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Two lineages, diploid and tetraploid, demonstrated in African species *Barbus* (Osteichthyes, Cyprinidae)

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Abstract

Enzymatic polymorphism was studied in nine enzyme systems of four species of large African *Barbus* (*B. bynni occidentalis* Boulenger, 1911; *B. sacratus* Daget, 1963; *B. petitjeani* Daget, 1962; and *B. wurtzi* Pellegrin, 1908), two species of small African *Barbus* (*B. guineensis* Pellegrin, 1913 and *B. cadenati* Daget, 1962), and two species of European *Barbus* (*B. barbus* Linnaeus, 1758 and *B. meridionalis*). Like European *Barbus*, the large African *Barbus* were found to express more than 20 enzymatic loci for these systems (21 to 23), whereas the small African *Barbus* only expressed 14. This result suggests that large African *Barbus* are tetraploid like European *Barbus* and that small African *Barbus* are diploid like the Asian species. The two groups of African *Barbus* do not appear to be phylogenetically closer to each other than they are to European *Barbus*. Consequently, tetraploidization must have occurred long ago in large African *Barbus*, and may correspond to the same event responsible for the origin of the European species.

Keywords : Tetraploidy, allozymes, Cyprinidae, *Barbus*, Africa.

Mise en évidence de deux lignées, diploïde et tétraploïde, chez les espèces africaines du genre Barbus (Osteichthyes, *Cyprinidae*).

Résumé

Le polymorphisme enzymatique a été étudié pour neuf systèmes enzymatiques chez quatre espèces de grands *Barbus* africains (*B. bynni occidentalis* Boulenger, 1911; *B. sacratus* Daget, 1963; *B. petitjeani* Daget, 1962 et *B. wurtzi* Pellegrin, 1908), deux espèces de petits *Barbus* africains (*B. guineensis* Pellegrin, 1913 et *B. cadenati* Daget, 1962) et deux espèces de *Barbus* européens (*B. barbus* Linné, 1758 et *B. meridionalis*). Les grands *Barbus* expriment pour ces systèmes, comme les *Barbus* européens plus de 20 locus enzymatiques (21 à 23) alors que les petits *Barbus* n'en expriment que 14. Ce résultat suggère que les grands *Barbus* africains sont tétraploïdes comme les *Barbus* européens et que les petits *Barbus* africains sont diploïdes comme les espèces asiatiques. Les deux groupes de *Barbus* africains ne semblent pas phylogénétiquement plus proches entre eux qu'ils ne le sont des *Barbus* européens. L'événement de tétraploïdie des grands *Barbus* est donc ancien et pourrait être le même que celui qui est à l'origine des espèces européennes.

Mots-clés : Tétraploïdie, allozymes, Cyprinidae, *Barbus*, Afrique.

INTRODUCTION

The Cyprinidae comprise more than 1,600 species distributed over all the continents except for Australia and South America. Most of the species are diploid, but some of them have been shown to be tetraploid, *e. g. Acrossocheilus sumatranus*, *Aulopyge hugeli*, *Carassius auratus*, *Cyprinus carpio*, *Tor putitora*, *Barbus barbus*, *B. meridionalis*, and *B. plebejus* (see reviews by Buth, 1984; Vasiliev, 1985). In Europe and Asia, the genus *Barbus* has the particularity of possessing both diploid and tetraploid species. At present, the name of the genus *Barbus sensu lato* is attributed to several hundred Eurasian and African species that show little resemblance to the European reference species *Barbus barbus* Linnaeus, 1758. Lévêque and Daget (1984) consider that the name *Barbus* should be reserved for European species and several North African species. The works of Howes (1987) support this remark insofar as the group of species comprising the reference species *Barbus barbus* can be separated from other Eurasian species on the basis of osteological characters. As indicated by Howes (1987), "Some authors have opted to recognize separate genera (*e. g. Puntius*, *Tor*, ...) for the Indian and South East Asian species, a solution which does little to elucidate relationships since these "genera" are not defined on derived characters. The definition of *Barbus* can only be approached through an adequate anatomical comparison of Eurasian and African species". In the absence of detailed revisions, the taxonomy of African Cyprinidae attributed to the genus *Barbus* remains confused. Nevertheless, external morphological characters can be used to distinguish two large groups (Daget and Iltis, 1965; Lévêque and Guégan, 1990) :

- large *Barbus* whose scales have many parallel stria, whose dorsal fin has nine to eleven branched rays and a last hard ray with no denticles, and which are sometimes longer than 50 cm;
- small *Barbus*, whose scales have a small number of divergent stria, whose dorsal fin has seven or eight branched rays, and which are rarely longer than 10 cm.

The European species resembling large African *Barbus* species, while nevertheless constituting a distinct line (the Siberian branch; Darlington, 1958) characterized by scales with divergent stria and denticles on the last hard ray of the dorsal fin (Boulenger, 1911; Pellegrin, 1921; Berrebi, 1981), appear to have a tetraploid karyotype (2N = 100). The Asian species are diploid (2N = 50 ± 2) or tetraploid (2N = 100) (Magtoon and Arai, 1989; Yu *et al.*, 1987). No karyological or electrophoretic study has yet been carried out on small or large African *Barbus*. In the present enzymatic study, we compared large and small African *Barbus* (*B. occidentalis*, *B. sacratus*, *B. petitjeani*, and *B. wurtzi versus B. guineensis* and *B. cadenati*) and European *Barbus* (*B. barbus* and *B. meridionalis*), which are known to be tetraploid (Klose *et al.*, 1969; Wolf *et al.*, 1969; Engel *et al.*, 1971; Sofradzija and Berberovic, 1973; Triantaphyllidis *et al.*, 1981). The objective of the study was to investigate phylogenetic relationships between these three groups of species. Although the existence of the two groups is indicated by osteological characteristics (Howes, 1987; table 4), neither group has been shown to be monophyletic. Determination of the phylogeny of *Barbus* should provide new elements for the debate on the biogeography and origin of these species.

MATERIALS AND METHODS

Immediately after capture, fish were dissected and liver and muscle samples were stored in liquid nitrogen. The samples were then transferred to the laboratory and stored at −80°C until analysis. All individuals studied (excepted the two European species) were deposited at the Muséum d'Histoire Naturelle de Paris under the numbers MNHN-1989-971, 1989-987 in the case of *B. bynni occidentalis*, MHNH-1989-986 for *B. petitjeani*, MNHN-1989-1015, 1989-1017, 1989-979 for *B. sacratus*, MNHN-1989-972, 1989-974 to 1989-976, 1989-1000 for *B. wurtzi*, MHNH-1989-1007 and MHNH-1989-1010 for both *B. guineensis* and *B. cadenati*. The number of specimens studied and their origins are given in *table* 1. Individuals of a single species taken from different locations were considered separately only if there was a significant difference in the allelic frequency at a locus. This was true of *B. wurtzi* 1 and *B. wurtzi* 2.

Electrophoresis was carried out according to the protocols described in Pasteur *et al.* (1987). Three buffers were used : (i) Poulik buffer to reveal adenylate kinases (E.C. 2.7.4.3), aspartate aminotransferases (E.C. 2.6.1.1), malate dehydrogenases (E.C. 1.1.1.37), and isocitrate dehydrogenases (E.C. 1.1.1.42); (ii) TC 6.7 to reveal lactate dehydrogenases (E.C. 1.1.1.27), superoxide dismutases (E.C. 1.15.1.1), phosphoglucose isomerases (E.C. 5.3.1.90) and phosphoglucomutases (E.C. 2.7.5.1); and (iii) TM 6.9 to study fumarases (E.C. 4.2.1.2). The system used for coding electrophoretic data minimizes information (Berrebi *et al.*, 1990), *i.e.* a locus that is absent in two different species is coded identically (ABS) in the two species. It is clear that diploid and tetraploid cannot be easily compared via their allozymes because duplicate loci are both homologue to the only one expressed in the diploid (for discussion, see Berrebi *et al.*, 1990).

Nei genetic distances were analyzed using the hierarchical clustering analysis program "Kitsch" (in the software package "Phylip" by J. Felsenstein, University of Washington). The value of the parameter P was fixed at 0, which made the processing analogous to UPGMA.

Table I. – Numbers and localities where the specimens investigated here were collected : BOC, *B. bynni occidentalis*; BSA, *B. sacratus*; BPE, *B. petitjeani*; BWU1 and BWU2, *B. wurtzy*; BBA, *B. barbus*; BME, *B. meridionalis*; BGU, *B. guineensis*; BCA, *B. cadenati*.

	SPECIES								
LOCALITIES	BOC	BSA	BPE	BWU1	BWU2	BBA	BME	BGU	BCA
BAFING at Sokotoro (GUINEA)	5		9						
SALA at Louka (GUINEA)								6	2
KONKOURE Télimélé road (GUINEA)				2					
ROKEL at Bumbuna (SIERRA LEONE)				2					
ROKEL at Kondembaya (SIERRA LEONE)		3							
MONGO at Moussaia (SIERRA LEONE)					3				
NIGER at Bamako (MALI)	3								
Hérault at Lodève (FRANCE)							5		
Aude at Carcassonne (FRANCE)						5			

Cladistic analysis was based on a matrix showing the presence (coded 1) or absence (coded 0) of alleles in each species or population. The matrix was then processed by the program "Clique" (also in "Phylip" by J. Felsenstein), which uses an algorithm of compatibility (Le Quesne, 1974; Farris, 1977). This program indicates the phylogenetic system that contains the largest number of compatible characters (alleles in this case), *i.e.* showing no contradictory information. Characters that are incompatible with the other characters are not used in constructing the network. Reversions (reverse mutations), convergences (gain or loss of alleles occurring independently in two different lineages), and mosaic evolution (gene exchange between two species that are not yet totally isolated genetically) can cause incompatibility between characters. This divergence of genetic information is called homoplasia.

RESULTS

A total of 25 different proteins corresponding to the same number of loci were studied. The alleles observed at these loci in each sample are given in *table* 2. Loci *Ldh*-3, *Mdh*-2, and *Mdh*-4 were only present in liver, and *Mdh*-3, *Mdh*-5, *Pgi*-1, *Pgi*-4, and *Pgi*-5 were only present in muscle. Two groups of species were clearly distinguished : those expressing at least 22 allozymes for the nine enzyme systems studied (large African *Barbus* and European *Barbus*), and those expressing 14 allozymes (small African *Barbus*).

Figure 1 shows a dendrogram obtained from a table of Nei distances (1972). Three groups appear, corresponding to : (a) small African *Barbus*, (b) large African *Barbus*, and (c) European *Barbus*. In large *Barbus*, it can be seen that *B. bynni occidentalis* is close to *B. sacratus* and *B. wurtzi* is close to *B. petitjeani*. The African and European tetraploid species appear to show more affinity to each other than to the diploid species. However, this diploid/tetraploid opposition is less marked in the results of cladistic analysis. *Figure* 2 shows the network obtained using the program "Clique." The nine populations or species studied still form the three groups defined above. Sixty out of the 69 alleles analyzed were found to be compatible, which means that the genetic information is of good quality (low homoplasia).

DISCUSSION

The two groups of African *Barbus* (large and small) defined by morphological characters (see Introduction) were also distinguished by genetic characters. The simplest hypothesis to explain the many gene duplications in large *Barbus* is that these species are tetraploid and small *Barbus* are diploid, as suggested by Buth (1984) for other cyprinids with the karyotype 2N = 100. A gene can be duplicated in two ways : tandem duplication or polyploidization. Tandem duplication results from a copying error during meiosis or unequal cross-over, *i.e.* one or more genes are copied several times next to each other (Ohta, 1988). Although this phenomenon has been observed in fishes, it appears to be rather infrequent (Buth, 1983). Polyploidization concerns the whole genome, *i.e.* the number of genes doubles all at once in a tetraploid. In the present case, the hypothesis of tetraploidization is the most plausible in large African *Barbus* because only a single event is involved instead of the many events occurring in tandem duplication. Indeed, although the loci studied are representative of the genome of these fishes (which must comprise 10,000 to 50,000 genes), several thousand additional genes are expressed in large African *Barbus*. The hypothesis of tetraploidy in large *Barbus* is also supported by the existence of tetraploids in the Cyprinidae, *e.g.* *Carassius auratus* (Ohno *et al.*, 1967; Bender and Ohno, 1968; Labat *et al.*, 1983), *Cyprinus carpio* (Ohno *et al.*, 1967; Bender and Ohno, 1968; Labat *et al.*, 1983), and specifically in the genus *Barbus* : *B. barbus* (Wolf *et al.*, 1969; Klose *et al.*, 1969),

Table 2. – Alleles observed at the 25 loci investigated in nine species or populations of the genus *Barbus* : BOC, *B. occidentalis*; BSA, *B. sacratus*; BPE, *B. petitjeani*; BWU1 and BWU2, *B. wurtzi*; BBA, *B. barbus*; BME, *B. meridionalis*; BGU, *B. guineensis*; BCA, *B. cadenati*. Allelic frequencies different of 100% are indicated in parentheses.

	SPECIES								
LOCI	BOC	BSA	BPE	BWU1	BWU2	BBA	BME	BGU	BCA
Mdh-1	120	120	120	120	120	100	100	100	100
Mdh-2	110	110	110	110	110	100	100	120	120
Mdh-3	110	110	110	110	110	100	100	090	090
Mdh-4	100	100	100	100	100	ABS	100	ABS	ABS
Mdh-5	100	100	100	100	110	100	100	099	099
Ldh-1	100	100	100	120	120	100	100	140	100
Ldh-2	100	100	100	100	100	100	100	100	100
Ldh-3	150	150	150	150	150	010	100	ABS	ABS
Idh-1	100	100	100	100	100	100	100	100	100
Idh-2	ABS	ABS	ABS	ABS	ABS	ABS	100	ABS	ABS
Fum	110	110	110	110	110	100	100	110	150
Sod-1	100	100	100	100	100	100	100	105	150
Sod-2	100	100	100	100	100	100	100	ABS	ABS
Aat-2	118	120	110	110	110	100	100	130	130
Aat-3	100	100	090	090	090	100	110	ABS	ABS
Pgi-1	100	100	100	100	100	100	100	120	120
Pgi-2	110	110	090	110	110	100	100	ABS	ABS
Pgi-3	110	110	105	105	105	100	100	100	100
Pgi-4	100	100	100	100	100	100	100	ABS	ABS
Pgi-5	ABS	ABS	ABS	ABS	ABS	100	ABS	ABS	ABS
Ak-1	110	ABS	ABS	ABS	ABS	100	ABS	ABS	ABS
Ak-2	100	100	100	100	100	100	100	100	100
Ak-3	100	100(92) 110(08)	100	100	100	ABS	ABS	ABS	ABS
Pgm-1	098	098	098	098	098	100	100	110	110
Pgm-2	090	090(83) 085(17)	090(95) 070(05)	070	090(50) 070(50)	ABS	100	ABS	ABS
TOTAL ALLOZYMES	23	22	22	22	22	21	22	14	14

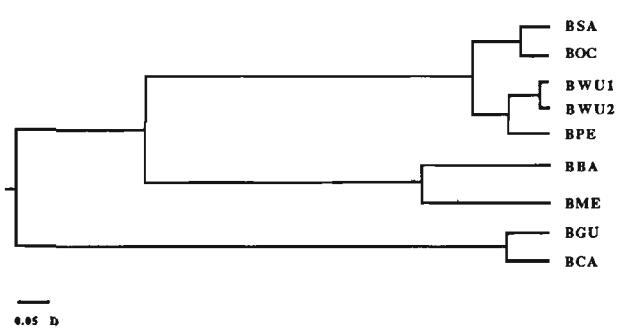

Figure 1. – Dendrogram obtained using the program "Kitsch", based on a matrix of genetic distances. The value of the parameter P was fixed at 0, which makes the processing very similar to an UPGMA : **BOC**, *B. bynni occidentalis*; **BSA**, *B. sacratus*; **BPE**, *B. petitjeani*; **BWU1** and **BWU2**, *B. wurtzi*; **BBA**, *B. barbus*; **BME**, *B. meridionalis*; **BGU**, *B. guineensis*; **BCA**, *B. cadenati*.

B. meridionalis (Sofradzija and Berberovic, 1973; Triantaphyllidis *et al.*, 1981; Berrebi *et al.*, 1988) and *B. plebejus* (Catandella *et al.*, 1977).

Thus, two well-differentiated lineages without particular relationships exist in Africa. Our results correspond to those of Howes (1987), who considers, on the basis of osteological data, that large African *Barbus* are similar to *Barbus sensu stricto*, unlike small African *Barbus*, which are more similar to certain Asian species. If two lineages of *Barbus* exist in Africa, one tetraploid and the other diploid, a question arises as to their phylogenetic relationships. The tetraploidization producing large African *Barbus* may thus correspond to the same event that produced European *Barbus*, which would explain the configuration of the phylogenetic systems obtained. If the small *Barbus* are taken as the reference species, the enzymatic systems showing duplication are very often the same in European *Barbus* and large African *Barbus* (MDH, LDH, SOD, AAT, PGI, and PGM). This

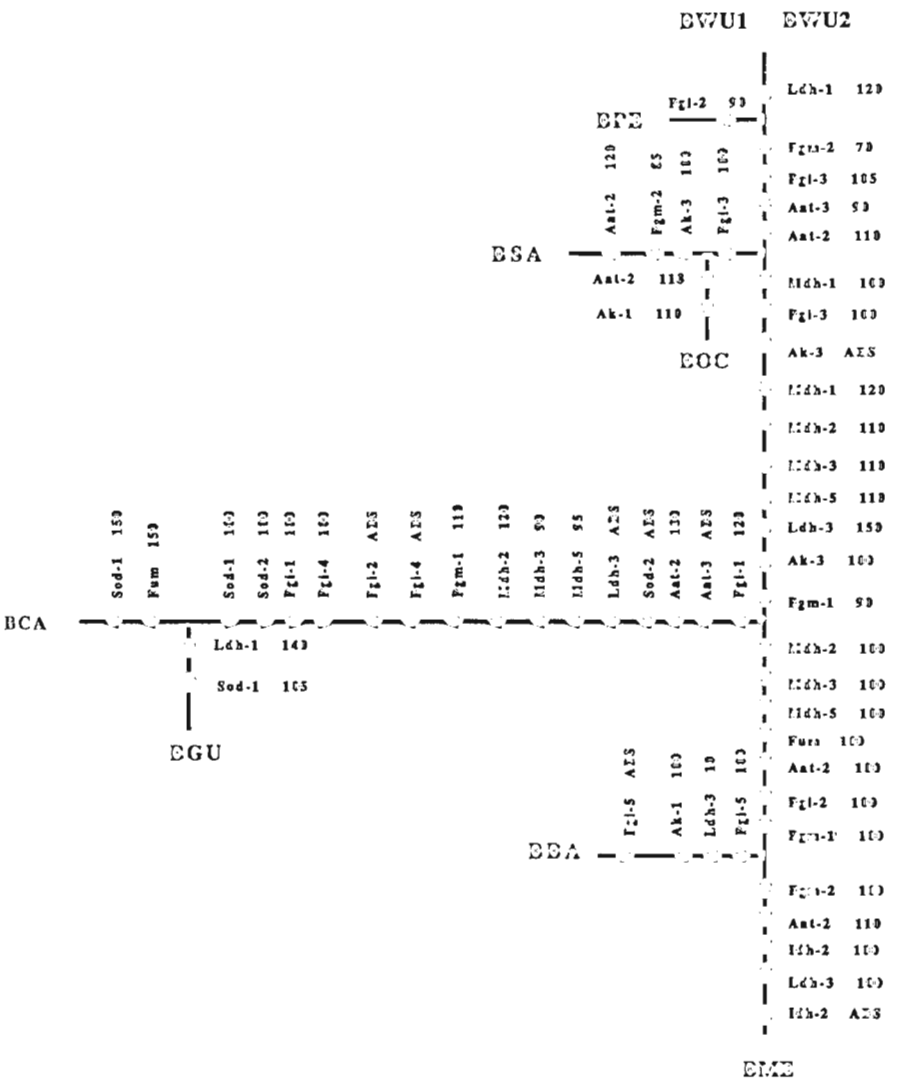

Figure 2. – Cladistic network obtained using the program "Clique", based on a matrix of allele presence (coded 1) or absence (code 2). Only the largest set of alleles compatible with each other was used to construct the system : **BOC**, *B. bynni occidentalis*; **BSA**, *B. sacratus*; **BFE**, *B. petitjeani*; **BWU1** and **BWU2**, *B. wurtzi*; **BBA**, *B. barbus*; **BME**, *B. meridionalis*; **BGU**, *B. guineensis*; **BCA**, *B. cadenati*

Table 3. – Nei's genetic distances (1972) between the nine species or populations of the genus *Barbus* studied : BOC, *B. bynni occidentalis*; BSA, *B. sacratus*; BFE, *B. petitjeani*; BWU1 and BWU2, *B. wurtzi*; BBA, *B. barbus*; BME, *B. meridionalis*; BGU, *B. guineensis*; BCA, *B. cadenati*.

	GENETIC DISTANCES							
SPECIES	BOC	BSA	BFE	BWU1	BWU2	BBA	BME	BGU
BSA	0.035	0						
BFE	0.224	0.178	0					
BWU1	0.274	0.218	0.124	0				
BWU2	0.238	0.183	0.093	0.010	0			
BBA	0.916	0.807	1.020	1.139	1.293	0		
BME	0.916	0.812	0.819	0.916	0.935	0.328	0	
BGU	1.427	1.264	1.271	1.273	1.263	1.021	1.139	0
BSP	1.427	1.264	1.271	1.427	1.417	0.916	1.022	0.128

could be due to kinship between the two tetraploid groups, which would support the hypothesis of a single tetraploidization event. However, another less parsimonious hypothesis cannot be excluded, namely that tetraploidization occurred several times in the species of this genus, in view of the fact that several independent polyploidizations have occurred in the Cyprinidae (Buth 1983, 1984). In this case, the resemblances in the expression of loci could be due to physiological constraints specific to these fishes.

Further genetic and karyological studies including European, African, and Asian species will allow a determination of the phylogeny of this group, making it possible to deduce its place of origin, and to reconstruct the dispersals of Cyprinidae of the genus *Barbus sensu lato* throughout the world.

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REFERENCES

Berrebi P., G. Lamy, G. Cattaneo-Berrebi, J.F. Renno, 1988. Variabilité génétique de *Barbus meridionalis* (Poisson, Cyprinidae) : une espèce quasi monomorphe. *Bull. Fr. Pêche Piscic.*, 310, 77-84.

Berrebi P., 1981. Contribution à l'étude du sous-genre *Labeobarbus* (genre *Barbus*, Poisson, Cyprinidae) du Maroc. *Bull. Inst. Sc. Rabat*, 5, 59-72.

Berrebi P., C. Lévêque, G. Cattaneo-Berrebi, J.F. Agnèse, J.F. Guégan, A. Machordom, 1990. Diploid and tetraploid African *Barbus* (Osteichthyes, Cyprinidae) : on the coding of differential gene expression. *Aquat. Living Resour.*, **3**, 313-323.

Boulenger G. A., 1911. Catalogue of fresh-water fishes of Africa in the British Museum. British Museum, London, t.2.

Buth D. G., 1983. Duplicate isozyme loci in fishes : Origins, distribution, phyletic consequences and locus nomenclature, in : Isozymes : Current topics in Biological and Medical Research, Vol. 10, M. C. Rattazi, J. G. Scandalios, G. S. Whitt, eds; Alan R. Liss, New York, 381-400.

Buth D. G., 1984. Allozymes of the Cyprinid Fishes, variation and application. Evolutionary genetics of Fishes. Bruce J. Turner ed. Plenum Publishing Corporation, 561-590.

Bender K., S. Ohno, 1968. Duplication of the autosomally inherited 6-phosphogluconate dehydrogeenase gene locus in tetraploid species of cyprinid fish. *Biochem. Genet.*, **2**, 101-107.

Cataudella S., L. Sola, R. Acame Muratori, E. Capanna, 1977. The chromosomes of 11 species of Cyprinidae and one Cobotidae from Italy, with some remarks on the problem of polyploidy in the Cypriniformes. *Genetica*, **47**, 161-171.

Daget J., A. Iltis, 1965. Poissons de Côte d'Ivoire (eaux douces et saumâtres). *Mém. IFAN*, Dakar, 74, 385 p.

Engel J., J. Faust, U. Wolf, 1971. Isoenzyme polymorphism of the sorbitol dehydrogenase and the NADP-dependent isocitrate dehydrogenases in the fish family Cyprinidae. Anim. *Blood Groups Biochem. Genet.*, **2**, 127-133.

Farris J. S., 1977. Phylogenetic analysis under Dollo's law. *Syst. Zool.*, **26**, 77-88.

Howes G. J., 1987. The phylogenetic position of the yugoslavian cyprinid fish genus *Aulopyge* Heckel 1841, with an appraisal of the genus *Barbus* Cuvier and Cloquet, 1816, and subfamily Cyprinidae. *Bull. Br. Mus. Nat. Hist., Zool. ser.*, **52**, 165-196.

Klose J., U. Wolf, H. Hitzeroth, H. Ritter, 1969. Polyploidization in fish family Cyprinidae, order Cypriniformes. II. Duplication of the gene loci coding for lactate dehydrogenase (E.C. : 1.1.1.27) and 6-phosphogluconate dehydrogenase (E.C. : 1.1.1.44) in various species of Cyprinidae,. *Humangenetik*, **7**, 245-250.

Labat R., R. Hafez, E. Quiller, 1983. Cytogenetic studies in some species of cyprinid fishes. *Roczniki Nank Rolniczych HT*, **100**, 89-93.

Le Quesne W. J., 1974. The uniquely evolved character concept and its cladistic application. *Syst. Zool.*, **23**, 513-517.

Lévêque C., J. Daget, 1984. Cyprinidae. *In* : Cheklist of the freshwater fishes of Africa. CLOFFA 1, 217-343. ORSTOM, Paris and MRAC Tervuren.

Lévêque C., J.F. Guégan, 1990. Les grands Barbus (Teleostei, Cyprinidae) d'Afrique de l'Ouest. *Rev. Hydrobiol. Trop.*, In press.

Matgoon W., R. Arai, 1989. Karyotypes of five *Puntius* and one *Cyclochelichthys* species (Pisces, Cyprinidae) from Thailand. *Bull. Nat. Sci. Mus. Tokyo*, Ser. A, **15**, 167-175.

Nei M., 1972. Genetic distance between populations. *Am. Nat.*, **106**, 283-292.

Ohno S., J. Muramoto, L. Christian, 1967. Diploid-tetraploid relationship among old world members of the fish family Cyprinidae. *Chromosoma*, **26**, 35-40.

Ohta T., 1988. Further simulation studies on evolution by gene duplication. *Evolution*, **42**, 375-386.

Pasteur N., G. Pasteur, F. Bonhomme, J. Catalan, J. Britton-Davidian J., 1987. Manuel de génétique par électrophorèse des protéines. Coll. tech. document., Lavoisier, Paris. 217 p.

Pellegrin J., 1921. Les poissons des eaux douces de l'Afrique du Nord française : Maroc, Algérie, Tunisie, Sahara. Mem. Soc. Sc. Nat. Maroc, II, 216 p.

Sofradzija A., L. Berberovic, 1973. The chromosome number of *Barbus meridionalis petenyi* Heckel (Cyprinidae, Pisces). *Bull. Sci. Acad. RSF Yougosl.*, **18**, 77-78.

Triantaphyllidis C. D., H. Damianakis, P. S. Economidis, J. Karakousis, 1981. Genetic variation in Greek barbel populations. I. Esterases, LDH, MDH, ME, and PGM in *Barbus meridionalis* (Pisces, Cyprinidae). *Comp. Biochem. Physiol.*, 70B, 278-293.

Vasiliev V. B., 1985. Evolutionnary karyology of fishes (*in* Russian). USSR. Academy of Sciences, Inst. of Evolution morphology and Ecology. Publ. Nanka, Moscou.

Wolf U., H. Ritter, N. Atkin, S. Ohno, 1969. Polyploidization in the fish family Cyprinidae, Order Cypriniformes, I. DNA content and chromosome set in various species of Cyprinidae. *Humangenetik*, **7**, 240-244.

Yu X., T. Zhou, K. Li, Y. Li, M. Zhou, 1987. On the karyosystematics of cyprinid fishes and a summary of fish chromosome studies in China. *Genetica*, **72**, 225-236.