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. bigornet, B. ahlabes and B. macrops from the Republic of Guinea (Western Africa), were investigated. Diploid chromosome (2n) and chromosome arm (NF) numbers were for B. bigornet 2n = 48 and NF = 96, for B. ahlabes 2n = 58 and NF = 98, and for B. macrops 2n = 70 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 130 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; BERRIET 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; OELLERS and SKELTON 1990; GO-LIJBERSTOV and KRYSANOV 1993; RAB et al. 1993; GUEGAN et al. 1995) investiga-
cations 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 (Mactoorn and Arai 1989, 1990; Arai and Mactoorn 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 Kysanov (1993) again. From a karyological view point, this African group of «small» barbels, similarly as nearly all other African ichthyofauna, it remains practically unknown. Table 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 bigomei* (Lévêque, Teugels and Thys van den Audenaerde, 1988), *B. ablabes* (Bleeker, 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 bigomei* (No. 83838-40 MNCN) from the Mongo river (Upper Little Scaries basin) at Marela, 1 specimen (female) of *Barbus ablabes* (No. 83857) from the Kaba River (Upper Little Scaries basin) at Kouloundala, and 1 specimen (sex unknown) of *Barbus macrops* (No. 83960) from the Samou river (Kankouré basin) at Débélet.

Chromosome preparations were made directly in field conditions according to the method described in Doussal De' Bazignan and Ozoir-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
<table>
<thead>
<tr>
<th>Species</th>
<th>Reference</th>
<th>N.F. of material</th>
<th>Locality</th>
<th>Chromosome number (2n)</th>
<th>haploid karyotype characteristics</th>
<th>chromosome arm number N.F.</th>
<th>Species of small African Barbus and related genera</th>
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<tr>
<td>Barbus bariiides</td>
<td>Boulenger, 1897</td>
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<td>12m, 13sm</td>
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<td>Barbus kenlenit</td>
<td>Boulenger, 1903, 1904</td>
<td>50</td>
<td>12m, 13sm</td>
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<td>Barbus paludinorus</td>
<td>Boulenger, 1914</td>
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<td>12m, 13sm</td>
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<td>Table 1 - Comparison of current and previously published data on the chromosome number (2n), haploid karyotype characteristics and chromosome arm number (N.F.) of species of small African Barbus and related genera</td>
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**Explanations:**
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organizer regions (NORs) were analyzed by the colloidal silver nitrate method of Howell and Black (1980). Chromosomes were classified according to Levand et al. (1964).

RESULTS

Barbus bigomei. - 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 bigomei (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 - subtelocentric and a - acrocentric chromosomes. Scale bars equal 5 \(\mu m\).
The colloidal silver nitrate method of staining was used and the karyotypes were classified according to LEVAN et al. (1980).

The number of chromosomes in the karyotype was determined. In B. macrolepis, the number of chromosomes was 2n = 48. The karyotype consists of a series of submetacentric chromosomes, with telomeric pairs in one middle-sized chromosome.

In B. macropterus, the number of chromosomes was 2n = 50. The karyotype consists of a series of submetacentric and a pair of metacentric chromosomes, with NORs in one middle-sized chromosome. The number of chromosomes in B. aureus was 2n = 50. The karyotype consists of a series of submetacentric and four pairs of metacentric chromosomes, with NORs in one middle-sized chromosome.

The karyotypes of these three species were analyzed and summarized in Table 1.

In B. macropterus and B. aureus, the small elements with their centromere located in the middle of the chromosome were arranged in a nearly terminal position. This is the effect of chromosome arm con

over and in spite of difficulties in preparing the karyotypes, the relative poor quality of the karyotypes may be attributed to the presence of telomeric and near-terminal elements. However, the presence of three barbels may permit the determination of the number of chromosome sets. This is more especially true when the first pair of metacentric chromosomes is distinctly larger, and it can be

A)  
-9

B)  
-9

C)  
-7

B. macrolepis (B): karyogram of B. macrolepis (C); karyogram of B. macropterus (C). Scale bars equal 5 μm.
Fig. 2 — The metaphase plates (A, C) and interphase nuclei (E) of Barbus bigomei (A, B), B. abdlakes (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 bigomei (A, B) and B. abdlakes (C, D) are framed and also enlarged in the insets. Interphase nuclei of B. macrops display two positive signals.
Karyotypes of three «small» Barbus

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 interspecific 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, inexpensive 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 «larger» 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 synonymy in Léveque 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, Capeota 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) «Capeota» species with 2n = 50 and NF = 54-58; and 4) «Capeota» 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. kerskenii 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 1 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-
lives at the group. Anyway, any other speculations about relationships between and within small African barbels and Asian Pantias 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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Karyotypes of three cyprinid fishes, Osteochilus hasselti, O. hasselti, and Labcobarbus lineata