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New evidence of hexaploidy in ‘ large ’ African *Barbus* with some considerations on the origin of hexaploidy

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Three species of ‘ large ’ African *Barbus* from the Republic of Guinea (West Africa), were characterized by the chromosome numbers (2N) of 148 (*B. bynni occidentalis* and *B. wurtzi*) and 150 (*B. petitjeani* Daget, 1962). All these species have a karyotype which corresponds to the evolutionary hexaploid level. The karyotype of *B. petitjeani* is composed of chromosomes clearly grouped into morphologically homomorphic sextets which may document the origin of hexaploidy via an autopolyploidic event. Present findings extend the known distribution of evolutionary hexaploidy in ‘ large ’ African *Barbus* to West Africa and show evidence of the pan-African distribution of this phenomenon.

Key words: karyology; *Barbus*; hexaploidy; West Africa.

INTRODUCTION

Many African species assigned to the genus *Barbus* involve two distinct groups—‘ small ’ and ‘ large ’ barbels differing especially in adult size and type of scale striation (Banister, 1973, 1987; Lévêque *et al.*, 1990; Skelton *et al.*, 1991). The cyprinid genus *Barbus sensu lato* is a polyphyletic assemblage in which a number of unrelated species and/or groups have been included. It is generally acknowledged that this cyprinid taxon requires a complete taxonomic reorganization of its status (Howes, 1987). The main phyletic lineages, within this conglomerate, need to be identified from different data-sets and concern the use of different methods. In this sense karyological studies (Vervoort, 1980; Rab, 1981; Vasil’ev, 1985; Collares-Pereira & Madeira, 1990; Oellerman & Skelton, 1990; Golubtsov & Krysanov, 1993; Rab *et al.*, 1993) have shown that only species with evolutionary tetraploid (2N = 100) and hexaploid (2N = 148–150) levels are assignable to the genus *Barbus sensu stricto* while those possessing a diploid chromosome number (2N = 48, 50) belong to different lineages of cyprinine cyprinids (*sensu* Howes, 1991). The evolutionary polyploidy feature in this group is, therefore, an important characteristic to promote a taxonomic reorganization of this fish group. However, knowledge about the distribution of evolutionary polyploidy, both geographical and among groups/lineages, in this group of fishes, especially in Africa, is far from complete.

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TABLE I. Collection data on specimens of *Barbus* karyologically investigated

Species	No. of individuals analysed	Collection number	No. of cells analysed per species	Locality
Barbus bynni occidentalis Boulenger, 1911	4	MNCN 83844-47 (3 females and 1 unknown)	47	Bafing River, at Sokotoro bridge (Senegal basin) Guinea
Barbus petitjeani Daget, 1962	2	MNCN 83841-42 (1 female and 1 male)	28	Bafing River, at Sokotoro bridge (Senegal basin) Guinea
Barbus wurtzi (ex. *V. wurtzi*) Pellegrin, 1908	3	MNCN 83815-17 (2 females and 1 male)	34	Kaba River, at Kouloundala bridge (Little Scarcies basin) Guinea

Recent findings based on karyological studies have shown that evolutionary hexaploid species of *Barbus* occur in southern (Oellerman & Skelton, 1990), and eastern (Golubtsov & Krysanov, 1993) Africa. The present report documents the occurrence of three other hexaploid species of *Barbus* in West Africa thus increasing the number of known hexaploid species and their range of distribution in Africa.

MATERIALS AND METHODS

The specimens used represent a part of a larger sample made during a joint French-Spanish field expedition to the Republic of Guinea (West Africa) in 1993. Specimens were collected in the field by electrofishing. All specimens analysed are deposited in the Museo Nacional de Ciencias Naturales, Madrid (Spain). The number of specimens karyotyped and their collection references are given in Table I.

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 deep frozen until analysis in the laboratory. Because cell suspensions were fixed with ethanol (instead of methanol generally used), the protocol was modified as follows. Suspensions were refixed in cold, freshly made methanol–acetic acid fixative at least five times. The chromosome preparations were made by dropping of cell suspensions either on to dry slides or, if unsuccessful, on to chloroform wetted slides. Slides were stained with 5% Giemsa and, if necessary and/or to get a better contrast, were slightly counter-stained with 50% silver nitrate. Chromosomes were classified according to Levan *et al.* (1964).

RESULTS

The diploid chromosome numbers (2N) were determined as 148 for *B. bynni occidentalis* (47 metaphases) and for *B. wurtzi* (34 metaphases), and 150 for *B. petitjeani* (28 metaphases). The karyotype of *B. petitjeani* is precisely classified as proposed in Fig. 1(a). Unfortunately, and certainly due to difficult conditions during the chromosome fixation in the field, it was impossible to karyotype more precisely *B. bynni occidentalis* and *B. wurtzi*, and we demonstrate only

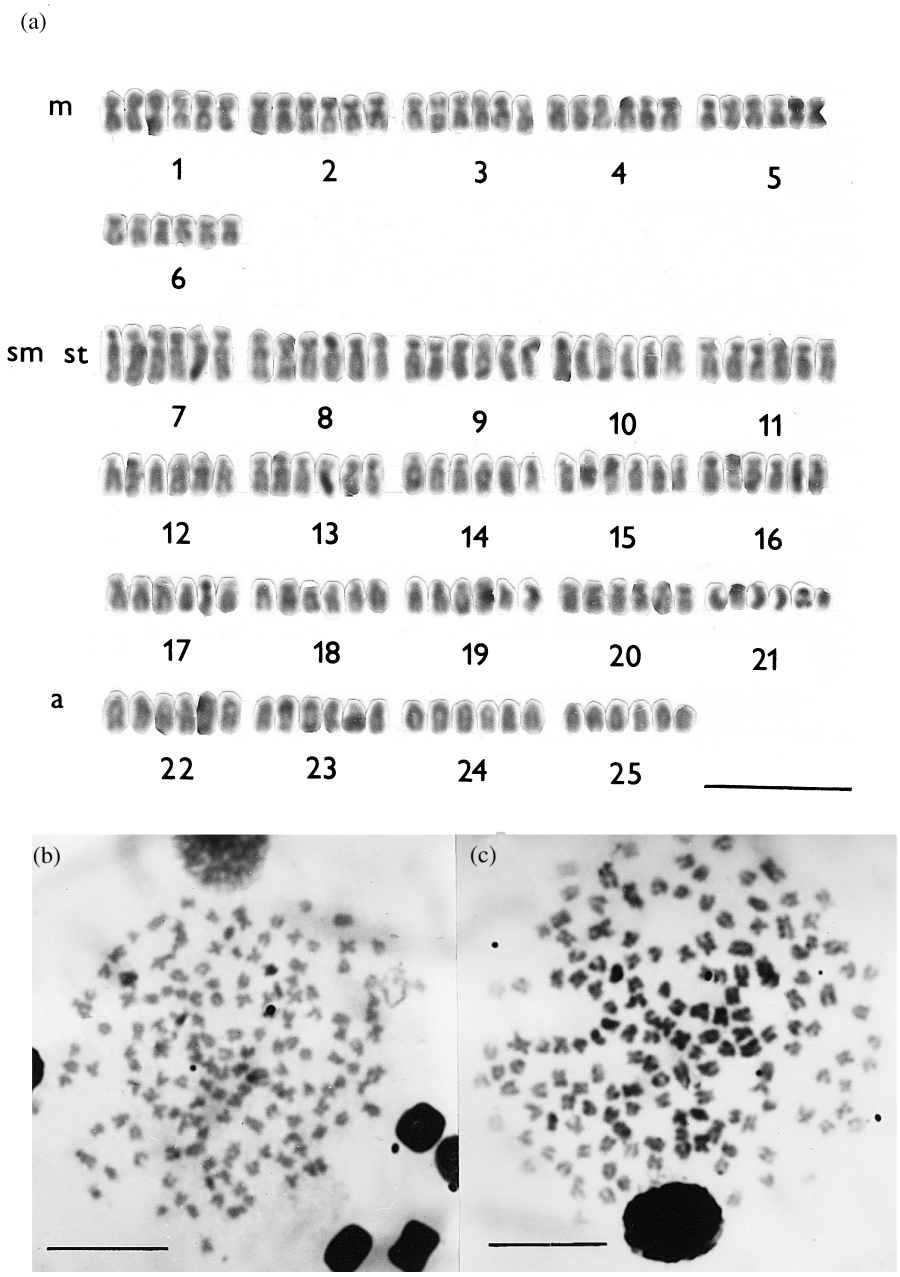

FIG. 1. Karyotype of *Barbus petitjeani* (a) and metaphase plates of *B. bynni occidentalis* (b) and *B. wurtzi* (c). Chromosomes in the karyogram of *Barbus petitjeani* are arranged in numbered sextets; m, metacentric; sm, submetacentric; st, subtelocentric; a, acrocentric chromosomes. Scale bars 5 μm.

chromosome spreads for these two latter species [Fig. 1(b), (c)]. The chromosome set of *B. petitjeani* comprises some elements which are easily arranged into distinct morphologically homomorphic sextets: six sextets of metacentric, 15 sextets of submetacentric to subtelocentric and four sextets of acrocentric

chromosomes [Fig. 1(a)]; scoring the metacentric chromosomes only as bi-armed and all others as uni-armed, we obtained an NF value of 186.

DISCUSSION

Up to now, nine species of large African *Barbus* (*B. aeneus* (Burchell, 1922), *B. bynni bynni* (Forskal, 1775), *B. capensis* Smith, 1840, *B. ethiopicus* Zolezzi, 1939, *B. intermedius* Rüppell, 1836, *B. kimberleyensis* Gilchrist & Thompson, 1913, *B. marequensis* Smith, 1841, *B. natalensis* Castelnou, 1861, *B. polylepis* Boulenger, 1907) and two species of *Varicorhinus* (*V. beso* Rüppell, 1836, *V. nelspruitensis* Gilchrist & Thompson, 1911) have been found to be hexaploid, i.e. with 148 to 150 chromosomes (Oellerman & Skelton, 1990; Golubtsov & Krysanov, 1993). Our findings increase the list of hexaploid species of large African *Barbus*. The polyploid status of these three species of *Barbus* has been suggested previously by Agnèse *et al.* (1990). In their paper, these authors concluded, on the basis of enzymatic systems analyses, that these barbels were evolutionary tetraploid similar to the European species *B. barbus* L. and *B. meridionalis* Risso, 1826. However, all three *Barbus* species present a chromosome number equivalent to the evolutionary hexaploid level. It is evident from this observation that even if such biochemical genetic analyses can separate diploid and polyploid lineages of barbels, they do not distinguish between tetraploid and hexaploid levels. Moreover, the species *B. wurtzi* was considered to belong to the genus *Barbus* by Lévêque & Guégan (1990) on the basis of both morphological and parasitological criteria, and not to the genus *Varicorhinus* in which it was previously placed. This present work reinforces the idea of a close resemblance between some African *Varicorhinus* and large *Barbus* species which was recently ' remis sur la sellette ' by Golubtsov & Krysanov (1993).

Golubtsov & Krysanov (1993) karyotyped *B. bynni bynni* (Forskal, 1775) from Ethiopia (Nile basin), in which they found 2N = 150. In this work, we have investigated another subspecies of *B. bynni* (Forskal, 1775), *B. b. occidentalis*, from the Bafing River (Senegal basin), whose chromosome number is 2N = 148. Unfortunately for reasons discussed above, we cannot compare the karyotypic formulae of both geographic forms. Thus, any speculations about the phylogenetic relationships and taxonomic status of both forms occurring in two distant areas of Africa would be premature. However, it has to be stressed that a certain level of intraspecific karyotypic variation has been shown in another polyploid barbel, the tetraploid *B. bocagei* (Steindachner, 1865) from the Iberian Peninsula (Collares-Pereira & Madeira, 1990), and in some tetraploid schizothoracine species of the *Diptychus* and *Schizothorax* genera from the Tian Shan Mountains in Kirgizia (Mazik *et al.*, 1989; Toktosunov & Mazik, 1991). These observations could be considered as a heuristic explanation for the observed difference in chromosome number between *B. bynni bynni* and *B. bynni occidentalis*.

In their investigation, Oellerman & Skelton (1990) hypothesized different ways of origin of hexaploidy in barbels: three solutions could imply autopolyploidy and one allopolyploidy. Conversely, Collares-Pereira & Coelho (1989) proposed another model of diploid/polyploid relationships in which the hexaploid level found in several lineages of cyprinine cyprinids could represent only a triploid

level. Generally, the karyological analysis of hexaploid chromosome complements, possessing high numbers of small chromosomes, is very difficult and intraspecific karyotypic variations often observed in polyploid cyprinids (see above) may result from this complication to distinguish between them clearly. However, the quality of chromosome preparations for *B. petitjeani* allowed us to karyotype both specimens in great detail. Surprisingly, the chromosome set of this species could be grouped easily into morphologically homomorphic sextets even if the absence of chromosome banding data in our study does not permit us to state that these sextets are really composed of six identical elements. This new evidence may suggest the origin of hexaploidy via an autopolyploidic event—even though an allopolyploidic event through hybridization of two species with morphologically similar or identical karyotypes cannot be definitely excluded. Examinations of gill parasites of West African barbels by Guégan & Lambert (1990) have shown that *B. petitjeani* living in a refuge area harbour parasites which are very primitive, and according to the rule that implies that primitive parasites are always associated with hosts whose rank is as primitive, *B. petitjeani* could represent a taxon which is less derived in relation to ancestral barbels. The karyotype structure, i.e. number of chromosome sextets in particular categories, of this barbel species thus certainly corresponds to karyotype structures, i.e. number of chromosome pairs in particular categories, observed in another cyprinid group (for further details see e.g. Vasil'ev, 1985; Yu *et al.*, 1989). On the other hand, the chromosomes in karyotypes of the two tetraploid European barbels, *B. meridionalis meridionalis* Risso, 1826 and *B. meridionalis petenyi* Risso, 1826 (which are, in fact, two distinct species according to Karakousis *et al.*, 1993) cannot be arranged into quadruplets (Rab *et al.*, 1993). All these remarks on karyotype structures of tetraploid and hexaploid species of *Barbus* may be important for future considerations on the origin of different ploidy levels among barbels and cyprinine cyprinids (*sensu* Howes, 1991).

The hexaploid African species of *Barbus* and *Varicorhinus* were reported to occur in southern (Oellerman & Skelton, 1990) and eastern (Golubtsov & Krysanov, 1993) Africa. Our findings report the occurrence of hexaploid barbel species also in West Africa and reinforce the assumption made by Golubtsov & Krysanov (1993) that the hexaploidy level in large African species of *Barbus* and related taxa is a pan-African phenomenon.

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