablabes* (C, D) are framed and also enlarged in the insets. Interphase nuclei of *B. macrops* display two positive signals.



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considered as a «marker» element for these species. Anyway, the deeper interspecific comparison of karyotypes either between African «small» *Barbus* and Asian *Puntius* species, or within «small» African barbels, is practically impossible because of the absence of chromosome banding data. The actual interspecies chromosomal homologies could be identified on the basis of chromosome banding techniques only and, as regards both cyprinid groups, there are only very few reports on karyotypes with banding methods (e.g. RISHI and ADARASH DEEP KAUR THIND 1992). Our observations on the number and location of NORs is, therefore, the one of the first attempt to characterize their karyotypes more precisely. We found that all three species exhibit single paired NORs. This could be the case also in a number of other «small» African barbels and such an information can be used for an a priori determination of ploidy level concerning species of African *Barbus*. which are not yet karyologically investigated. The number of NORs in the tetraploid European *Barbus* is 6 (RAB *et al.* 1993; COLLARES-PEREIRA, pers. comm.) and as many as 8 in the hexaploid African *Barbus* (work in progress). The determination of ploidy level by means of checking the number of NORs using very simple silver staining (HOWELL and BLACK 1980) is simple, quick, unexpensive and can be performed on any fixed cell smears (FLASHANS *et al.* 1992).

Our present results in demonstrating the diploid status of three new *Barbus* species confirm the viewpoint that «small» African barbels usually classified into this «catch-all» genus do not belong phylogenetically to the true polyploid genus *Barbus* sensu stricto. The southern Asian genus *Puntius* (and/or related genera) is probably phylogenetically closer to this African group of «small» *Barbus* group than to the «large» African *Barbus* group. GOLUBSTOV and KRYSANOV (1993) clearly stated: «Although the idea to relate small African of *Barbus* with this group is not new (see synonyms in LÉVÊQUE and DAGET 1984), karyological data seem to be most serious evidence supporting this hypothesis...».

MAGTOON and ARAI (1989) have reviewed the available karyological data of the species classified into genus *Puntius* sensu lato (i.e. species classified variously either to genera *Puntius, Capoeta* or *Barbodes*). They recognized the presence of four groups within species of *Puntius* karyotyped so far: 1) $2n = 48$ and low NF = 52 to 54; 2) $2n = 50$ and NF more than 82; 3) *«Capoeta»* species with $2n = 50$ and NF = 54 - 58, and 4) *«Capoeta»* species with $2n = 50$ and NF = 82 to 98. African species of *«Barbus»* karyotyped so far (Tab. 1) have both $2n = 48$ (3 species) and 50 (all others) but NF is always higher than 92 except for *B. kerstenii* where NF = 84. Another remarkable contrast concerns the distribution of the two diploid chromosome numbers $2n = 48$ and 50. The formula $2n = 48$ was found in 3 species of *Puntius* which is about 9% of the total number of karyotyped species but also in 3 species of African «Barbus» representing almost 24% of karyotyped taxa. This comparison indicates that the $2n = 48$ formula might be widely shared by African representa-

tives of the group. Anyway, any other speculations about relationships between and within «small» African barbels and Asian *Puntius* from a cytotaxonomical point of view are premature and speculative and, undoubtedly, we do need more applicable data for these African cyprinids.

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