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Taxonomic status and genetic differentiation among West African populations of the *Chrysichthys auratus* complex (Pisces, Siluriforme), based on protein electrophoresis

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Abstract. Thirteen populations of *Chrysichthys auratus longifilis* (Pfaff) and *C. filamentosus* (Boulenger) from five West African drainages were studied using enzymatic protein electrophoresis to estimate genetic differentiation and verify their taxonomic status. Twenty-seven alleles were observed at 19 loci. Only five loci were polymorphic. Genetic distance and cladistical analyses based on presences/absences of alleles did not separate the populations in groups corresponding to the two taxa. The maximum genetic distances between two populations ($D = 0{\cdot}112$) is lower than the level of maximum divergence observed between conspecific populations of species of the genus in other studies using the same enzymatic systems ($D = 0{\cdot}289$ in *C. maurus*; $D = 0{\cdot}304$ in *C. nigrodigitatus*). These results confirm those of Risch (1986a) who, using morphological data, considered the two taxa to be synonyms. Genetic variability in several of these populations is also low ($0{\cdot}00 < H < 0{\cdot}064$), some of them being monomorphic at the 19 loci studied. The reasons for this low variability are discussed.

Introduction

Until the mid-1980s four species or subspecies of the complex *Chrysichthys auratus* (Geoffroy Saint-Hilaire, 1809) have been named: *C. auratus auratus* (Geoffroy Saint-Hilaire, 1809) present in the River Nile and Lake Turkana; *C. auratus tilhoi* (Pellegrin, 1909) in the Lake Tchad system; *C. auratus longifilis* (Pfaff, 1933) in the Rivers Niger, Volta and Sénégal; and *C. filamentosus* (Boulenger, 1912) in the coastal rivers of Liberia to Cabinda (Angola); Risch (1986b). Risch (1986a) carried out an exhaustive study of all the species and subspecies of the genus *Chrysichthys* using meristical and morphometrical characters. This work suggested that the three subspecies of *C. auratus* and *C. filamentosus* appear to be a single species: *C. auratus* (Geoffroy Saint-Hilaire, 1809). Some morphological characters exhibit variability, for example the length of the first ray of the dorsal fin, and were employed to group some populations according to their affinities (sahelian, lagoonal or intermediate populations), but Risch (1986a) did not define any subspecies.

Fishes of the genus *Chrysichthys* are commercially important, as are most Siluriforms. In particular *C. auratus* is abundant in the Sénégal, Volta and Niger systems as well as in all the lagoons of the Ivory Coast, where it is consumed. The aim of this study is to investigate the taxonomic status of some West African populations of the '*C. auratus*' complex using genetic methods and to describe the importance and the distribution of genetical variation in these populations.

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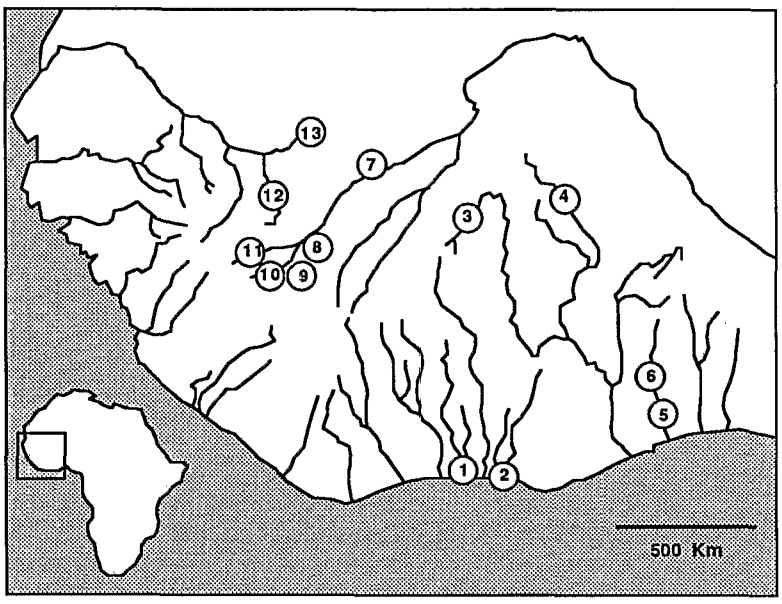

Figure 1. Localities where the presumptive taxa where caught: (1) Ebrié lagoon, (2) Aby lagoon, (5,6) Mono, for *C. filamentosus*; (3,4) Volta, (7,8,9,10,11) Niger system, (12,13) Sénégal system, for *C. auratus longifilis*.

Materials and methods

Specimens were collected in 13 localities (Table 1, Fig. 1). 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 −30°C until analysis a few weeks later. For each population, some specimens, after dissection, were kept in alcohol and deposited at the Muséum d'histoire Naturelle de Paris under the numbers MNHN-1989-1766 to MNHN-1989-1816. Numbers of specimens scored and their origins are given in Table 1.

Electrophoresis was carried out according to the protocols described in Pasteur, Pasteur, Bonhomme, Catalan & Britton-Davidian (1988), which were adapted from those of Selander, Smith, Yang, Johnson & Gentry 1971. A total of 19 loci representing 13 enzyme systems plus muscle proteins were consistently interpretable and were used in the analyses reported here (Table 2). We have designated the isozyme systems by the initials of the category of enzymes being studied, followed by a number if there was more than one locus. These numbers are always in the same order: from the most anodic to the most cathodic (for example, *Aat-1* and *Aat-2*). For each polymorphic locus, the most common allozyme has been called 100, others allozymes 110 or 90 if they were faster or slower respectively. Genetic variability (H) and genetic distances (D) were measured according to Nei (1978) and Nei (1972) respectively.

Phylogenetic reconstructions were undertaken using two approaches: using allelic frequencies to calculate (1) genetic distances between populations and (2) in production of character states. These two coding approaches were compared to assess their utility at this taxonomic level.

(1) *Treatment using genetic distances*. Nei's (1972) genetic distance was used to build a distance matrix between samples. This matrix was then treated by KITSCH programme (PHYLIP Package from J. Felsenstein, University of Washington) using a Fitch & Margoliash (1967) algorithm to generate a phenogram (UPGMA) taking account of the distance matrix. This programme assumes the same evolutionary rate in all lineages (molecular clock).

Table 1. System, rivers and localities where the presumptive taxa where caught. *n*: number of specimens studied

Code number	System	River or lagoon	Locality	Presumptive taxa	*n*
1	Comoé	Ebrie lagoon	N'Djen	*C. filamentosus*	4
2		Aby lagoon		*C. filamentosus*	37
3	Volta	Black Volta	Bobo Dioulasso road	*C. auratus longifilis*	4
4	Volta	White Volta	Ouagadougou	*C. auratus longifilis*	22
5	Mono	Mono	Achinedji	*C. filamentosus*	18
6	Mono	Mono	Anié	*C. filamentosus*	6
7	Niger	Niger	Bamako	*C. auratus longifilis*	24
8	Niger	Sankarani	Sélingué	*C. auratus longifilis*	14
9	Niger	Sankarani	Mandiana	*C. auratus longifilis*	16
10	Niger	Niandan	Baro	*C. auratus longifilis*	3
11	Niger	Milo	Boussoulé	*C. auratus longifilis*	5
12	Sénégal	Bakoye	Kokofata	*C. auratus longifilis*	26
13	Sénégal	Baoulé	Missira	*C. auratus longifilis*	24

Table 2. List of enzymes and other proteins examined, EC number, locus, tissue specificity and buffer system used according Pasteur *et al.* (1988): PC 6·3, phosphate-citrate pH 6·3; TG 8·5, tris-glycine pH 8·5; TC 6·7, tris-citrate pH 6·7; TM 6·9, tris malate pH 6·9. Loci are numbered according Agnèse (1989) and Agnèse *et al.* (1989)

Enzyme	EC number	Locus	Buffer	Tissue
Aspartate aminotransferase	2·6·1·1	*Aat-1*	PC 6·3	M
		Aat-2	PC 6·3	M
Adenylate kinase	2·7·4·3	*Ak*	PC 6·3	M
Amylase		*Amy*	TG 8·5	F
Esterase	3·1·1·1	*Es-1*	TM 6·9	F
		Es-2	TM 6·9	F
Glyoxalase	4·4·1·5	*Glo*	TC 6·7	F
Isocitrate dehydrogenase	1·1·1·42	*Idh-1*	TC 6·7	F
		Idh-2	TC 6·7	F
Lactate dehydrogenase	1·1·1·27	*Ldh-1*	TC 6·7	F
		Ldh-2	TC 6·7	F
Malate dehydrogenase	1·1·1·37	*Mdh-1*	PC 6·3	M
		Mdh-2	PC 6·3	M
Nothing dehydrogenase		*Ndh*	TC 6·7	F
6 phosphogluconate dehydrogenase	1·1·1·44	*6Pgd*	TC 6·7	F
Phosphoglucose isomerase	5·3·1·9	*Pgi*	TM 6·9	F
Phosphoglucomutase	2·7·5·1	*Pgm*	TM 6·9	F
Sarcoplasmic protein		*Pt*	PC 6·3	M
Superoxide dismutase	1·15·1·1	*Sod*	TC 6·7	F

(2) *Treatment using character states*. The allelic frequencies matrix is transformed into a presence/absence of alleles matrix: when an allele is present at least once in a sample, it is represented by a '1' in the new matrix; when it is absent, it is represented by a '0'. This new matrix was treated by MIX program (PHYLIP Package), which uses the Wagner parsimony method (Eck & Dayhoff 1966; Kluge & Farris 1969). This programme gives the shortest (the most parsimonious) phylogenetic network, taking account of the presence/absence matrix.

As the MIX programme is sensitive to the order of the populations in the matrix, several treatments were made each time in order to check the stability of the networks obtained.

χ^2 tests were performed to check whether the genotype frequencies were in agreement with Hardy-Weinberg expecta.

Results

Gene frequencies observed for polymorphic loci appear in Table 3. Genetic interpretation of gels was conducted using the knowledge of genetic systems previously studied in fishes. *Aat-1, Aat-2, Sod, Es-2, Ndh, Ldh-1, Ldh-2, Mdh-1, Mdh-2, Glo, Idh-1, Pt, 6Pgd,* and *Ak* were fixed in all samples.

The *Ndh* locus is a separate locus from *Ldh* or *Adh*, two loci often showing activity even when specific substrate is not added, because *Ndh* activity is not affected by Pyruvate or Pyrazole (which inhibited *Ldh* or *Adh* respectively).

One locus, *Amy*, was monomorphic in all samples but with two different alleles: *Amy 100* is observed in all samples except in the Bakoye (12) and in the Baoulé (13) where only *Amy 90* is present.

Two loci were polymorphic at low frequencies, with the more frequent allele being the same in all samples and having a frequency equal to or greater than 95% (except in small samples where the low number of specimens can overestimate the importance of some alleles). These were *Es-1* for which the allele 95 was observed only in Aby lagoon (2) (3%), and *Pgm* for which the allele 90 was scored in the Black Volta (3) (25% four species scored), Sankarani at Sélingué (8) (9%, 11 specimens scored), Sankarani at Mandiana (9) (3%), Niandan (10) (17%, three specimens scored), Bakoye (12) (6%), and Baoulé (13) (3%).

Only two loci showed a high level of polymorphism.

(1) Pgi *with three alleles: 90, 100 and 110*. Populations of Ebrié Lagoon (1), Aby Lagoon (2), Black Volta (3), White Volta (4), Bakoye (12), and Baoulé (13) were 100/100 homozygote. Samples from Mono (5 and 6) were 110/110 homozygote. In the Niger, the three alleles were present; 90 only in the Milo (11) (17%); 100 always the most frequent in all samples; 110 present in all samples except in the Milo.

(2) Idh-2 *with four alleles: 80, 95, 100 and 110*. Samples from the Volta (3 and 4), and Sénégal system (12 and 13) were 95/95 homozygote. The allele 110 has been observed from the two lagoons: Ebrié (1) (25%), Aby (2) (18%). In these two samples, the allele 100 is the most frequent whereas everywhere else it is the allele 95 (except the Milo (11) where 95 and 100 had the same frequency).

Within samples, no significant deviation from Hardy-Weinberg proportions was observed at polymorphic loci.

The mean heterozygosity calculated over 19 loci (Nei 1978) was 0·024. This genetic diversity varied between samples: three samples are monomorphic at all the loci, White Volta (4), Mono at Anié (6), and Baoulé at Missira (13); in general, samples from the Niger drainage (7 to 11) had greater diversity (0·033 to 0·063).

Genetic distances calculated over 19 loci (Table 4) between all examined samples are summarized in a dendrogram (Fig. 2). Samples are not aggregated according to their supposed taxon: *C. filamentosus* (populations 1, 2, 5, 6) and *C. auratus longifilis* (populations 3, 4, 7, 8, 9, 10, 11, 12, 13). A grouping of samples from the Niger system is observed (Niger 7,

Table 3. Allele frequencies at the five polymorphic loci and (H) heterozygosity observed in the 13 populations investigated

		Populations (Code numbers)												
ocus	Alleles	Ebrié (1) $n = 4$	Aby (2) $n = 37$	Volta Black (3) $n = 4$	Volta White (4) $n = 22$	Mono Achinedji (5) $n = 18$	Mono Anié (6) $n = 6$	Niger Bamako (7) $n = 24$	Sankarani Sélingué (8) $n = 14$	Sankarani Mandiana (9) $n = 16$	Niandan Baro (10) $n = 3$	Milo Boussoulé (11) $n = 5$	Bakoye Kokofata (12) $n = 26$	Baoule Missira (13) $n = 24$
.my	90												1·00	1·00
	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00		
s-1	95		0·03											
	98	1·00	0·97	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
lh-2	80		0·03											
	95		0·05	1·00	1·00	0·93	1·00	0·78	0·78	0·77	0·63	0·50	1·00	1·00
	100	0·75	0·74			0·07		0·22	0·22	0·23	0·37	0·50		
	110	0·25	0·18											
gi	90											0·17		
	100	1·00	1·00	1·00	1·00			0·68	0·93	0·88	0·83	0·83	1·00	1·00
	110					1·00	1·00	0·32	0·07	0·12	0·17			
gm	90			0·25					0·09	0·03	0·17		0·06	
	100	1·00	1·00	0·75	1·00	1·00	1·00	1·00	0·91	0·97	0·83	1·00	0·94	1·00
		0·026	0·025	0·026	0·000	0·007	0·000	0·042	0·035	0·034	0·064	0·053	0·006	0·000

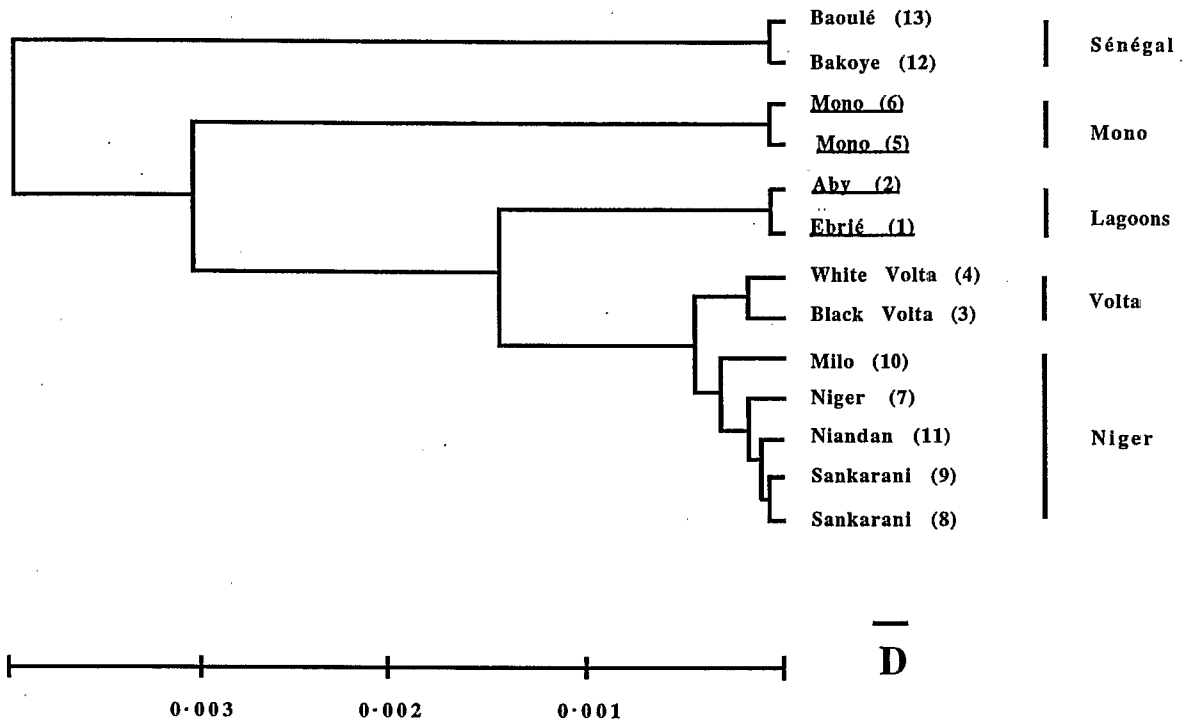

Figure 2. Dendrogram obtained with the KITSCH program (PHYLIP package), from the distances matrix, showing the genetic relationships between the thirteen samples of *Chrysichthys auratus longifilis* and *C. filamentosus* (underlined).

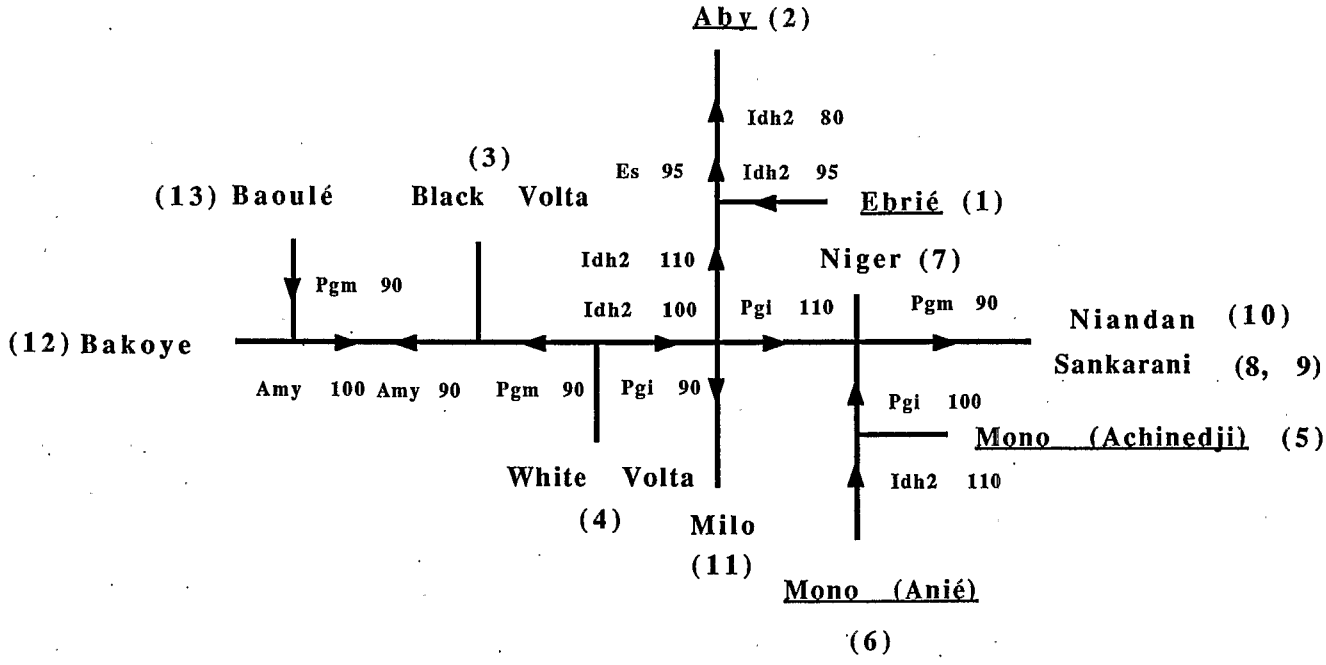

Figure 3. Most parsimonious phylogenetic network obtained with the MIX program (PHYLIP package), from the presence/absence matrix, showing the cladistic relationship between the 13 samples of *Chrysichthys auratus longifilis* and *C. filamentosus* (underlined). Samples from the Sankarani (8 and 9) and the Mono (10) are not differentiated by presence or absence of allele.

Sankarani 8 and 9, Niandan 10 and Milo 11), then samples from the Volta system (Black 3 and White 4), samples from the two lagoons (Ebrié 1 and Aby 2), samples from the Mono system (5 and 6) and then those of the Sénégal system (Bakoye 12 and Baoulé 13).

Figure 3 represents the network obtained using the presence/absence (1/0) matrix. The two samples of the Mono (5 and 6) are grouped with samples from the Niger system (7, 8, 9, 10, 11). Among these, only the sample from the Milo (11) is differentiated (absence of the allele *Pgi 110* and presence of *Pgi 90*). Then there are samples from the two lagoons (1 and 2) characterized by the allele *Idh-2 110*, and samples from the Volta system (3, 4) and the Sénégal system (12, 13) characterized by the presence of *Amy 90* (and the absence of *Amy 100*).

Discussion

Sample clusters in the cladogram are different from those observed in the genetic distance analysis. This is probably on account of the low genetical divergence between some populations. Phenogram is influenced by gene frequencies while cladogram depends on presence or absence of alleles. In our case, at this taxonomic level, the cladistic method seems to be more sensitive that the phenetic one. Nevertheless, genetic distance analysis and cladistical analysis do not show any grouping of the samples into two taxa corresponding to *C. auratus longifillis* and *C. filamentosus*. Samples from the lagoons (1 and 2) and from the Mono (5 and 6) ('*C. filamentosus*') are as divergent to each other as they are from the other samples corresponding to *C. auratus longifilis*. Moreover, the level of genetic differentiation between all these samples is very low ($D_{max} = 0{\cdot}112$) compared with the level of maximal divergence observed between conspecific populations of species of the genus in other studies using the same enzymatic systems: $D = 0{\cdot}289$ in populations of *C. maurus* from the Ivory Coast and Guinea (Agnèse 1989); $D = 0{\cdot}304$ in *C. nigrodigitatus* from Mali (Niger system) and the Ivory Coast (Agnèse, Pasteur & Lévêque 1989). These results confirm those of Risch (1986a), who concluded that there was only one species: *C. auratus*.

Table 4. Nei's genetic distances (1972) between the 13 populations studied

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
(1) Ebrié Lagoon												
(2) Aby Lagoon	0·000											
(3) Black Volta	0·044	0·040										
(4) White Volta	0·048	0·044	0·003									
(5) Mono Achinedji	0·095	0·091	0·055	0·059								
(6) Mono Anié	0·101	0·097	0·054	0·058	0·000							
(7) Niger Bamako	0·032	0·029	0·008	0·012	0·026	0·028						
(8) Sankarani Sélingué	0·027	0·024	0·003	0·004	0·049	0·050	0·004					
(9) Sankarani Mandiana	0·026	0·023	0·004	0·006	0·044	0·045	0·002	0·000				
(10) Niandan Baro	0·020	0·017	0·010	0·009	0·045	0·047	0·004	0·002	0·002			
(11) Milo Boussoulé	0·012	0·010	0·015	0·019	0·058	0·061	0·009	0·006	0·005	0·004		
(12) Bakoye Kokofata	0·102	0·098	0·054	0·057	0·112	0·112	0·064	0·058	0·059	0·066	0·071	
(13) Baoulé Missira	0·101	0·097	0·054	0·058	0·112	0·011	0·064	0·058	0·059	0·066	0·071	0·000

Recently, a survey of the branchial parasites (monogeneans) of the species of the genus *Chrysichthys* has shown that these parasites are specific to their host (Euzet, Agnése & Lambert 1989), i.e. each species of fishes has a special set of parasite species. Populations of *C. auratus* from Ivory lagoons, and the Volta, Niger or Sénégal systems have the same parasites. These results are congruent with ours and those of Risch (1986a).

Nevertheless, it would be interesting to study samples of *C. auratus* from the Tchad and Nile systems to confirm in its totality the taxonomic revision proposed by Risch (1986a), and from rivers of Cameroun and Congo to evaluate genetic diversity of the species throughout its distribution.

In our samples, the average heterozygosity observed ($H = 0{\cdot}024$) is lower than those observed by Nevo, Beiles & Ben-shlomo (1984) for 183 species of fishes ($H = 0{\cdot}051$). Several phenomena can reduce considerably genetic diversity, including small isolated populations and/or severe bottle-necking (when a population has had its effective population size drastically reduced, the population is said to have gone through a bottleneck).

In small isolated populations, low levels of heterozygosity have been observed, for example in cave populations of *Astyanax mexicanus* (Avise & Selander 1972). Nevertheless, populations of *C. auratus* in the systems studied here cannot be considered to be small or isolated populations on a permanant basis because in all the localities investigated, many individuals are fished.

The low heterozygosity observed in the Sénégal system ($H = 0{\cdot}003$) could be explained in part by the annual reduction in habitable space for fauna which results in a considerable reduction of the population sizes of fish species, i.e. a severe bottleneck effect. Indeed, during the dry season (April – June), the Bakoye and Baoulé Rivers become totally dry for hundreds of kilometres from their source. These variations in the effective size of the populations could have reduced heterozygosity by strong stochastic effects (genetic drift). In contrast, the Niger, Volta, and Mono systems, which also have a lower period, never become totally dry. The lagoons are not affected by the dry period.

A similar bottleneck action on variability has been observed by McAndrew & Majumdar (1983) in a population of *Sarotherodon mossambicus* ($H = 0{\cdot}002$) obtained from 10 breeders. Krieg & Guyomard (1985) also supposed that low heterozygosity observed in domestic populations of trout (*Salmo trutta* L.) could have resulted from a bottleneck effect.

Abban & Skibinski (1988) observed a low heterozygosity ($H = 0{\cdot}001$) in *Schilbe mystus* (Siluriform, Schilbeidae), from the Volta system. These authors suggested that the low variability in that species could be the result not only of the regular reduction in habitat space (drying periods), but also of human activities which could directly influence the genetic structure of fish populations: intensive fishing using poisons, especially during low water periods or indirect effects of larvicides deposited in rivers to eliminate populations of *Simulium damnosum*, the vector of Onchocerciasis, which could result in the increased susceptibility of fish to trapping. These explanations might also account for the low variability observed in samples of *C. auratus* from the Volta system. However, with our results and those of Abban & Skibinski (1988), it is difficult to evaluate exactly what caused the low variability observed. The use of other genetical techniques such as, for example, the study of mitochondrial DNA using restriction enzymes (Avise, Reeb & Saunders 1987) or sequencing (Desmarais 1989), might be useful for a better understanding of genetical diversity of African fish species.

Acknowledgments

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