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Morphometric and genetic characterization of sympatric populations of *Clarias gariepinus* and *C. anguillaris* from Senegal

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A sample of African *Clarias* catfishes from the Senegal River was studied using morphometry, allozyme variation, microsatellites and RFLPs of mitochondrial DNA. They all confirmed the presence of two species, *C. gariepinus* and *C. anguillaris*. The two species were closely related genetically and no diagnostic loci were found in allozymes and microsatellites studies. Two of the 11 haplotypes of mtDNA observed were shared by both species. Three of the four assays (morphometry, allozymes and microsatellites) allowed a precise characterization of both. One specimen occupied an intermediate position in the analysis of the data; it was considered an F_1 hybrid whose possible origin is discussed.

Key words: morphometry; allozymes; microsatellites; mitochondrial DNA; hybrid; catfish.

INTRODUCTION

The catfish genus *Clarias* Scopoli, 1777 has a widespread distribution and is found in Africa and south-east Asia (Teugels, 1997). Some species are of great economic importance both in fisheries and aquaculture. The African catfish, *C. gariepinus* (Burchell, 1822), has been introduced in Europe, America and south-east Asia for aquaculture purposes.

Various authors (Debouche *et al.*, 1979; Teugels, 1982*a*; Benech *et al.*, 1993) have published on the identification problems between *Clarias gariepinus* and the closely related and partially sympatric, *C. anguillaris* (Linnaeus, 1758). Numerous erroneous identifications have been published. These have been annotated by Teugels (1982*b*, 1986). The only reliable difference between these species is the number of gill rakers on the first branchial arch. This number displays a positive allometry and also shows clinal geographic variation which, however, was not significantly different for conspecific populations but highly significant between the two species (Teugels, 1982*a*). A preliminary allozyme study (Teugels *et al.*, 1992) indicated that the two species are genetically close and that a more detailed study is needed to distinguish them genetically.

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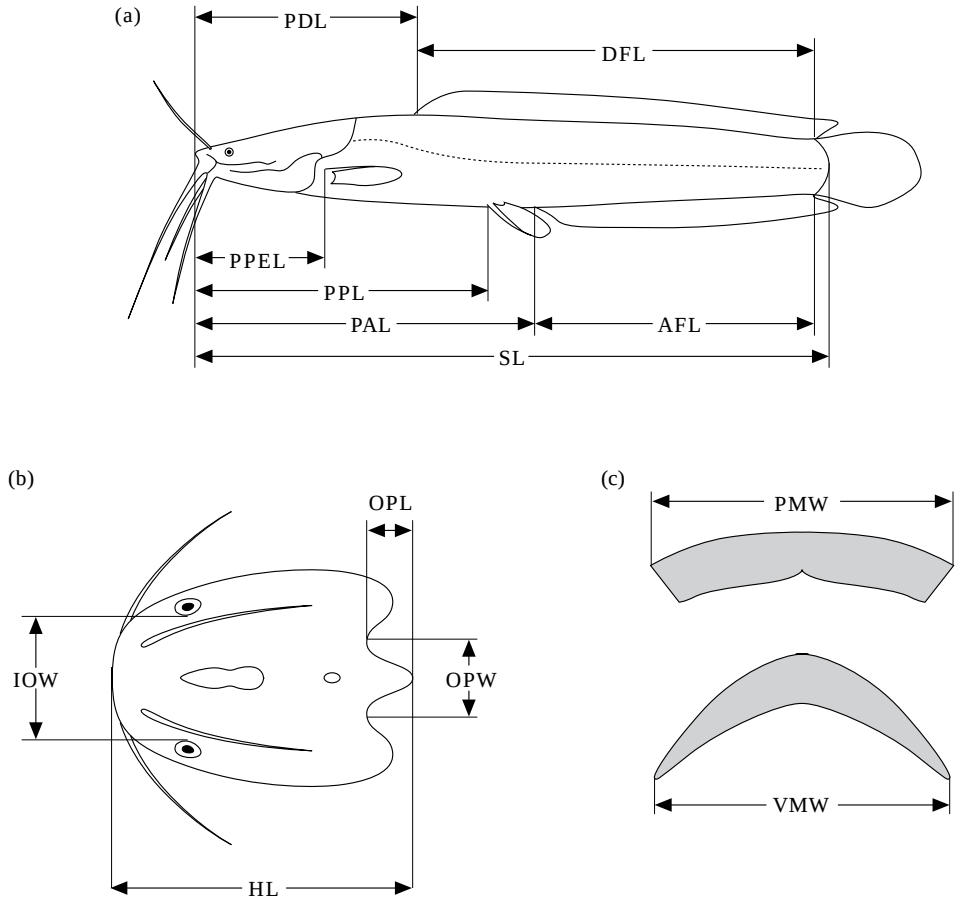

FIG. 1. Schematic illustration of morphometric characters taken on *Clarias* specimens. (a) Body measurements: SL, standard length; PDL, predorsal length; PAL, preanal length; PPL, prepelvic length; PPEL, prepectoral length; DFL, dorsal fin length; AFL, anal fin length. (b) Head measurements: HL, head length; IOW, interorbital width; OPL, occipital process length; OPW, occipital process width. (c) Toothplate measurements: PMW, premaxillary toothplate width; VMW, vomerine toothplate width.

In this paper, *Clarias* specimens from the Senegal River have been characterized using four different approaches: morphometry, allozymes, microsatellites and mitochondrial DNA studies.

MATERIALS AND METHODS

Forty-nine *Clarias* specimens collected with a throw-net in one of the Dagana canals (16°30′ N, 15°30′ W) of the Senegal River basin in Senegal were examined. Eye, liver and muscle tissues were dissected for each specimen and preserved in liquid nitrogen. In addition, muscle tissues were preserved in a 95% ethanol. The specimens were labelled individually and deposited in the Musée Royal de l'Afrique Centrale, Tervuren, Belgium.

MORPHOMETRIC ANALYSIS

Thirteen measurements were taken on each specimen using dial calipers (Fig. 1). These characters (except for standard length) were selected on their diagnostic value as

TABLE I. Enzymes analysed in the *Clarias* sample from Senegal, migration buffers used and genetic interpretations

Enzyme	Abbrev.	EC no.	Buffer	Locus	Tissue
Adenilate kinase	AK	2.7.4.3	MC2	*AK**	M
Aspartate aminotransferase	AAT	2.6.1.1	MC2	*AAT-1**	L, M
				*AAT-2**	L, M
Creatine kinase	CK	2.7.3.2	MC2	*CK-1**	M
				*CK-2**	E
Fructose biphosphatase	FBP	3.1.3.11	MC2	*FBP-1**	L
Fumarase hydratase	FH	4.2.1.2	MC2	*FH**	M
Glycerol-3-phosphate dehydrogenase	G3PDH	1.1.1.8	Tris-PO4	*G3PDH**	M
Glucose-6-phosphate isomerase	GPI	5.3.1.9	Ridgeway	*GPI-1**	M
				*GPI-2**	E, L
Isocitrate dehydrogenase	IDHP	1.1.1.42	Tris-PO4	*IDHP-1**	M
			MC2	*IDHP-2**	L
L-lactate dehydrogenase	LDH	1.1.1.27	Ridgeway	*LDH-1**	M
				*LDH-2**	L, M
Malate dehydrogenase	MDH	1.1.1.37	MC2	*MDH-1**	M
				*MDH-2**	L
				*MDH-3**	M
Malic enzyme (NADP dependent form)	ME	1.1.1.40	Tris-PO4	*ME-1**	M
				*ME-2**	L, M
Mannose-6-phosphate isomerase	MPI	5.3.1.8	Tris-PO4	*MPI**	M
Phosphogluconate dehydrogenase	PGDH	1.1.1.44	Tris-PO4	*GPDH**	M
Phosphoglucomutase	PGM	5.4.2.2	Ridgeway	*PGM**	M
Superoxyde dismutase	SOD	1.15.1.1	Ridgeway	*SOD-1**	L
				*SOD-2**	L
Xanthine dehydrogenase	XDH	1.2.1.37	Ridgeway	*XDH**	L

E, eye; L, liver; M, muscle.

demonstrated by Teugels (1982*a*, 1986). For each fish the number of gill rakers on the complete first branchial arch was counted. Due to their poor condition, only four specimens were examined for their number of gill rakers. Morphometric data obtained were submitted to principal component analysis. Data were log transformed. In this analysis, as suggested by Humphries *et al.* (1981) and Bookstein *et al.* (1985), the first principal component was interpreted as a size factor and the others as shape factors. In order to exclude the effect of size difference between the samples, the first component was not considered.

ALLOZYME ELECTROPHORESIS

Twenty-five loci representing 16 enzyme systems were analysed in all samples (Table I). Tissue extraction, migration buffers and staining procedures were as described by Guyomard & Krieg (1983) and Krieg & Guyomard (1985). Locus and allele nomenclature followed the recommendations of Shaklee *et al.* (1990). Alleles were designated by their electrophoretic mobility relative to the most frequent allele in the complete Dagana sample.

MICROSATELLITES

DNA was extracted from each sample using the phenol–chloroform method. Seven different primer sets for *Clarias gariepinus* described by Galbusera *et al.* (1997) were used (Cga 01, 02, 03, 05, 06, 09 and 10). The conditions of PCR and final concentrations of the reagents were those given by Galbusera *et al.* (1997). One additional marker has been

developed besides the published microsatellite markers: it has been named Cga 07 (Genbank submission number: U30868). The microsatellite array consists of a perfect run of 13 GT-dinucleotides. The sequences of the forward and the reverse primer are respectively TTGTGTAACTCACATTACCGG and GTTTAGAAAATGTTTACG CCG. At an annealing temperature of 62° C, the primer set amplifies specific PCR products of 154–188 bp in length, but some unspecified bands of around 210 bp have been amplified as well. In a sample of 38 individuals (the same population as in Galbusera *et al.*, 1997), 11 alleles could be detected indicating a relatively high level of polymorphism at this microsatellite locus.

mtDNA RFLP ANALYSIS

DNA was prepared from liver as described in Estoup *et al.* (1993). Two adjacent mtDNA regions were amplified: one encompassing the complete ND5/6 region (approximately 2·4 kb) was amplified with the primers of Cronin *et al.* (1993). The other segment (approximately 2·1 kb) comprised the cytochrome b gene and the control region (D-loop) and was amplified using the complementary primer of the 3′-end primer of the ND5/6 region and the HN20 primer (Bernatchez & Danzmann, 1993). PCR conditions were as described in Bernatchez *et al.* (1995). Restriction digests were done on aliquots of 2–7 µl of PCR. Resulting fragments (more than 50 bp) were electrophoretically separated in 1·2% agarose gel stained with ethidium bromide. The following restriction enzymes were analysed: *Alu* I, *Hae* III, *Hha* I, *Hinf* I, *Hpa* II, *Mbo* I, *Msp* I, *Mva* I, *Rsa* I, *Taq* I.

DATA TREATMENT

Morphometric data were submitted to a principal components analysis. Deviation from Hardy–Weinberg and genotypic linkage equilibria were checked by an exact test using GENEPOP (Rousset & Raymond, 1995). Unbiased expected heterozygosities and standard genetic distances were calculated from allozymes and microsatellite data as described in Nei (1978) using BIOSYS (Swofford, 1983). Microsatellite data were analysed by different population genetics programs (GENEPOP and BIOSYS). Both allozyme and microsatellite data were submitted to a correspondence analysis to assess the overall relationship between samples. The input of multi-locus data consisted of two unweighted individual × allele matrix, where each individual is described for each allele by the values 2, 1 or 0 according to whether it shows 2 (homozygote), 1 (heterozygote), or 0 copies of the considered allele (see She *et al.*, 1987 for more details). Individuals were defined by their allelic counts at the 25 (allozymes) or eight (microsatellites) loci.

A matrix of presence/absence of restriction sites was generated from the mtDNA RFLP patterns. Haplotype trees were constructed from the presence/absence matrix by Wagner parsimony and restriction site maximum likelihood methods using the PHYLIP package (Felsenstein, 1993).

RESULTS

MORPHOMETRIC ANALYSIS

Variation in gill raker number on the first branchial arch identified two groups (Fig. 2): in the first group there was a strong correlation between standard length and gill raker number, corresponding to the value previously reported for *C. gariepinus* (Teugels, 1982*a*). In the second group, there was a low correlation, corresponding to that previously reported for *C. anguillaris* (Teugels, *l.c.*). Specimen 2 showed a relatively high number of gill rakers (57) but did not correspond to the range found in *C. gariepinus*. According to the regression equation, its expected number (68) was considerably higher than the observed one.

The gill raker count analysis allowed 15 specimens to be identified as *C. gariepinus* and 33 as *C. anguillaris*. Only those specimens for which a complete

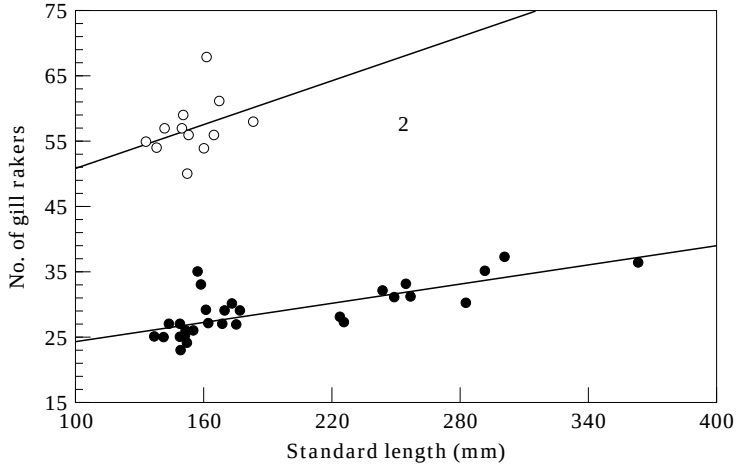

FIG. 2. Number of gill rakers on the first branchial arch related to the standard length for sympatric populations of *Clarias gariepinus* (○) and *C. anguillaris* (●) from Senegal. The position of specimen 2 is also indicated.

data set was available (25 for *C. anguillaris* and 10 for *C. gariepinus*), were considered for further analysis (Table II). The problematic specimen 2 was not included in this Table. The most discriminating characters in this Table are related to the width of toothplates.

A principal component analysis was carried out on 13 log transformed metric variables using the matrix of covariance (Fig. 3). The second component was defined mainly by the width of the premaxillary and the vomerine toothplates and by the length of the occipital process. The third component was defined mainly by the length of the occipital process, the width of the premaxillary toothplate and the anal fin length. The PCA allowed distinction between the two species corresponding to those identified on gill raker count (all *C. gariepinus* except one were located on the positive sector of the second axis where all *C. anguillaris*, except five, were situated on the negative sector of the second axis). Specimen 2 was located in the *C. anguillaris* polygon, close to the origin of both axis.

ALLOZYME ANALYSIS

Among the 25 loci studied, 13 were polymorphic (Table III). Principal component analysis was done using presence/absence data on 35 alleles in 38 specimens (Fig. 4). Two groups were defined clearly, corresponding to the two specific assemblages identified using morphometric analysis. Specimen 2 showed an intermediate position between the two groups. This was considered to be different from both species. Forty-four per cent of polymorphic loci (P) were found in both species. Mean observed heterozygosity (H) varied from 0·095 in *C. anguillaris* to 0·152 in *C. gariepinus*. Despite this high genetic variability, no diagnostic loci were found. Nei's genetic distance (1978) between the two species was 0·162. In both species, allelic frequencies observed at the polymorphic loci were not significantly different from those expected under Hardy–Weinberg equilibrium (GENEPOP Program, Hardy–Weinberg exact test). In contrast,

TABLE II. Measurements and basic statistics for sympatric populations of *Clarias gariepinus* and *C. anguillaris* from Senegal

Clarias anguillaris	*n*	Min.	Max.	Mean	S.D.
Standard length (SL) (mm)	32	137	364		
Head length (HL) (% SL)	32	29·2	32·9	30·9	0·9
Predorsal length (% SL)	32	32·6	38·1	35·4	1·6
Preanal length (% SL)	32	54·4	59·4	57·6	1·3
Prepelvic length (% SL)	31	46·5	51·9	49·2	1·5
Prepectoral length (% SL)	31	22·5	25·5	23·9	0·8
Dorsal fin length (% SL)	28	58·8	68·6	63·5	2·1
Anal fin length (% SL)	32	39·1	45·6	42·2	1·5
Interorbital width (% HL)	32	36·6	42·8	39·6	1·4
Occipital process length (% HL)	32	12·8	19·9	17·1	1·5
Occipital process width (% HL)	31	17·3	22·4	19·7	1·2
Premaxillary toothplate width (% HL)	32	24·9	31·8	28·8	1·6
Vomerine toothplate width (% HL)	32	22·2	27·1	24·5	1·4
Clarias gariepinus					
Standard length (SL) (mm)	12	133	183		
Head length (HL) (% SL)	12	30·8	32·9	31·6	0·6
Predorsal length (% SL)	12	32·2	38·1	35·7	1·5
Preanal length (% SL)	12	56·0	59·9	57·9	1·3
Prepelvic length (% SL)	11	46·1	51·4	48·8	1·8
Prepectoral length (% SL)	12	22·7	25·9	23·9	0·8
Dorsal fin length (% SL)	10	59·3	65·1	62·6	1·9
Anal fin length (% SL)	12	39·3	46·0	43·0	2·3
Interorbital width (% HL)	12	33·0	39·8	37·5	1·9
Occipital process length (% HL)	12	14·6	19·4	17·5	1·4
Occipital process width (% HL)	12	18·3	22·4	20·9	1·2
Premaxillary toothplate width (% HL)	12	20·0	23·9	22·3	1·0
Vomerine toothplate width (% HL)	12	20·3	22·7	21·5	0·7

when allelic frequencies were compared between the two species (regardless of the inclusion of specimen 2 as *C. gariepinus*) using Fisher's exact tests, the F_{st} value (0·4799) was highly significant.

MICROSATELLITE ANALYSIS

The eight loci studied were polymorphic in both species (Table IV). Principal component analysis was done using presence/absence data on 89 alleles in 45 specimens (Fig. 5). All specimens situated in the negative sector of the second axis belonged to *C. gariepinus*; all specimens situated in the positive sector of the second axis belonged to *C. anguillaris*. Again, specimen 2 occupied an intermediate position between the two species.

No diagnostic locus between the two species was found. There were significant heterozygote deficiencies (GENEPOP Program, Hardy–Weinberg exact test) compared to the expected values for loci Cga01, Cga02, Cga06 and Cga09 in the *C. anguillaris* population and for locus Cga01 and Cga03 in the *C. gariepinus* population regardless of inclusion of specimen 2 as *C. gariepinus* in the analysis. These departures from Hardy–Weinberg equilibrium may have a wide range of causes: firstly the assumptions of equilibrium (no migration, mutation or selection) may not have been fulfilled; secondly, it is possible that the samples

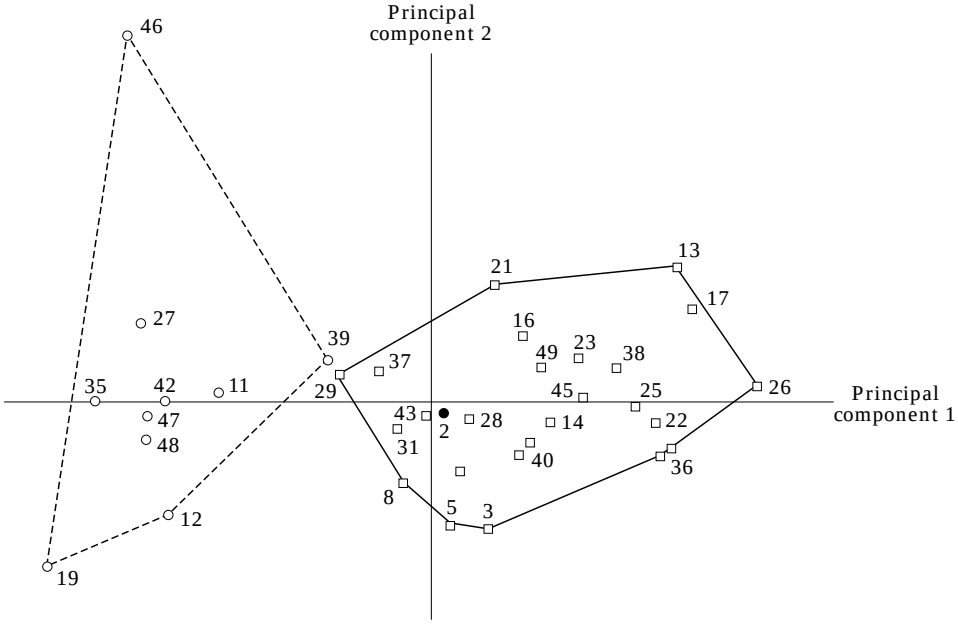

FIG. 3. Plot of the first and second principal components taken from a principal component analysis of 12 metric variables on sympatric populations of *Clarias gariepinus* (○, – – –) (n=10) and *C. anguillaris* (□, ——) (n=25). Specimen 2 is represented by a black spot.

belonged to different reproductively isolated populations (Wahlund effect); finally the deviations can be due to null alleles (Pemberton *et al.*, 1995). Nei's genetic distance (1978) observed between the two species was 0·808.

mtDNA RFLP ANALYSIS

Ten haplotypes of mtDNA were observed in 22 specimens (Table V). Two of them were characteristic for the specimens previously identified as *C. anguillaris*, six were typical for *C. gariepinus* specimens and two were in common to both species (Fig. 6). Specimen 2 was not included in this analysis.

DISCUSSION

The morphometric analysis of the *Clarias* sample studied demonstrated clearly the presence of two species as defined by Teugels (1982*a*, 1986). Two of the three genetic approaches (allozymes and microsatellites) reached the same conclusion. Although no diagnostic loci were detected, both species were characterized by a number of private alleles: the allozyme study showed eight private alleles for *C. gariepinus* and 84 for *C. anguillaris*. The microsatellite approach revealed 19 and 26 respectively. Nevertheless, due to rather low sample sizes (between 15 and 32 specimens), the private status of these alleles should be considered with caution. Thus, the two approaches provided a refined genetic characterization of both species.

The allozyme results were comparable with those previously observed in populations of both species from Niger and Côte d'Ivoire (Teugels *et al.*, 1992). For other African catfishes lower P and H values have been reported: P varied

TABLE III. Allelic frequencies observed for allozymes in *Clarias gariepinus* and *C. anguillaris* from Senegal

Population	Locus	*Ck-1**			*FBP-1**			*FH**			*IDHP-2**					
	Allele	100	115	(*n*)	100	0	(*n*)	100	120	(*n*)	40	87	100	115	125	(*n*)
C. gariepinus		0·941	0·145	(17)	1·00	0·00	(17)	0·118	0·882	(17)	0·118	0·029	0·706	0·000	0·147	(17)
C. anguillaris		0·059	0·855	(31)	0·982	0·018	(28)	0·984	0·016	(32)	0·000	0·000	0·983	0·017	0·000	(29)

	Locus	*LDH-2**				*MDH-2**			*MDH-3**				*MEP-1**			
	Allele	50	100	150	(*n*)	83	100	(*n*)	57	66	100	(*n*)	80	100	110	(*n*)
C. gariepinus		0·971	0·029	0·000	(17)	0·563	0·438	(16)	0·176	0·118	0·706	(17)	0·433	0·533	0·033	(15)
C. anguillaris		0·323	0·661	0·016	(31)	1·000	0·000	(31)	0·000	0·422	0·578	(32)	0·155	0·845	0·000	(29)

	Locus	*PGDH**					*PGM**			*PGI1**			*PGI-2**				*MPI**		
	Allele	100	150	210	230	(*n*)	100	112	(*n*)	0	100	(*n*)	150	210	230	(*n*)	100	105	(*n*)
C. gariepinus		0·265	0·059	0·676	0·000	(17)	1·00	0·00	(17)	0·294	0·706	(17)	0·000	0·906	0·094	(16)	0·765	0·235	(17)
C. anguillaris		0·563	0·031	0·391	0·016	(32)	0·828	0·172	(32)	0·016	0·984	(31)	0·034	0·906	0·000	(29)	1·000	0·000	(32)

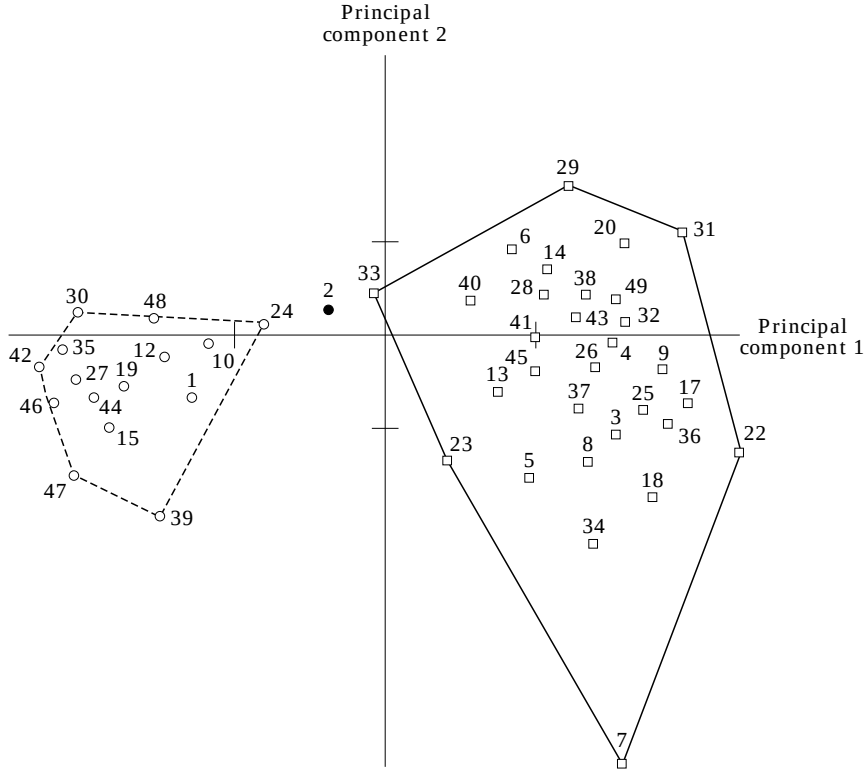

FIG. 4. Plot of the first and second principal components taken from a principal component analysis of a matrix of presence/absence of allozymes on sympatric populations of *Clarias gariepinus* (○, – – –) (n=17) and *C. anguillaris* (□, ——) (n=32). Specimen 2 is represented by a black spot.

from 0 to 10% and H from 0·000 to 0·081 in *Chrysichthys auratus* (Geoffroy Saint-Hilaire) (Agnèse, 1991); P varied from 5 to 21% and H from 0·017 to 0·080 in *Chrysichthys nigrodigitatus* (Lacépède) (Agnèse *et al.*, 1989); P=16% and H=0·001 in *Eutropius niloticus* (Rüppell) (=*Schilbe mystus*; De Vos, 1995) (Abban & Skibinski, 1988). Only *Schilbe mystus* (L.) (=*S. intermedius*; De Vos, 1995) showed comparable values: P=55% and H=0·118 (Abban & Skibinski, 1988).

The genetic distance observed was comparable with that previously reported between both species (Teugels *et al.*, 1992) but it was very low compared with those observed for other African catfishes. D varies from 0·271 to 0·916 in the genus *Chrysichthys* (Agnèse, 1989), D=0·361 between *Bagrus docmac* (Forsshäll) and *B. bajad* (Forsshäll) (Agnèse, 1989), D varies from 0·88 to 1·14 between *Eutropius niloticus* and *Schilbe mystus* (Abban & Skibinski, 1988). Genetic distances obtained were comparable with intraspecific genetic distances observed in other African catfishes [D varied from 0·01 to 0·304 between populations of *Chrysichthys nigrodigitatus* (Agnèse *et al.*, 1989); from 0·003 to 0·112 between populations of *Chrysichthys auratus* (Agnèse, 1991); from 0·007 to 0·276 between populations of *Chrysichthys maurus* (Valenciennes) (Agnèse, 1989)].

The mtDNA analysis demonstrated a number of private haplotypes for each species; however, two were in common to both. Also, the different haplotypes of

TABLE IV. Allelic frequencies observed for microsatellites in 15 specimens of *Clarias gariepinus* and 32 specimens of *C. anguillaris* from Senegal

Locus/allele	8	9	10	11	15	16	14	15	16	17	18	19					
Cga01																	
C. gariepinus	0·344	0·094	0·156	0·031	0·063	0·063	0·000	0·000	0·000	0·000	0·250	0·000					
C. anguillaris	0·063	0·063	0·000	0·000	0·188	0·172	0·094	0·078	0·313	0·016	0·000	0·016					
Locus/allele	15	16	17	18	19	23	24	25									
Cga02																	
C. gariepinus	0·281	0·500	0·031	0·031	0·031	0·031	0·000	0·094									
C. anguillaris	0·375	0·172	0·219	0·000	0·000	0·109	0·094	0·031									
Locus/allele	38	39	40	41	42	43	44	45	46	47	48	49	54				
Cga03																	
C. gariepinus	0·031	0·063	0·031	0·094	0·063	0·000	0·031	0·063	0·156	0·375	0·031	0·031	0·031				
C. anguillaris	0·000	0·000	0·000	0·047	0·000	0·031	0·109	0·281	0·203	0·141	0·078	0·109	0·000				
Locus/allele	64	66	67	68	71	73	74	75									
Cga05																	
C. gariepinus	0·844	0·000	0·000	0·000	0·000	0·000	0·000	0·156									
C. anguillaris	0·078	0·094	0·344	0·094	0·063	0·141	0·172	0·016									
Locus/allele	30	31	32	33	34												
Cga06																	
C. gariepinus	0·000	0·156	0·813	0·031	0·000												
C. anguillaris	0·094	0·094	0·203	0·516	0·094												
Locus/allele	42	43	44	45	46	48	49	50	51	52	53	54	55	56	57	60	61
Cga07																	
C. gariepinus	0·031	0·063	0·031	0·219	0·031	0·031	0·094	0·094	0·231	0·000	0·031	0·000	0·000	0·063	0·031	0·000	0·000
C. anguillaris	0·203	0·031	0·078	0·219	0·047	0·000	0·000	0·031	0·063	0·016	0·047	0·156	0·047	0·016	0·000	0·016	0·031
Locus/allele	53	54	55	56	57	58	59	61	62	71							
Cga09																	
C. gariepinus	0·313	0·125	0·000	0·063	0·125	0·219	0·000	0·125	0·031	0·000							
C. anguillaris	0·016	0·016	0·063	0·109	0·125	0·359	0·109	0·078	0·000	0·125							
Locus/allele	15	16	17	18	19	20	21	24	25	26	28	29	32	33			
Cga10																	
C. gariepinus	0·250	0·031	0·031	0·219	0·219	0·219	0·031	0·000	0·000	0·000	0·0294	0·000	0·000	0·000			
C. anguillaris	0·000	0·000	0·078	0·000	0·000	0·047	0·000	0·344	0·219	0·156	0·031	0·031	0·031	0·063			

Alleles are named following the number of repeats.

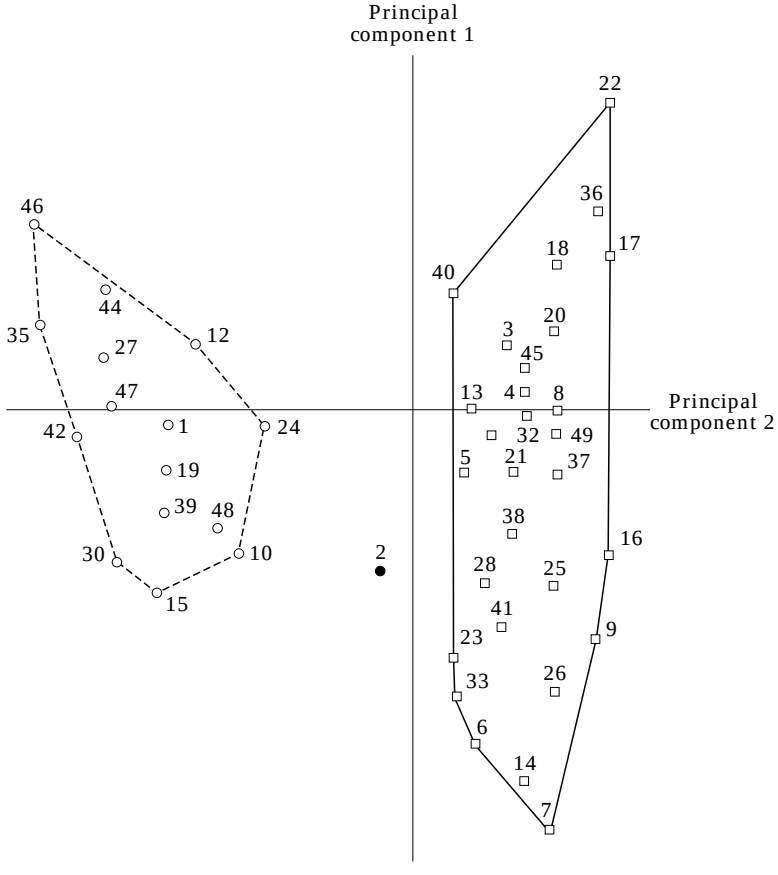

FIG. 5. Plot of the first and second principal components taken from a principal component analysis of a matrix of presence/absence of microsatellite alleles on sympatric populations of *Clarias gariepinus* (○, – – –) (n=16) and *C. anguillaris* (□, ——) (n=32). Specimen 2 is represented by a black spot.

one species were not clustered in the same part of the tree (Fig. 6). Thus, different haplotypes belonging to one species do not have a common origin. Due to this, therefore, unambiguous characterization was not possible with this approach.

Only allozymes and microsatellites allowed clear species discrimination. Nevertheless, as both species are very close (no diagnostic loci have been found), multifactorial analysis was the only method which permitted the recognition of two distinct groups. Without this analysis, it would be impossible to make a non-subjective discrimination between the two species.

As indicated above, specimen 2 occupied an intermediate position between *C. anguillaris* and *C. gariepinus* in three of the four assays. The possibility of the presence of a third species in our sample was excluded. The ichthyological diversity of the Senegal River Basin, one of the most explored basins in Africa, is fully known. Furthermore, Teugels (1982*a*, 1986) recognized only two valid species (*C. anguillaris* and *C. gariepinus*) in the morphologically well-defined subgenus *Clarias* (*Clarias*). Sufficient evidence was available to consider this specimen as an hybrid between the two species. Therefore, this specimen was an

TABLE V. Restriction site profiles and frequencies of mtDNA haplotypes (ND5/6 and D-loop regions) observed in *Clarias gariepinus* and *C. anguillaris* samples

mtDNA haplotypes	Restriction sites														Number of speciment	
															G	A
1	1101111	01111	101010111	10	1	111011110	1	1	0111	01011	111	111011011	11101010	101	1	0
2	1101111	00111	101010111	11	1	111011110	1	1	0111	01011	111	111011010	11101010	101	5	2
3	1101111	00111	101110111	11	1	011011110	1	1	0111	01011	111	111011010	10101010	101	1	6
4	1101111	01111	101010111	10	1	011011110	1	1	0111	01011	111	111011011	11101010	101	1	0
5	1101111	00111	101010111	10	1	111011110	1	1	0111	01011	111	111011011	11101010	101	1	0
6	1101111	00111	101010111	10	1	111111110	1	1	0111	01011	111	111011011	11101010	101	1	0
7	1101111	01111	101010111	10	1	111011110	1	1	0111	01011	111	111101010	11101010	101	1	0
8	1101111	01111	101010111	11	1	111011110	1	1	0111	01011	111	111011010	11101010	101	1	0
9	1101111	10111	101010111	10	1	011011110	1	1	0111	01011	111	111011011	11101010	101	0	1
10	1101111	10111	101010111	10	1	111011110	1	1	0111	01011	111	111011100	11101010	101	0	1

All the restriction sites obtained with the seven restriction enzymes are reported; order of enzymes: *Alu* I, *Hae* III, *Rsa* I, *Hpa* II, *Mbo* I, *Hinf* I, *Taq* I (for each enzyme, ND5/6 is given first).

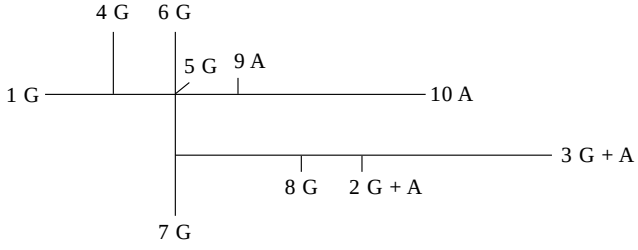

FIG. 6. Genetic relationships between the 10 mtDNA haplotypes observed: two are typical for *C. anguillaris* (A), seven are typical for *C. gariepinus* (G), two are common haplotypes (G+A). Bar=1 character.

F_1 rather than an F_n hybrid because it was positioned both morphometrically and genetically between the polygons of the two species (Figs 3, 4 and 5).

It is not possible to say whether specimen 2 was a natural hybrid (wild) or an artificial hybrid (fish culture). The collecting site, one of the Dagana channels, is man-made. It is connected with the Senegal River from which fish can enter freely but cannot return to the river due to the presence of canal locks. During the reproductive season, the fish can no longer perform their lateral migrations towards the inundated plains which were their natural reproduction sites (Bruton, 1979). All this favoured hybridization. Finally, there was the possibility that the specimen escaped from a neighbouring fish culture station.

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