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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.

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, preparal 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
Clarias (Clarias) gariepinus	Nile Basin Lake Manzala (Nile delta, Egypt) Chobra (Cairo, Egypt) Lake Victoria (Kendu Bay, Kenya)	Manz G Chob G Victo G	37/20 9/10 23/34	3 3 3
	Orange Basin Orange River (Vanderkloof Dam, South African Republic) Komati Basin	SoAf G	130	4 (
	Sand Kiver Dam (Swaziland) Senegal Basin Senegal River (Dagana, Senegal)	Swaz G	10/17	9
	Sankarani River (Selingue, Mali) Chao Basin	Seli G	2/2	L
Clarias (Clarias) anguillaris	Chari River at Ndjamena (Chad) Chari delta at Hadide (Chad) Senegal Basin	Djam G Had G	3/—	0 0
	Senegal River (Dagana, Senegal) Niger Basin	Sene A	25/32	9
	Sankarani River (Selingue, Mali) Niger River (Bamako, Mali) Chad Basin	Seli A Bama A	25/30 9/10	L 88
	Chari River at Ndjamena (Chad) Chari delta at Hadide (Chad)	Djam A Had A	4/5	9 10
Hoterohrandus longiflis	Layo, 1993 (Ivory Coast) Layo, 1994 (Ivory Coast) Ebrie 1 agon	Layol A Layo2 A	14/15	==
C (Clarioides) alhommetatus	Layo (Ivory Coast)	Heter	-/13	11
	Chari delta at Hadide (Chad)	Albo	-/33	10

^{*}M, Morphometric; A; allozymes.

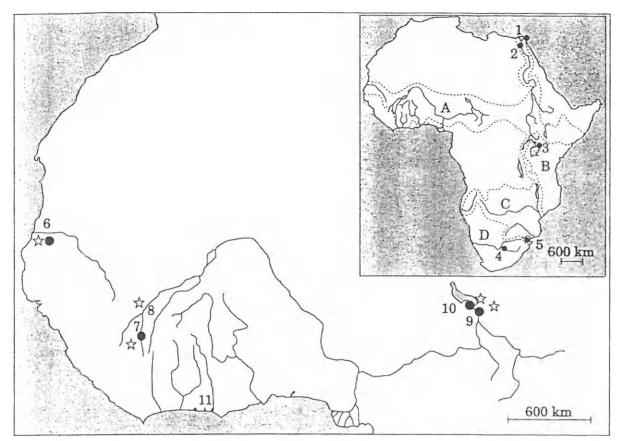


Fig. 1. Geographical locations of Clarias gariepinus (•) and C. anguillaris (\$\phi\$) populations sampled. Collection sites are numbered as indicated in Table I. The ichthyofaunal provinces sampled in this study are: A, Nilo-Sudan; B, East Coast; C, Zambesi; D, Cape of Good Hope.

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 1/2N, where 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

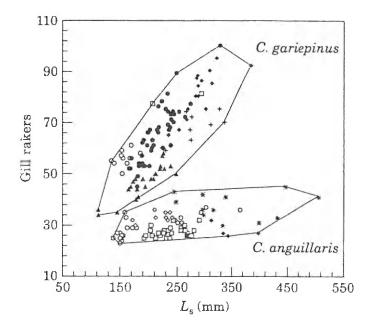


Fig. 2. Number of gill rakers on the first branchial arch in relation to the standard length (L_S) for all specimen examined of C gariepinus and C anguillaris. \bigcirc , Senegal: \square , Selingue; \diamondsuit , Bamako; \triangle , Cairo; \blacksquare , Lake Manzalla; \blacksquare , Hadide; \spadesuit , Ndjamena; \blacktriangle , Lake Victoria; +, Swaziland; *, Layo.

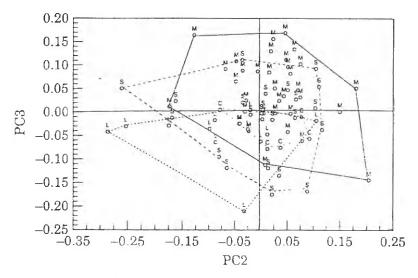


Fig. 3. Plot of second and third axis of a principal component analysis using 13 log-transformed metric variables for four populations of *C. anguillaris*. S. Senegal; M. Mali; C. Chad; L. Ivory Coast.

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

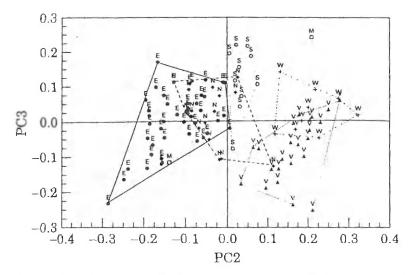


Fig. 4. Plot of second and third axes of principal component analysis using 13 log-transformed metric variables for five populations of *C. gariepinus*. S, Senegal; M, Mali; N, Chad; E, Egypt; W, Swaziland; V, Lake Victoria.

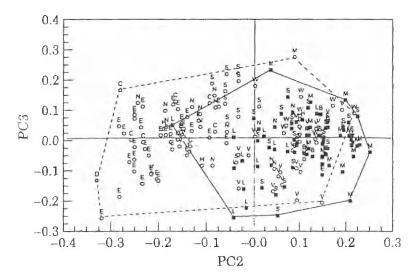


Fig. 5. Plot of second and third axes of principal component analysis using 13 log-transformed metric variables for all specimens examined of *C. anguillaris* (\blacksquare ; —) and *C. gariepīnus* (\bigcirc ; ——). B, Bamako; C, Cairo; E, Lake Manzala; H, Hadide; L, Côte d'Ivoire; M, Selingue; N, Chad; 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

AAT-1* *90	_	0.297										
*150 *160 *75 *115 *0 *200 *200 0.028 *300 *15 *20 *15 *80 *80 *80 *80 *80 *80 *80 *80					1 1	1						- 1
*75 *115 *0 *200 *200 0.028 *300 *15 *20 0.075 *85 *80 *120 0.925 0.850 *150 *300 0.028 *300 0.050 *20 0.050				1 ,	1 1	1 1	1 1		1 +			
*115 *0 *200 *200 0.028 *0 *15 *20 0.075 *85 *80 *120 0.925 0.850 *120 0.925 0.850 *150 0.028 *10 0.050 0.100 *200 0.050				1						-		-
*200 0.028 *300			11		0.946 0.018	- 1	٦	- 1	-	-		
*15										0.094		1 -
*20 0.075 *65 *80 *120 0.925 0.850 * *50 *150 *150 *150 *200 0.050 0.100 *200 *25 0.050] [0.367	1 1	1 1	11	1.1	1			
*80				-					}		0.038	0.061
** 120 0.925 0.850 ** \$50							1				0.000	
*150			-	0.267	0.016	0.017	0.400	11	0.100	0.063		1 1
*0 0.050 0.100 *200			1	1	1	1	1	1	1	}	-	-
*25 0.050 -				0.067	0.016	0.017		0.100	-		1	1 1
0.775 0.900				0.067					0.033	- sp-aaan-	-	
0.025			11	0.033	0.034	0.050	11	11			.	1
00100					1 1					[0.984
*120		ĺ						1	0.033	0.059	Γ	Ţ

Table II. Continued

Locus	Alfele	Manz G	Chob G	Victor G	SoAf G	Sene	Seli	Djam G	Sene A	Seli A	Banna A	Djum A	Layol	Lilyo2 A	Heter	Albo
DHP-2*	*40		1			0.118										ļ
	()9*	0.105	0-150	0.030											199.0	
	*87					0.029										
	*110	ž										,				-
	*115	i	to						0.016							
	*125	0.368	0.550	ï		0.147		0.071								
I-HOT	0	i	0.050								0.050					
	*200											0.100				-
	* - 160				0.982											
LDH-2*	*50	_	_	_	_	179-0	-	-	0.328	0-333	0.350	;	0-367	0-324		
*I-HQM	*200														_	_
MDH-2*	*83	0.375	0.250			0.563	_	0.467	_	-	_	_	_	_		
MDII-3*	*20	i														-
	*57					0.176										
	99*			Ţ	:	0.118			0.438			-	1			
	*105		-	-		1	1					7	-		-	
MEP-I*	*30		ì												,	0-133
	* \$0		1	0.015			×			0.017					0.038	
	09*		ì	1										Ţ		0-534
	*70	:												-	0.962	
	08*	-	0.050	0.924	0.900	0.433	-1-	0.067	0.100	0.500	0.050	0.100	629.0	0.571		
	06*	1	1		1	5										0.333
	*110	0.059	1	ĺ	I	0.030	ŀ	10		ř	ï		-	1.		1
	*120	1	1	ĺ		-		0.100		ř		-	1	-	ł	
MEP-2*	*105	i	-	į.	- 4 11	1		-				1	Table 1	Ė	1	_

snoo	Allele	Allele Manz	Chob G		Victo SoAf G G	Sene	Seli	Diam G	Sene	Seli	Bama	Djam	Djum Layol	Layo2 A	Heter	Albo
*IdN	#67									0.017		100				
	*			0-242												
	*105	0.100				0-235	0.250	001-0						0.300		
PGDII*	*10															0.037
	*30															CSS
	*50	0-150				0.050		00.300	0.031	0.103	0.050					0-111
	19*										0.050					
	*75	0-725	0.050	_	_	0.676	0.250	0.533	90+0	0.362	0.300		0.500	0.500		
	* *								0.010		0.050					
PGM*	*50			0.015												
	0× *	0.025		0.632											C.XX.	-
	×	0.025	0.00-0						0-172	0-067	0.050					
*C-OOS	07.	0 - 125	0.250													
	*50	0.050	0.050													
	*	0-150	0-150	-												
	*	0.09-0	0.500				0-250 0-767	10.767		050-0	0.050	0.52-0				-
*IIIX	*105															-
H (%)		~	12.2	ĵ.∞	٢,	15.4	6	14.3	1.6	7-4	 	4.6	7.1	9-3	۲.	+
195 (%)		36	4 x	28	39	07	12	36	20	77	00	91	16	S.	7.	-

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

Manz G Chob G Victo G SoAf G Scnc G Scli G Djam G Scnc A ScliA Bama A Djam A Layol A Layo2 A Heter

													0-7133
												0.5476	1-1802
											0.0050	0.5321	1-1622
										0.0432	0.0413	0.5634	1.0465
									0.0282	0.0227	0.0230	0.5514	1.1776
								$0.000 \cdot 0$	0.0226	0.01111	0.0112	0.5282	1.1392
							6600-0	0.0164	0.0339	0.0253	0.0248	0.5115	1-1255
						0.1478	0.1355	0-1278	0.1577	0.1497	0.1531	0.5602	1.0453
					0.0650	0.1231	0.1168	0.0851	0.1445	0.1293	0.1277	8819.0	1-2323
				0.0394	0.0575	0.1274	0.1256	0.1064	0.1881	0.1143	0.1183	0.5310	1-1883
			0.2144	0.2875	0.2053	0.3709	0.3476	0.3506	0.4097	0.3103	0.3324	0.6186	1-2087
		0.1625	0.1873	0.2660	0.1883	0.2397	0.2183	0.2229	0.2812	0.1867	0.1985	0.6392	1-1773
	0.2287	0.1695	0.0994	0.1244	0.0804	0.2550	0.2445	0.2167	0.2849	0.2520	0.2645	0.5232	1-1676
0-0077	0.2239	0.1760	6080-0	0.0860	0.0606	0.2227	0-2115	0-1820	0.2440	0.2236	0-2315	0.5212	1-1448
Chob G	Victo G	SoAf G	Sene G	Seli G	Djam G	Sene A	Seli A	Bama A	Djam A	Layol A	Layo2 A	Heter	Albo

Population codes are given in Table 1

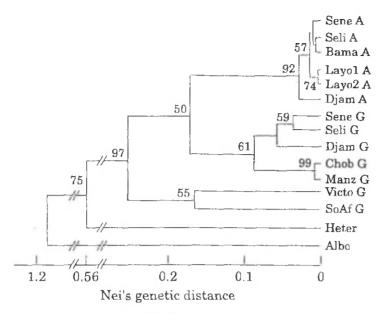


Fig. 6. UPGMA tree derived from standard Nei's genetic distances between C. gariepinus, C. anguillaris, H. longifilis and C. albopunctatus samples. Bootstrap values estimated from 1000 replications are reported when higher than 50%.

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 Clurias 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 ichthvofaunal 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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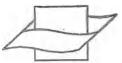
References

Agnèse, J. F., Teugels, G. G., Galbusera, P., Guyomard, R., Volckaert, F. & Zouros, E. (1997). Four independent approaches to characterize sympatric catfish populations of *Clarias gariepinus* and *C. anguillaris* (Siluroidei; Clariidae) from Senegal: morphometry, allozymes, microsatellites and RFLP of mitochondria DNA. *Journal of Fish Biology* 50, 1143-1157.

- Beadle, L. G. (1981). The Inland Waters of Tropical Africa. An Introduction to Tropical Limnology, 2nd edn. New York: Langman Inc.
- Bookstein, F. L., Chernoff, B., Elder, R., Humphries, J., Smith, G. & Strauss, R. (1985).

 Morphometrics in evolutionary biology. Academy of Natural Sciences
 Philadelphia, Special Publication 15, 1-277.
- Chakraborty, R. & Leimar, O. (1987). Genetic variation within a subdivided population. In *Population Genetics and Fishery Management* (Ryman, N. & Utter, F., eds), pp. 89–120. Seattle, WA: University of Washington Press.
- Felsenstein, J. (1973). Maximum-likelihood estimation of evolutionary trees from continuous characters. American Journal of Human Genetics 25, 471–492.
- Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package), version 3.5C. Seattle, WA: University of Washington.
- Galbusera, P., Volckaert, F. A., Hellemans, B. & Ollevier, F. (1996). Isolation and characterisation of microsatellite markers in the African catfish *Clarias gariepinus* (Burchell, 1822). *Molecular Ecology* 5, 703-705.
- Gorman, G. C., Kim, Y. J. & Rubinoff, R. (1976). Genetic relationships of three species of *Bathygobius* from the Atlantic and Pacific sides of Panama. *Copeia* 2, 361-364.
- Grove, A. T. (1985). The physical evolution of the river basins. In *The Niger and Its Neighbours*. Environmental History and Hydrobiology, Human Use and Health Hazard of the Major West African Rivers (Grove, A. T., ed.), pp. 21-60. Netherlands: Balkena Publishers.
- Guyomard, R. & Krieg, F. (1983). Electrophoretic variation in six populations of brown trout (Salmo trutta L.). Canadian Journal of Genetics and Cytology 25, 403-413.
- Humphries, J. M., Bookstein, F. L., Chernoff, B., Smith, G. R., Elder, R. L. & Poss, S. G. (1981). Multivariate discrimination by shape in relation to size. *Systematic Zoology* 30, 291-308.
- Krieg, F. & Guyomard, R. (1985). Population genetics of French brown trout (Salmo trutta L.): large geographical differentiation of wild populations and high similarity of domesticated stocks. Génétique Sélection et Evolution 17, 225-242.
- Nei, M. (1987). Molecular Evolutionary Genetics. New York: Columbia University Press.
- Raymond, M. & Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86, 248-249.
- Roberts, T. R. (1975). Geographical distribution of African freshwater fishes. Zoological Journal of the Linnean Society 57, 249-319.
- Rognon, X., Andriamanga, M., McAndrew, B. J. & Guyomard, R. (1996). Allozyme variation in natural and cultured populations in two tilapia species: *Oreochromis niloticus* and *Tilapia zillii*. *Heredity* 76, 640-650.
- Shaklee, J. B., Allendorf, F. W., Morizot, D. C. & Whitt, G. S. (1990). Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fishery Society* 119, 2-15.
- Sneath, P. H. & Sokal, R. R. (1973). Numerical Taxonomy. San Francisco, CA: W. F. Freeman and Co.
- Swofford, D. L. & Selander, R. B. (1989). BIOSYS-1: A Computer Program for the Analysis of Allelic Variation in Population Genetics and Biochemical Systematics. Champaign, IL: Illinois Natural History Survey.
- Teugels, G. G. (1982). Preliminary results of a morphological study of five nominal species of the subgenus *Clarias* (Pisces; Clariidae). *Journal of Natural History* 16, 439-464.
- Teugels, G. G. (1983). Notes on the status of *Clarias ngamensis* Castelnau 1861, C. mellandi Boulenger 1905, C. prentissgrayi (Fowler 1930) and C. lamotei Daget and Planquette 1967 (Pisces, Clariidae) with the rehabilitation of *Dinotopteroides* Fowler 1930 as a subgenus of *Clarias*. Cybium 7, 15-28.
- Teugels, G. G. (1986). A systematic revision of the African species of the genus *Clarias* (Pisces; Clariidae). Annales du Musée Royal de l'Afrique Centrale 247, 1-199.

- Teugels, G. G. (1996). Taxonomy, phylogeny and biogeography of catfishes (Ostariophysi, Siluroidei): an overview. In *The Biology and Culture of Catfishes* (Legendre M. & Proteau, J.-P., eds). *Aquatic Living Resources* 9 (hors série), 9-34.
- Teugels, G. G., De Nayer, B. & Legendre, M. (1990). A systematic revision of the African catfish genus *Heterobranchus* (Pisces; Clariidae). Zoological Journal of the Linnean Society 98, 237-257.
- Teugels, G. G., Guyomard, R. & Legendre, M. (1992). Enzymatic variation in African clariid catfishes. *Journal of Fish Biology* 40, 87-96.
- Van der Bank, F. H., Grobler, J. P. & Du Preez, H. H. (1992). A comparative biochemical genetic study of three populations of domesticated and wild African catfish (Clarias gariepinus). Comparative Biochemistry and Physiology 101B, 387-390.



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POSTER ABSTRACTS

variable. The observation that some groups have disappeared as a result of fishing pressure indicates that such generalizes are not applicable to all species.

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Novel antibiotics from parrot-fish pyjamas

This study searched for glycoproteins and their potential batericidal properties in the pyjamas of the queen parrotfish (*Scarus vetula*). It was found that the mucous cocoon is an extensive di-sulphate bonded network consisting exclusively of small glyco-proteins of about 21 kDa apparant molecular weight. N-terminal amino acid sequencing resulted in an unique sequence of the first 25 amino acids. Sonicated samples of mucus inhibit growth of *Escherichia coli*, strains lilly and 8623, *Micrococcus luteus*, *Aeromonas hydrophila* and *Vibrio anguilarium*. The dialysed new protein (pyjamicine) had an antibiotic effect on *Micrococcus luteus* and *Aeromonas hydrophila*.

Volckaert, F. A. M., Hellemans, B. (Katholieke Universiteit Leuven, Zoological Institute, Naamsestraat 59, B-3000 Leuven, Belgium) & Legendre, M. (ORSTOM, Jakarta 12540, Indonesia).

The genetic variability of some catfishes of the genus *Pangasius* and *Clarias* in SE Asia as measured with DNA microsatellites

Ten species-specific DNA microsatellites were developed for the catfishes *Clarias batrachus* and *Pungasius hypophthalnus* by means of a gDNA bank enriched for di- and tetranucleotides. Several of these markers, as well as markers previously developed specifically for *C. gariepinus*, were applicable (i.e. observing a high level of genetic variability) in related species of the genus *Clarias* and *Pangasius*. A significant level of genetic variability was observed in catfish populations collected in the field or by sampling alive in fish markets from the Mekong Delta (Vietnam) and Java Island (Indonesia), apart from the reported taxonomic diversity. Estimates of family and genetic variability point to well-structured populations with the typical features of freshwater fish populations.

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Developing management strategies for the domestic fishery of the Seychelles

The level of fishing within the inshore region of the Seychelles means a form of regulation is now required in order to sustain present catch levels and conserve the future biological status of the resource. This research aims to develop and evaluate a number of alternative management strategies for the domestic fishery. An existing research framework has been adapted to collect information on the biological, technical, economic and socioeconomic attributes of the domestic fishery. This framework forms the basis of a preliminary institutional analysis of the domestic fishery and provides specific information on the decision-making arrangements of the fishers. The latter will be used to help predict the behaviour of fishers in response to different management strategies. These data will be combined to develop a bio-socioeconomic simulation model of the fishery, which will be used to investigate the likely outcomes from new management strategies (e.g. maximum employment, sustainability of the resource base). The results of this study can then be used to develop guidelines for a management plan of the domestic fishery.

