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Morphometric and allozyme variation in the African catfishes *Clarias gariepinus* and *C. anguillaris*

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This study investigated morphological characters and electrophoretic polymorphism at 25 protein loci in nine wild populations of the African clariid catfish *Clarias gariepinus* and seven wild populations of *C. anguillaris*. Two other clariid species, *Clarias albopunctatus* and *Heterobranchus longifilis*, were used as outgroups in the allozyme study. Morphometric and allozyme data are congruent for the Nilo-Sudanian populations of *C. gariepinus* and *C. anguillaris*. Both approaches also distinguished two groups amongst the *C. gariepinus* populations, one containing Nilo-Sudanian populations and the other including Lake Victoria and southern African populations. However, allozyme data suggest that *C. gariepinus* is not a monophyletic group and show that *C. albopunctatus* is more divergent from *C. gariepinus* and *C. anguillaris* than it is from *H. longifilis*, stressing the need for a revision of clariid systematics. The variation observed in *C. gariepinus* is discussed in terms of palaeogeographical events and its use in aquaculture. © 1998 The Fisheries Society of the British Isles

Key words: Africa; Clariidae; morphometry; allozymes; variation.

INTRODUCTION

Clariidae or walking catfishes occur naturally in Asia Minor, Africa and South-east Asia. They are recognized by an elongated body with spineless and long dorsal and anal fins, four pairs of circumoral barbels and especially by the presence of a suprabranchial airbreathing organ. At present, 14 genera including 92 species are known (Teugels, 1996).

Some clariid species are of great economic importance in fisheries and are intensively used in fish culture in many parts of the world. *Clarias gariepinus* (Burchell, 1822) is one of them. Its natural geographical distribution ranges from southern Turkey to the Orange River in South Africa. In a systematic revision of the African species of the genus *Clarias*, Teugels (1986) placed this species in the nominate subgenus *Clarias* (*Clarias*), together with *C. anguillaris* (Linnaeus, 1758). Except for central and southern Africa, both species have an almost sympatric distribution. They are morphologically very similar, and the

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only character that can be used to identify them easily is the number of gill rakers on the first branchial arch: 24–110 in *C. gariepinus* and 16–50 in *C. anguillaris* (Teugels, 1982, 1986). In both species, and especially in *C. gariepinus*, this number is related to size; clinal variation has also been reported for this characteristic (Teugels, 1982). If gill raker number is affected by environmental variation, its discriminating validity may be questionable.

To obtain genetic evidence for the validity of the two species, Teugels *et al.* (1992) examined electrophoretic variation at 13 protein loci in two West African populations of both species. The results showed that sympatric populations were differentiated significantly and that morphological and genetic clustering were congruent. More recently, Agnèse *et al.* (1997) described genetic variation at 25 protein loci, eight microsatellite loci and two mtDNA segments in two sympatric samples from the Senegal River. The three approaches confirmed that both species were genetically closely related (Nei's standard genetic distance based on 25 loci=0·16).

This paper extends the morphometrical and the allozyme study to nine populations of *Clarias gariepinus* and seven populations of *C. anguillaris* sampled throughout the distributional ranges of these species in order to quantify their intra- and interspecific variation and to retrace the genetic relationships between populations of both species over a large geographical scale.

MATERIALS AND METHODS

SAMPLING

Table I lists populations of *C. gariepinus* and *C. anguillaris* that were sampled, from localities illustrated in Fig. 1. All specimens examined were deposited at the Musée Royal de l'Afrique Centrale, Tervuren, Belgium, except for the sample from South Africa, which was not preserved. Species identifications followed Teugels (1986). Two other clariid catfishes, *Heterobranchus longifilis* Valenciennes, 1840 and *C.* (*Clarioides*) *albopunctatus* Nichols & La Monte, 1953 were used for outgroup comparison in the allozyme study. Their identification was based on Teugels *et al.* (1990) and Teugels (1986), respectively.

MORPHOMETRY

In the morphometric analysis, 13 measurements were made with dial callipers on each specimen following Agnèse *et al.* (1997). Measurements included: standard length, head length, interorbital width, occipital process length, occipital process width, premaxillary toothplate width, vomerine toothplate width, predorsal length, preanal length, prepelvic length, prepectoral length, dorsal-fin length and anal-fin length. For each specimen, the number of gill rakers on the complete first branchial arch was counted. Results obtained were log transformed and subjected to principal component analysis using the covariance matrix (STATISTICA package: Statsoft inc., v. 3.1 and v. 5.0). To minimize the effect of size differences between samples, the first component, which is considered to be the size factor as suggested by Humphries *et al.* (1981) and Bookstein *et al.* (1985), was not used.

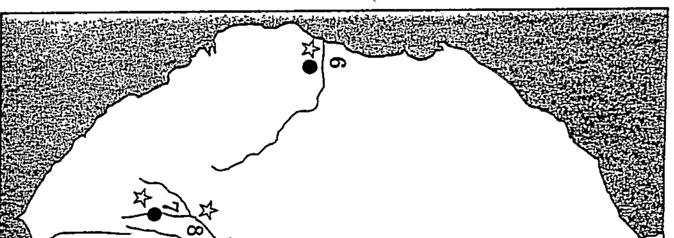
ENZYME ELECTROPHORESIS

Twenty-five loci representing 16 enzyme systems (Agnèse *et al.*, 1997) were scored by starch gel electrophoresis. Tissue extraction, migration buffer and staining procedures are described by Guyomard & Krieg (1983) and Krieg & Guyomard (1985). The locus and allele nomenclature recommended by Shaklee *et al.* (1990) were used. Exact tests for Hardy–Weinberg equilibrium, genotypic linkage disequilibrium and genetic

Table I. Origin (river system and location) and size of the 18 samples of African catfish

Species	Origin	Population codes	Sample size M/A*	Location number
Clarias (*Clarias*) *gariepinus*	Nile Basin			
	Lake Manzala (Nile delta, Egypt)	Manz G	37/20	1
	Chobra (Cairo, Egypt)	Chob G	9/10	2
	Lake Victoria (Kendu Bay, Kenya)	Victo G	23/34	3
	Orange Basin			
	Orange River (Vanderkloof Dam, South African Republic)	SoAf G	—/30	4
	Komati Basin			
	Sand River Dam (Swaziland)	Swaz G	9/—	5
	Senegal Basin			
	Senegal River (Dagana, Senegal)	Sene G	10/17	6
	Niger Basin			
	Sankarani River (Selingue, Mali)	Seli G	2/2	7
	Chad Basin			
	Chari River at Ndjamena (Chad)	Djam G	13/15	9
	Chari delta at Hadide (Chad)	Had G	3/—	10
Clarias (*Clarias*) *anguillaris*	Senegal Basin			
	Senegal River (Dagana, Senegal)	Sene A	25/32	6
	Niger Basin			
	Sankarani River (Selingue, Mali)	Seli A	25/30	7
	Niger River (Bamako, Mali)	Bama A	9/10	8
	Chad Basin			
	Chari River at Ndjamena (Chad)	Djam A	4/5	9
	Chari delta at Hadide (Chad)	Had A	1/—	10
	Ebrie Lagoon			
	Layo, 1993 (Ivory Coast)	Layo1 A	14/15	11
	Layo, 1994 (Ivory Coast)	Layo2 A	/17	11
Heterobranchus longifilis	Ebrie Lagoon			
	Layo (Ivory Coast)	Heter	—/13	11
C. (*Clarioides*) *albopunctatus*	Chad Basin			
	Chari delta at Hadide (Chad)	Albo	—/33	10

*M, Morphometric; A; allozymes.



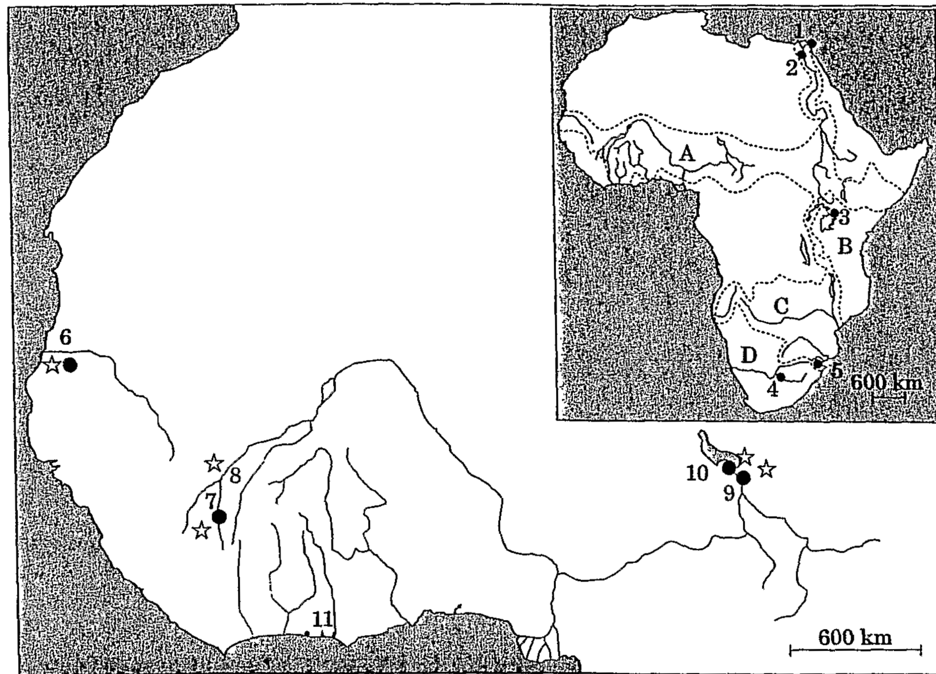


FIG. 1. Geographical locations of *Clarias gariepinus* (●) and *C. anguillaris* (☆) populations sampled. Collection sites are numbered as indicated in Table I. The ichthyofaunal provinces sampled in this study are: A, Nilo-Sudan; B, East Coast; C, Zambesi; D, Cape of Good Hope.

differentiation among populations were performed using the program GENEPOP (Raymond & Rousset, 1995). Unbiased estimates and standard deviation of gene diversity and standard genetic distances were calculated according to Nei (1987) using BIOSYS-1 (Swofford & Selander, 1989). Phenograms were generated using PHYLIP (Felsenstein, 1993) with the UPGMA cluster analysis (Sneath & Sokal, 1973) and the restricted maximum likelihood (ML) method (Felsenstein, 1973). Bootstrap values were computed over 1000 replications. The coefficient of population differentiation (G_{ST}) was estimated as described in Chakraborty & Leimar (1987). A bias correction of $1/2\check{N}$, where $\check{N}$ is the harmonic mean over population sizes (Chakraborty & Leimar, 1987) was applied.

RESULTS

MORPHOMETRIC VARIATION

Two groups (Fig. 2) can be distinguished by the number of gill rakers on the first branchial arch: in the first, identified as *C. anguillaris*, gill raker number increased slightly with the standard length; in the second group, identified as *C. gariepinus*, the number of gill rakers increased considerably with increasing standard lengths. The small-sized specimens from Lake Victoria were identified as *C. gariepinus* because no large specimens with a reduced number of gill rakers (i.e. *C. anguillaris*) have ever been reported from this lake (Teugels, 1986).

The results of the morphometric analysis for the different populations of *C. anguillaris* are presented in Fig. 3. As no difference could be found between specimens from Selingue and Bamako in Mali, they were considered as one group in this analysis. The same was done for specimens from Hadide

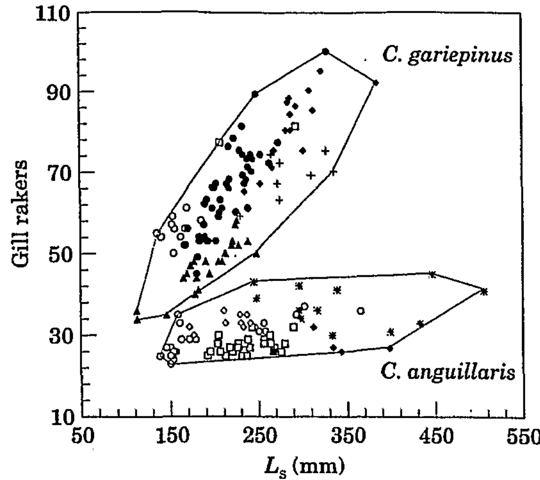


FIG. 2. Number of gill rakers on the first branchial arch in relation to the standard length (L_S) for all specimen examined of *C. gariepinus* and *C. anguillaris*. ○, Senegal; ☐, Selingue; ◇, Bamako; △, Cairo; ●, Lake Manzalla; ■, Hadide; ◆, Ndjamena; ▲, Lake Victoria; +, Swaziland; *, Layo.

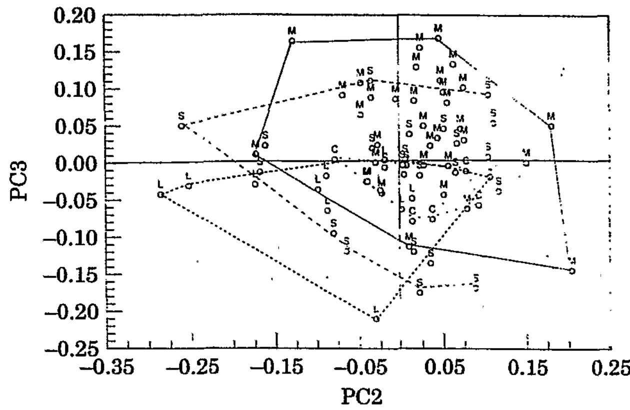


FIG. 3. Plot of second and third axis of a principal component analysis using 13 log-transformed metric variables for four populations of *C. anguillaris*. S, Senegal; M, Mali; C, Chad; L, Ivory Coast.

and Ndjamena in Chad. The four remaining populations almost completely overlapped and could not be distinguished from each other either on the second or on the third component. The small size of the polygon from Chad is undoubtedly related to the small sample size (n=5).

For *C. gariepinus*, the specimens from Hadide and Ndjamena in Chad were considered as a single group. In the same way, the specimens from Chobra and Lake Manzala in Egypt completely overlapped and were also considered to represent a single group. In contrast to *C. anguillaris*, the populations of *C. gariepinus* (Fig. 4) display a considerable morphometric variation. Most surprisingly, the population from Egypt, situated on the negative sector of the second component, is entirely separated from the Lake Victoria one, completely located on the positive sector of the second component. This second component

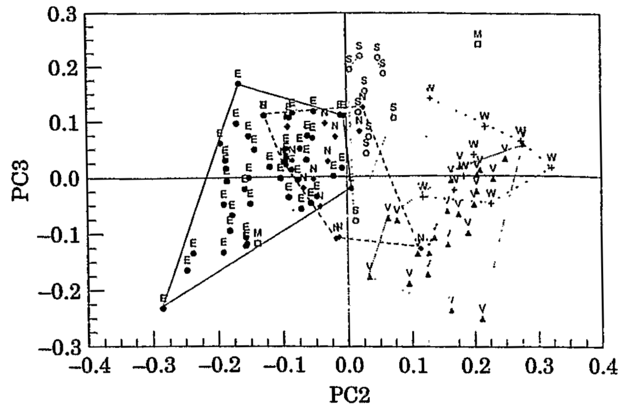


FIG. 4. Plot of second and third axes of principal component analysis using 13 log-transformed metric variables for five populations of *C. gariepinus*. S, Senegal; M, Mali; N, Chad; E, Egypt; W, Swaziland; V, Lake Victoria.

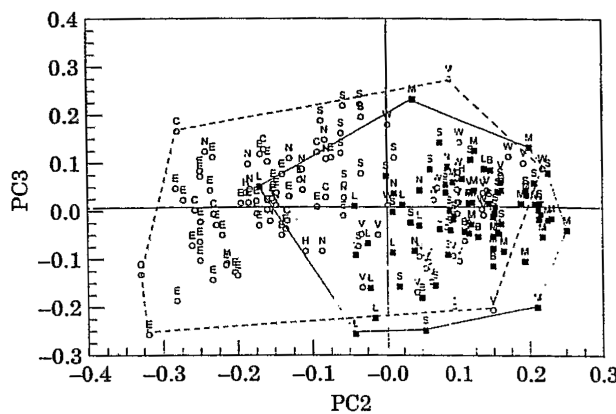


FIG. 5. Plot of second and third axes of principal component analysis using 13 log-transformed metric variables for all specimens examined of *C. anguillaris* (■: ——) and *C. gariepinus* (○: – – –). B, Bamako; C, Cairo; E, Lake Manzala; H, Hadide; L, Côte d'Ivoire; M, Selingue; N, Chad; S, Senegal; V, Lake Victoria; W, Swaziland.

is merely defined by the width of the premaxillary toothplate, the width of the occipital process, the length of the occipital process and the dorsal-fin length. Populations from Senegal and Chad mostly overlap, and an important overlap was also noted between the populations from Egypt and Chad. The Lake Victoria population partly overlapped with the population from Swaziland, and the partial overlap with the Chad population should also be noted.

An overall morphometric analysis of all populations examined, showed that both species completely overlapped (Fig. 5), but interestingly almost all *C. anguillaris* overlapped with the *C. gariepinus* specimens from Lake Victoria and Swaziland located on the positive sector of the second PCA component. In this analysis, the second component was defined by the width of the premaxillary toothplate, the width of the occipital process, the dorsal-fin length and the anal-fin length.

ALLOZYME VARIATION

Out of 25 loci scored, 23 were polymorphic (Table II). Only one significant deviation from the Hardy–Weinberg (H–W) equation was found at the 5% level (P=0·015) out of 80 probability tests. Multiple tests by locus and by population using Fisher's method did not yield any significant deviation from H–W (P-values >0·49). Exact tests for linkage disequilibrium between loci gave nine significant values at the 5% level out of 472 tests done (about 24 significant tests were expected on the basis of type I error). Significant tests were distributed randomly across populations. No multiple test by pair of loci was significant.

Eighteen and 16 loci exhibited variation in *C. gariepinus* and *C. anguillaris* populations, respectively, with the number of alleles ranging from two to seven. Only four loci exhibited variation in *C. albopunctatus* and in *H. longifilis* populations, with two or three alleles at these loci. More than 10 loci were diagnostic between *C. albopunctatus* and *H. longifilis* (12 loci), *C. gariepinus* (11) or *C. anguillaris* (13). Six discriminating loci were found between *H. longifilis* and *C. anguillaris* or *C. gariepinus*, and none between *C. anguillaris* and *C. gariepinus*. However, these two species could be distinguished genetically by the occurrence of private alleles and large allele frequency differences. In the Sudanian sampling sites (i.e. Senegal, Chad and Niger Basins) where both species occur sympatrically, the individual genotypes were consistent with the morphological phenotypes.

All populations were polymorphic (Table II). Polymorphism (under the 0·95 criterion) ranged from 12 to 48% in *C. gariepinus*, and from 16 to 28% in *C. anguillaris*. Gene diversities within populations ranged from 5·3% to 15·4% in *C. gariepinus* with an average value of 11·2% and from 4·6 to 9·1% in *C. anguillaris* with an average value of 7·5%. *Heterobranchus longifilis* and *C. albopunctatus* exhibited lower values (8 and 12% for the polymorphism rate and 3·3 and 4·1% for the observed heterozygosity, respectively).

Nei's standard genetic distances (Table III) ranged from 0·008 (Manz G and Chob G) to 0·29 (SoAf G and Seli G) in *C. gariepinus*. In *C. anguillaris*, they varied from 0·005 (Layo1 A and Layo2 A) to 0·043 (Layo1 A and Djam A). The mean genetic distance were 0·207 ± 0·081 between both species and 0·147 ± 0·075 and 0·022 ± 0·012 within *C. gariepinus* and *C. anguillaris*, respectively. The largest divergence was between *C. albopunctatus* and the other three species (genetic distances ranging from 0·713 to 1·232). *Heterobranchus longifilis* appeared to be more closely related to *C. gariepinus* and *C. anguillaris* than to *C. albopunctatus*. Genetic differentiation tests were highly significant (P<0·001) for all pairs of populations, except for Layo 1 and 2.

A UPGMA phenogram derived from standard Nei's genetic distances (Fig. 6) showed that *C. gariepinus* populations did not form a single cluster compared to *C. anguillaris*. One group, including Lake Victoria and South African populations, was substantially divergent from the others. The remaining *C. gariepinus* populations formed a single cluster with respect to *C. anguillaris*. The congruence between tree topology and hydrographical origin was high. Note that the topology was not supported by high bootstrap values, except for the *C. anguillaris* cluster. The Lake Victoria and the South African populations also clustered together and diverged from all the other samples in the ML tree (results not shown). Finally, in all cases *C. albopunctatus* branched first, followed by

TABLE II. Allele frequencies at polymorphic loci (common allele, *100, omitted), polymorphism (P95) and average heterozygosity (*H*) rates in 15 populations of African catfishes; population codes are given in Table I, CK-2 and SOD-1 are monomorphic

Locus	Allele	Manz G	Chob G	Victo G	SoAf G	Sene G	Seli G	Djam G	Sene A	Seli A	Bama A	Djam A	Layo1 A	Layo2 A	Heter	Albo
*AAT-1**	*90	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
*AAT-2**	*0	0·025	0·050	—	—	—	—	—	—	—	—	—	—	—	—	—
	*150	—	—	0·059	—	—	—	—	—	—	—	—	—	—	—	1
	*160	—	—	—	0·297	—	—	—	—	—	—	—	—	—	—	—
*AK**	*75	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
CK-1	*115	—	—	0·956	—	0·059	—	—	0·946	1	1	1	1	1	—	—
*FBP**	*0	—	—	—	—	—	—	—	0·018	—	—	—	—	—	—	—
	*200	0·028	—	0·036	—	—	—	—	—	—	—	—	—	0·094	—	—
	*300	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
*FH**	*0	—	0·150	—	—	—	—	0·367	—	—	—	—	—	—	—	—
	*15	—	—	0·613	—	—	—	—	—	—	—	—	—	—	—	—
	*20	0·075	—	—	—	—	—	—	—	—	—	—	—	—	—	0·061
	*65	—	—	—	—	—	—	—	—	—	—	—	—	—	0·038	—
	*80	—	—	0·387	0·983	—	—	—	—	—	—	—	—	—	—	—
	*120	0·925	0·850	—	0·017	0·882	1	0·267	0·016	0·017	0·400	—	0·100	0·063	—	—
*G3PDH**	*50	—	—	—	0·103	—	—	—	—	0·018	—	—	—	—	—	—
	*150								—	—	—	—	—	—	1	1
	*300	0·028	—	—	—	—	—	—	—	—	—	—	—	—	—	—
*GPI-1**	*0	0·050	0·100	—	0·065	0·294	—	0·400	0·016	0·017	—	0·100	—	—	—	—
	*200	—	—	0·250	0·016	—	—	0·067	—	—	—	—	—	—	—	—
*GPI-2**	*25	0·050						—	—	—	—	—	—	—	—	—
	*50	0·775	0·900	—	0·813	—	—	0·067	—	—	—	—	0·033	—	1	—
	*75	0·025	—	0·015	—	—	—	—	0·034	—	—	—	—	—	—	—
	*110	—	—	—	—	0·094	—	0·033	—	0·050	—	—	—	—	—	—
	*210	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·984
*IDHP-1**	*90	—	0·100			—	—	—	—	—	—	—	—	—	—	—
	*120	—	—	—	—	—	—	—	—	—	—	—	0·033	0·059	—	—

Table II. *Continued*

Locus	Allele	Manz G	Chob G	Victo G	SoAf G	Sene G	Seli G	Djam G	Sene A	Seli A	Bama A	Djam A	Layo1 A	Layo2 A	Heter	Albo
*IDHP-2**	*40	—	—	—	—	0·118	—	—	—	—	—	—	—	—	—	—
	*60	0·105	0·150	0·030	—	—	—	—	—	—	—	—	—	—	0·667	—
	*87	—	—	—	—	0·029	—	—	—	—	—	—	—	—	—	—
	*110	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
	*115	—	—	—	—	—	—	—	0·016	—	—	—	—	—	—	—
	*125	0·368	0·550	—	—	0·147	—	0·071	—	—	—	—	—	—	—	—
*LDH-1**	*0	—	0·050	—	—	—	—	—	—	—	0·050	—	—	—	—	—
	*200	—	—	—	—	—	—	—	—	—	—	0·100	—	—	—	1
	* − 160	—	—	—	0·982	—	—	—	—	—	—	—	—	—	—	—
*LDH-2**	*50	1	1	1	1	0·971	1	1	0·328	0·333	0·350	—	0·367	0·324	—	—
*MDH-1**	*200	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
*MDH-2**	*83	0·375	0·250	—	—	0·563	1	0·467	1	1	1	1	1	1	—	—
*MDH-3**	*20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
	*57	—	—	—		0·176	—	—	—	—	—	—	—	—	—	—
	*66	—	—	—	—	0·118	—	—	0·438	—	—	—	—	—	—	—
	*105	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
*MEP-1**	*30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·133
	*50	—	—	0·015	—	—	—	—	—	0·017	—	—	—	—	0·038	—
	*60	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·534
	*70	—	—	—	—	—	—	—	—	—	—	—	—	—	0·962	—
	*80	—	0·050	0·924	0·900	0·433	—	0·067	0·100	0·200	0·050	0·100	0·679	0·571	—	—
	*90	—	—						—	—	—	—	—	—	—	0·333
	*110	0·029	—	—	—	0·030	—	—	—	—	—	—	—	—	—	—
	*120	—	—	—	—	—	—	0·100	—	—	—	—	—	—	—	—
*MEP-2**	*105	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1

Table II. *Continued*

Locus Allele Manz G Chob G Victo G SoAf G Sene G Seli G Djam G Sene A Seli A Bama A Djam A Layo1 A Layo2 A Heter Albo

*MEP-2**	*105	—	—	—	—	—	—

TABLE II. *Continued*

Locus	Allele	Manz G	Chob G	Victo G	SoAf G	Sene G	Seli G	Djam G	Sene A	Seli A	Bama A	Djam A	Layo1 A	Layo2 A	Heter	Albo
*MPI**	*67	—	—	—	—		—	—	—	0·017	—	—	—	—	—	—
	*83	—	—	0·242	—	—	—	—	—	—	—	—	—	—	—	—
	*105	0·100	—	—	—	0·235	0·250	0·100	—	—	—	—	—	0·300	—	—
*PGDH**	*10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·037
	*30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·852
	*50	0·150	—	—	—	0·059	—	0·300	0·031	0·103	0·050	—	—	—	—	0·111
	*67	—	—	—	—	—	—	—	—	—	0·050	—	—	—	—	—
	*75	0·725	0·950	1	1	0·676	0·250	0·533	0·406	0·362	0·300	—	0·500	0·500	—	—
	*85	—	—	—	—	—	—	—	0·016	—	0·050	—	—	—	1	—
*PGM**	*50	—	—	0·015	—	—	—	—	—	—	—	—	—	—	—	—
	*80	0·025	—	0·632	—	—	—	—	—	—	—	—	—	—	0·885	1
	*112	0·025	0·050	—	—	—	—	—	0·172	0·067	0·050	—	—	—	—	—
*SOD-2**	*20	0·125	0·250						—	—	—	—	—	—	—	—
	*50	0·050	0·050	—	—	—	—	—	—	—	—	—	—	—	—	—
	*55	0·150	0·150	1	1	—	—	—	—	—	—	—	—	—	—	—
	*117	0·600	0·500				0·250	0·767	-	0·050	0·050	0·250				1
*XDH**	*105												—	—	—	1
H (%)		13	12·2	8·9	5·3	15·4	9	14·3	9·1	7·4	8·1	4·6	7·1	9·3	3·3	4·1
P95 (%)		36	48	28	20	40	12	36	20	24	28	16	16	28	8	12

TABLE III. Nei's standard genetic distances between the populations of *C. gariepinus*, *C. anguillaris*, *H. longifilis* and *C. albopunctatus*

	Manz G	Chob G	Victo G	SoAf G	Sene G	Seli G	Djam G	Sene A	SeliA	Bama A	Djam A	Layo1 A	Layo2 A	Heter
Chob G	0·0077													
Victo G	0·2239	0·2287												
SoAf G	0·1760	0·1695	0·1625											
Sene G	0·0809	0·0994	0·1873	0·2144										
Seli G	0·0860	0·1244	0·2660	0·2875	0·0394									
Djam G	0·0606	0·0804	0·1883	0·2053	0·0575	0·0650								
Sene A	0·2227	0·2550	0·2397	0·3709	0·1274	0·1231	0·1478							
Seli A	0·2115	0·2445	0·2183	0·3476	0·1256	0·1168	0·1355	0·0099						
Bama A	0·1820	0·2167	0·2229	0·3506	0·1064	0·0851	0·1278	0·0164	0·0080					
Djam A	0·2440	0·2849	0·2812	0·4097	0·1881	0·1445	0·1577	0·0339	0·0226	0·0282				
Layo1 A	0·2236	0·2520	0·1867	0·3103	0·1143	0·1293	0·1497	0·0253	0·0111	0·0227	0·0432			
Layo2 A	0·2315	0·2645	0·1985	0·3324	0·1183	0·1277	0·1531	0·0248	0·0112	0·0230	0·0413	0·0050		
Heter	0·5212	0·5232	0·6392	0·6186	0·5310	0·6188	0·5602	0·5115	0·5282	0·5514	0·5634	0·5321	0·5476	
Albo	1·1448	1·1676	1·1773	1·2087	1·1883	1·2323	1·0453	1·1255	1·1392	1·1776	1·0465	1·1622	1·1802	0·7133

Population codes are given in Table I

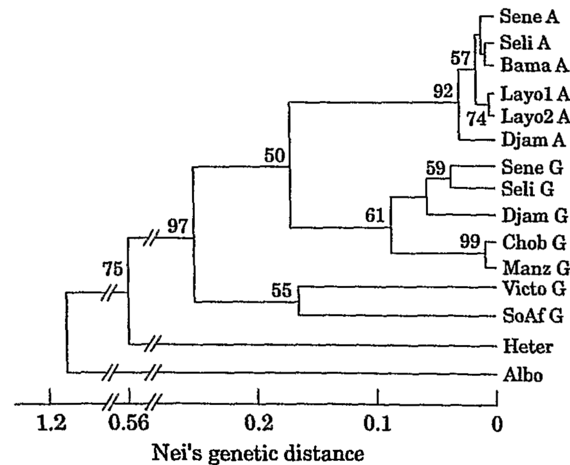


FIG. 6. UPGMA tree derived from standard Nei's genetic distances between *C. gariepinus*, *C. anguillaris*, *H. longifilis* and *C. albopunctatus* samples. Bootstrap values estimated from 1000 replications are reported when higher than 50%.

H. longifilis and, the cluster grouping both *C. gariepinus* and *C. anguillaris* populations, which is supported by a high bootstrap value (97%).

Corrected G_{ST} values were approximately 0·15 in *C. anguillaris* (Layo 1 and 2 were pooled), 0·44 with and 0·20 without the Lake Victoria and South African populations in *C. gariepinus*.

DISCUSSION

PARTIAL CONGRUENCE BETWEEN MORPHOMETRIC AND ALLOZYME DATA

The allozyme and morphometric data are congruent on several points. They confirm the occurrence of two distinct sympatric species in the Nilo-Sudanian region: *C. anguillaris* and the Nilo-Sudan populations (including West Africa and Nile regions) of *C. gariepinus*. They also point out that differentiation between populations is rather low in this area. Climatological and geological events during the Late Quaternary largely explain their uniformity (Roberts, 1975; see below). Both approaches also distinguish the Nilo-Sudanian populations of *C. gariepinus* from the Lake Victoria and southern African populations (Swaziland in the morphometric analysis, South Africa in the allozyme study).

However, the two approaches show a major discrepancy: In the morphometric study (measurements and gill raker counts) two groups, *C. anguillaris* and *C. gariepinus*, can be discerned, while in the allozyme study the *C. gariepinus* populations do not form a monophyletic group with respect to *C. anguillaris* since this species separates clearly the Nilo-Sudanian populations of *C. gariepinus* from the Lake Victoria and South African ones. Although the clusters obtained with the allozyme data do not show high bootstrap values, this topology is supported by preliminary mtDNA RFLP data (Krieg & Guyomard, unpubl. data). If this topology is correct, the validity of the high number of gill rakers to assign all the *C. gariepinus* populations to a single species with respect to the other *Clarias* species is questionable. It is noteworthy that large numbers

of gill rakers can also be found in some other clariid species (Teugels, 1983; Teugels *et al.*, 1990). Nevertheless, this character remains valid for the identification of sympatric populations in the Nilo-Sudanian region. Even if the gill raker number is not included, allozyme and morphometric data do not show the same trend. The former set of data clusters all the Nilo-Sudanian populations, while in the morphometric analysis, *C. anguillaris* seems to be closer to the Lake Victoria and Swaziland populations. Further research including additional characters and populations, in particular those originating from the area between Lake Victoria and the Orange River, is required to understand the discrepancy between morphometric and genetic data and to clarify the phylogenetic relationships between *C. gariepinus* and *C. anguillaris*.

Our allozyme data also show that *C.* (*Claroides*) *albopunctatus* is more divergent from *C.* (*Clarias*) *gariepinus* and *C.* (*Clarias*) *anguillaris* than is *H. longifilis*. In a similar study, Teugels *et al.* (1992) showed that *C.* (*Anguilloclarias*) *ebriensis* is also less closely related to *C.* (*Clarias*) *anguillaris* and *C.* (*Clarias*) *gariepinus* than *H. longifilis*. These findings should be paralleled with the fact that the subgenera *C.* (*Clarias*) and *C.* (*Dinotopteroides*) appear to be closely related to *H. longifilis* for some morphological and osteological features (Teugels, 1983; Teugels *et al.*, 1990). This confirms the need for a revision of clariid systematics involving both morphological and genetic approaches and is the subject of forthcoming research.

INTRASPECIFIC RELATIONSHIPS AND PALEOGEOGRAPHY

The ichthyofauna of tropical Africa has been relatively uniform with regard to its geographical distribution, at least until the Miocene (Roberts, 1975; Beadle, 1981); some species, including *Clarias* spp., were widely distributed. After the Miocene, tectonic movements led to the formation of the Rift Valleys and important changes of the hydrographic systems in this area. The Rift Valleys have resulted in particular in the isolation of two distinct regions which differ greatly in their present fish fauna composition: the Nilo-Sudan and the East coast ichthyofaunal provinces, this second province including Lake Victoria (Roberts, 1975). Since the level of genetic variation within populations is high in *C. gariepinus*, it can be assumed that the large genetic divergence found between the Nilo-Sudanian and Lake Victorian populations did not result from a recent founder effect, but appears to reflect an ancient divergence, probably due to the separation of the two provinces. If one assumes a molecular clock hypothesis and apply the substitution rate proposed by Gorman *et al.* (1976) for allozymes to our data, one obtains a coalescence time of 3·9 Myear between Lake Victoria and Nilo-Sudanian populations. This value is roughly in agreement with the dating of the formation of the Rift Lakes (Beadle, 1981).

The partial morphometric overlapping and genetic clustering of the Swaziland or Orange River Basin populations and the Lake Victoria population could suggest that they descended from a common ancestor, different from the Nilo-Sudanian one. This hypothesis is plausible since the ichthyofaunae of the East Coast and Zambesi provinces are closely related. *Clarias gariepinus* is assumed to have invaded the Cape Province from the Zambesi Basin (Roberts, 1975). However, this cluster is not supported by very high bootstrap values and

an alternative scenario of colonization of the Cape Province from the Zaire Basin *via* the Zambesi or Cunene Rivers cannot be excluded (Roberts, 1975).

Climatological and geological events during the recent Quaternary are likely to explain most of the geographical variation observed in morphological characters and protein loci for the Nilo-Sudanian populations of both species. During the pluvial phases of this period, lakes and rivers expanded dramatically over most of the Nilo-Sudanian region and connections were established between the hydrographic basins of this region (Roberts, 1975; Beadle, 1981; Grove, 1985). Presently, the upper reaches of the major basins of the major West African river systems (i.e. Senegal, Niger and Chad) are in contact during heavy rainy seasons (Beadle, 1981). These connections may have allowed individuals to migrate from one basin to another, as indicated by the lack of substantial differentiation between populations and the high level of gene diversity within populations. It is noteworthy that the pattern of differentiation of *C. gariepinus* populations in the Nilo-Sudanian region probably reflects the chronology of separation between the different basins of this region (Roberts, 1975; Beadle, 1981; Grove, 1985), as supported by the similar pattern of differentiation among tilapia species (Rognon *et al.*, 1996).

IMPLICATION FOR THE DOMESTICATION OF *C. GARIEPINUS*

Despite the relatively small number of natural populations of *C. gariepinus* examined here, this species already exhibits a very high level of polymorphism and a strong geographical structuring of its genetic diversity. Other divergent populations may be identified when additional populations, particularly in other ichthyofaunal provinces, are investigated.

Domesticated stocks of *C. gariepinus* have been founded and propagated in various countries for aquaculture. Despite the increasing commercial importance of this species, little is known about its gene diversity since few of these cultured stocks have been analysed (Teugels *et al.*, 1992; Van der Bank *et al.*, 1992). The rational utilization of genetic resources requires an assessment of their genetic diversity in comparison with wild stocks. This can be achieved with the methods used here and also more powerful molecular techniques recently developed (Galbusera *et al.*, 1996). Depending on the results of these investigations, the founding of new fish-farmed stocks or the restoration of genetic variation in existing ones may be warranted. However, cultured stocks should be established without transferring fish between ichthyofaunal provinces, in order to avoid genetic contamination of the native gene pool.

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