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Phylogenetic Relationships Between Eight African Species of Mormyriform Fish (Teleostei, Osteichthyes): Resolution of a Cryptic Species, and Reinstatement of *Cyphomyrus* Myers, 1960

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Key Word Index—Mormyridae; Gymnarchidae; elephantfish; allozyme; isozyme; electric organ discharge; phylogeny.

Abstract—Phylogenetic relationships between seven elephantfish species from five genera (Mormyridae) that are endemic to southern Africa, and the Nile knifefish of the sister family, Gymnarchidae, were determined using biochemical genetic data, behaviour (the waveforms of the electric organ discharge), morphology, and habitat and food preferences. The results confirmed the existence of an undescribed species of *Hippopotamyrus*, and that *H. ansorgii* and *H. discorhynchus* are not congeners. These distinctions had been suggested by differences in electric organ discharge waveforms and are here substantiated by morphological and genetic data. We recommend the reinstatement of *Cyphomyrus discorhynchus* (Peters, 1852). Genetic distance (D, Nei, 1972) values suggest confamilial genera (D average of 1.09 for all species studied, and 0.715 for southern African species), with the exception of the sympatric and congeneric species: *H. ansorgii* and *H.* sp. (D = 0.423). Phylogenies based on morphological and electrophoretic data are congruent. Copyright © 1996 Elsevier Science Ltd.

Introduction

Fishes of the order Mormyriformes are endemic to Africa and include two families, the monotypic Gymnarchidae, and at least 188 species of Mormyridae (Gosse, 1984). Mormyridae have unusually large brains relative to body weight, comparable to that of humans (Fessard, 1958; Bell and Szabo, 1986). Mormyridae and *Gymnarchus* both use their electric sense for orientation, prey detection and communication, and are both electrogenic and electroreceptive (Lissmann, 1958; Szabo, 1961, 1967; reviews, Hopkins, 1986; Kramer, 1990, 1994; Moller, 1995). Some mormyrids are popular with aquarists and serve as favourite bait for catching tigerfish (*Hydrocynus vittatus*), and some have been utilised to monitor changes in drinking water quality (Grove and Moller, 1979; Geller, 1984; Kunze and Wetzstein, 1988; Kunze, 1989). Mormyrids can be trained by rewarding them with food whenever an appropriate action is executed after a previously recorded electric organ discharge is played back (Kramer, 1979; Graff and Kramer, 1992).

Electric organ discharge (**EOD**) waveforms are species-specific and may be used for species recognition. Kramer (in press) discusses electroreception and communication in fishes, and found distinct **EOD** differences between the species from the genus *Hippopotamyrus*, and also between two phenotypes of *H. ansorgii*, which suggest that sympatric species exist for the latter. More species than are presently recognised might exist, and the systematic classification of these fish need review. The last major revision of the phylogenetic relationships of mormyrids was based on morphology and anatomy (Taverne, 1971a,b; 1972). The only

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previous study using allozyme data compared evolutionary relationships in West African mormyrids (Agnèse and Bigorne, 1992), and confirmed that electrophoretic studies can clarify evolutionary history and systematic relationships within the Mormyridae.

While allozyme data may provide insights into the interrelationships of higher taxa (above the species level), they should not be used to the exclusion of other information, i.e., data on anatomy, behaviour, development patterns, karyotypes, etc. (Shaklee and Whitt, 1981). The unstable nomenclature of some of the mormyrids inspired this multidisciplinary study of eight species which is based on a combined examination of 26 protein-coding loci, **EOD** waveforms, some food and habitat preferences, and morphological characters frequently used in identification keys.

Materials and Methods

Seven mormyrid species (Table 1) were collected from the Upper Zambezi River near Katima Mulilo (17°29'S, 24°18'E) and identified using the key by Skelton (1993). The **EOD** waveforms were recorded in the field in the original stream water (conductivity, 86–92 μS/cm at 17–24°C in September 1993; 55–70 μS/cm at 24.1–25.8°C in March 1994) immediately after their capture. A pair of low-impedance carbon electrodes oriented head-to-tail were connected to a differential preamplifier (1 Hz–100 kHz), and the output digitised (8 bit vertical resolution) using a digital storage oscilloscope (up to 10 MHz conversion rate) connected to a computer. **EOD**s stored on disk were corrected to 25°C using a Q_{10} value of 1.5 (Kramer and Westby, 1985). Table 1 shows the sample sizes for **EOD** analysis.

Muscle and liver samples were stored in liquid nitrogen and transported to the laboratory. For small species, the whole fish was frozen (e.g. the dwarf stonebasher, *Pollimyrus castelnaui*). Morphological data (Jubb, 1967; Bell-Cross and Minshull, 1988; Skelton, 1993; Kramer, unpublished findings; Kramer *et al.*, in prep.), **EOD** waveforms (Kramer, in press, and unpublished findings) and habitat and food preferences (Gilmore, 1979; Van der Waal, 1985; Heeg and Kok, 1988; Marrero and Winemiller, 1993) were used in combination with the electrophoretic data. Dorsal and anal fin rays, scale counts along the lateral line, and pericaudal scales were useful morphological characters to distinguish groups of mormyrids and to determine affinities. The morphological data was transformed to produce a cladogram by phylogenetic analysis using parsimony (**PAUP**, version 2.4, Swofford, 1985) as follows: a) dorsal fin rays more than 182 = 0 (species #1), 62–68 = 1 (species #2), 30–36 = 2 (species #3) and less than 27 = 3 (species #4–8); b) anal fin rays absent = 0 (species #1), 18–21 = 1 (species #2) and more than 21 = 2 (species #3–8); c) scales on the lateral line more than 90 = 0 (species #1), 80–90 = 1 (species #2), 46–75 = 2 (species #3–6, 8) and less than 41 = 3 (species #7); and caudal peduncle absent = 0 (species #1), scales around the caudal peduncle less than 21 = 2 (species #3–8) and 22–26 = 2 (species #2). Species are designated numerically (Table 1).

Gymnarchus niloticus from Nigeria was selected as an outgroup species. The precise geographical origin in Nigeria is unknown; all were less than 10 cm and obtained in one batch from a dealer near Frankfurt/Main airport. This species was chosen since the phylogenetic relationships between the southern African mormyrid genera are not known. *Gymnarchus niloticus* differs from other mormyriforms by lacking ventral, anal, and tail fins, and by its peculiar mode of locomotion by its long, undulating dorsal fin.

Tissue extracts were prepared and analysed by starch gel electrophoresis as described in Van der Bank *et al.* (1992). Locus nomenclature (Table 2) followed Shaklee *et al.* (1990) and Dando (1974) for **GPI**. Loci were numbered beginning at the anodal end of the gel, and cathodally migrating allozymes were designated by a minus sign. The following buffer systems were used: **HC**—a continuous histidine, citrate buffer, pH 6.5, (Kephart, 1990); **MF**—a continuous Tris, boric acid, EDTA buffer, pH 8.6, (Markert and

TABLE 1. SPECIES STUDIED, AUTHORITIES, COMMON NAMES, SAMPLE SIZES FOR ELECTROPHORETIC ANALYSIS AND SAMPLE SIZES FOR EOD ANALYSIS IN BRACKETS (SEX CONFIRMED BY HISTOLOGY OR GONAD INSPECTION)

Species	Authority	Common name	*n*
1 *Gymnarchus niloticus*	Cuvier, 1829	Nile knifefish	15 (30)
2 *Mormyrus lacerda*	Castelnau, 1861	Western bottlenose	10 (10)
3 *Hippopotamyrus discorhynchus*	(Peters, 1852)	Zambezi parrotfish	10 (8)
4 *Hippopotamyrus ansorgii*	(Boulenger, 1905)	Slender stonebasher	2 (4) (2♀)
5 *Hippopotamyrus* sp.			9 (14) (2♀; 3♂)
6 *Marcusenius macrolepidotus*	(Peters, 1852)	Bulldog	8 (85) (47♀; 30♂)
7 *Petrocephalus catostoma*	(Günther, 1866)	Churchill	10 (37) (9♀; 13♂)
8 *Pollimyrus castelnaui*	(Boulenger, 1911)	Dwarf stonebasher	10 (14)

TABLE 2. LOCUS ABBREVIATIONS, ENZYME COMMISSION NUMBERS (E.C. NO.), TISSUE AND BUFFERS GIVING THE BEST RESULTS ARE LISTED AFTER EACH PROTEIN. SEE MATERIAL AND METHODS FOR ABBREVIATIONS OF BUFFERS USED

Protein	Locus	E.C. No.	Tissue	Buffer
Adenylate kinase	AK	2.7.4.3	L,M	MF,P,HC
Alcohol dehydrogenase	ADH	1.1.1.1	L	MF,P
Aspartate aminotransferase	mAAT	2.6.1.1	L,M	MF
	sAAT		L,M	MF
Creatine kinase	CK-A	2.7.3.2	M	MF,TC
Esterase	EST	3.1.1.-	L,M	MF,HC,P
General protein	PROT-1,-2,-3		L,M	MF
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1.2.1.12	M	MF
Glycerol-3-phosphate dehydrogenase	G3PDH	1.1.1.8	L,M	MF,RW
Glucose-6-phosphate isomerase	GPI-A	3.5.1.9	M	MF
	GPI-B		L,M	MF
L-Lactate dehydrogenase	LDH-A	1.1.1.27	L,M	RW
	LDH-B		M	RW
Malate dehydrogenase	sMDH	1.1.1.37	L,M	P
Malic enzyme	sMEP	1.1.1.38	L,M	MF
Mannose-6-phosphate isomerase	MPI	5.3.1.8	L,M	MF,TC
Peptidase:		3.4.-.-		
Substrate: Glycyl-L-leucine	PEPA		L,M	MF
Leucyl-glycyl-glycine	PEPB		L,M	RW
Leucyl-tyrosine	PEP-LT1,-LT2,-LT3		L,M	MF
Peroxidase	PER	1.11.1.7	L,M	MF,P
Phosphoglucomutase	PGM	5.4.2.2	L,M	MF
Superoxide dismutase	sSOD	1.15.1.1	L,M	MF

L = liver, M = muscle.

Faulhaber, 1965); **P**—a discontinuous Tris, citric acid (gel 8.7), NaOH, boric acid (electrode pH 8.2) buffer (Poulik, 1957); **RW**—a discontinuous Tris, citric acid, (gel pH 8.7), lithium hydroxide, boric acid (electrode pH 8.0) buffer (Ridgway *et al.*, 1970); and **TC**—a continuous Tris, citric acid (pH 6.9) buffer system (Whitt, 1970).

Statistical analysis of allozyme data was executed using **BIOSYS-1** (Swofford and Selander, 1981) and a computer program written by W.S. Grant (University of the Witwatersrand, Johannesburg). Genetic distances, **D** (Nei, 1972), $\mathbf{D_{78}}$ (Nei, 1978), $\mathbf{D_R}$ (Rogers, 1972) and $\mathbf{D_C}$ (the chord distance coefficient of Cavalli-Sforza and Edwards, 1967), were calculated. Nei's (Nei, 1972, 1978) nonmetric coefficients measure biological properties, whereas $\mathbf{D_R}$ and $\mathbf{D_C}$ have no biological premise and satisfy the triangle inequality. The latter matrix ($\mathbf{D_C}$) has the added advantage that it incorporates some realistic assumptions about the nature of evolutionary change in gene frequencies (Rogers, 1986). These genetic distances were used to produce phenograms generated by the unweighted pair group arithmetic average clustering method (**UPGMA**) of Sneath and Sokal (1973).

For the cladistic analysis, we followed recommendations of Mickevich and Mitter (1981, 1983) and Mickevich and Johnson (1976). The latter authors argued that the presence or absence of an allele is of more fundamental evolutionary importance than its frequency. We used this approach with the **MIX** procedure in **PHYLIP** (Felsenstein, 1993), with loci treated as characters and allele combinations as character-states. We used **PAUP** (Swofford, 1985) and a transformed data set, using the method of Green (1986), to construct a cladogram. **FREQPARS** (Swofford and Berlocher, 1987) was used to infer a parsimony tree directly from allelic frequencies, and **FITCH** (in **PHYLIP**, using **D** values) was used to produce cladograms. The cladogram produced by **FREQPARS** was scaled by the numbers of character-state changes across loci, and **CONSENSE** (in **PHYLIP**) was used to produce a consensus tree. The different clustering methods were used to produce dendrograms because there is still some debate as to the best way to analyse this type of data (e.g. Sober, 1993; Hillis *et al.*, 1993).

Results

Figure 1 depicts **EOD** waveforms for the mormyriform species studied. The outgroup, *G. niloticus*, was the only species with a "wave" discharge, that is, similar to a sine wave of constant frequency (depending on the individual, about 220–400 Hz). All other species of the sister family Mormyridae, generate "pulse" **EOD**s of short duration, at irregular intervals and usually lower rates (see Kramer, 1990).

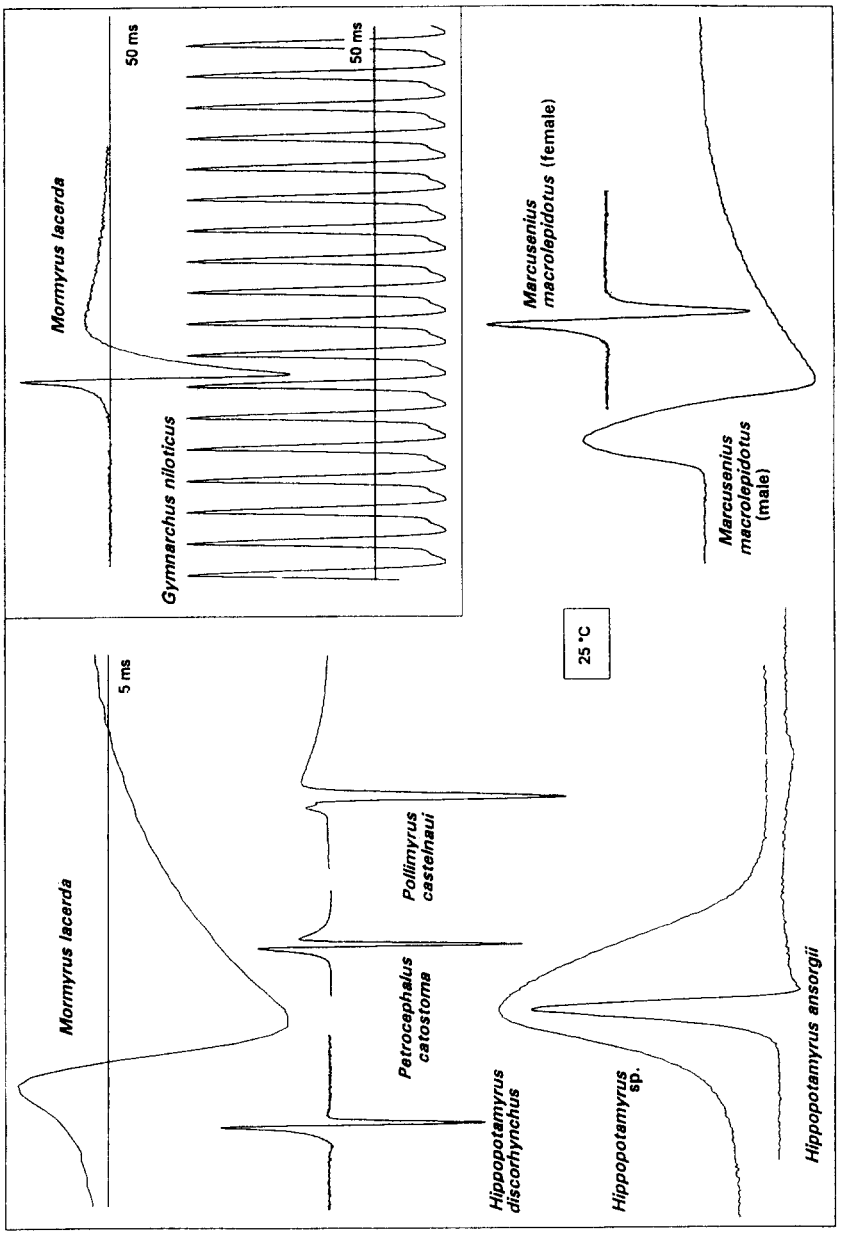

FIG. 1. ELECTRIC ORGAN DISCHARGES (**EODS**) FOR SEVEN MORMYRID SPECIES FROM SOUTHERN AFRICA AND OF *GYMNARCHUS NILOTICUS* (GYMNARCHIDAE) FROM WEST AFRICA (HEAD-TO-TAIL POTENTIAL DIFFERENCES IN VOLT; HEAD-POSITIVITY IS UPWARDS; ABSCISSA TIME; AND TIME BARS ARE USED TO INDICATE THE 0-V-BASELINE WHERE NECESSARY). All **EODS** are corrected to 25°C using a Q_{10} of 1.5, and scaled to the same oscilloscope sweep speed (time bar, 5 ms), except insert (time bar, 50 ms), which shows the difference between wave and pulse **EODs**.

The **EOD** activity of the southern African mormyrids studied is in agreement with the general pattern of **EOD** activity established for western and central African species. However, the southern African species' **EOD** waveforms are distinct, and in some cases unique. For example, the **EOD** of *M. lacerda* was of exceptionally long duration, longer than any other mormyrid **EOD** known before. **EOD** duration of the other species was in a more conventional range, except in adult *M. macrolepidotus* males that had **EOD**s up to nine times that of the average duration of females (N = 47). This is the first clear case of **EOD** sexual dimorphism in a mormyrid (Kramer, in press, and unpublished findings). Although individual variation in **EOD** waveforms occurred, no qualitative differences were found within the species studied. Sexual dimorphism in *H. ansorgii* and *H.* sp. does not apply since in *H.* sp. fish from both sexes were present, as confirmed by gonad histology (Table 1).

The **EOD**s of all mormyrids studied up to now (review Kramer, 1990) have little or no D.C. component, because positive potentials are balanced by negative ones, with the exception of *Mormyrus* species that all seem to have a more prominent head-negative **EOD** phase (also seen in *M. lacerda* of the present study, and *M. macrolepidotus* males (Kramer, unpublished findings)). Almost pure D.C. pulses like those generated by *H. ansorgii* and *H.* sp. of the present study were never seen in adult mormyrids before, although similar **EOD**s are known from larvae of the West African species *Pollimyrus adspersus* (erroneously considered *isidori* by all or most members of the "electric fish community" (J.D. Crawford, pers. comm. 1995)) up to an age of about 60 days, when their distinct larval electric organ degenerates and the developing adult organ becomes functional (Kirschbaum, irschbaum, 1977; Westby and Kirschbaum, 1977, 1978; Postner and Kramer, 1995).

A tree (Fig. 4) produced using dorsal and anal fin rays, scale counts along the lateral line, and around the caudal peduncle shows that the clade containing species #4–8 (*P. castelnaui, P. catostoma, H. ansorgii, H.* sp. and *M. macrolepidotus*) was separated from the monophyletic groups: *H. discorhynchus, M. lacerda* and the outgroup, respectively. Anatomical and morphological similarities between *P. castelnaui* and *P. catostoma* (Table 6) and between *H. ansorgii* and *H.* sp. are useful as quantitative characters to separate these groups, and *M. macrolepidotus*. The latter species differs from the others in having a well-developed chin, and the only conspicuous morphological difference between *H. ansorgii* and *H.* sp. is the number of scales around the caudal peduncle (16 and 20, respectively; Kramer *et al.*, in prep.).

Locus abbreviations, enzyme commission numbers, tissues and buffers giving the best results are listed in Table 2 and electrophoretic data (relative mobilities of alleles and allele frequencies) are presented in Table 3. Figure 2 shows isozyme and allozyme differences between species. Allele frequencies conformed to expected Hardy-Weinberg proportions at all loci, with $\chi^2 < 3.841$ (the critical value at $P < 0.05$ and the smallest degrees of freedom). Several loci (guanine deaminase (E.C. 3.5.4.3), isocitrate dehydrogenase (E.C. 1.1.1.42) and L-iditol dehydrogenase (E.C. 1.1.1.14) **sMDH-2** and **PEPA-2**) could not be scored satisfactorily in all samples. Allozyme phenotypes of putative heterozygotes were congruent with those expected on the basis of the quaternary structure of the enzyme (Ward, 1977).

The mean number of alleles per locus ranged from 1–1.23 (with a maximum of four alleles at **MPI** in *P. castelnaui*), and the average percentage of polymorphic loci ranged from 0–23.08%. The average heterozygosity values per locus ranged from 0–8.3%. Genetic distances, **D**, $\mathbf{D_{78}}$ and $\mathbf{D_C}$ values (Table 4), and $\mathbf{D_R}$ values were also calculated from allele frequencies, based on information at all 26 protein

TABLE 3. ALLELE FREQUENCIES AND RELATIVE MOBILITIES OF ALLELE PRODUCTS DETECTED AT 26 PROTEIN CODING LOCI IN EIGHT MORMYRIFORM SPECIES DESIGNATED NUMERICALLY (TABLE 1)

Locus	Species							
allele	1	2	3	4	5	6	7	8
AK								
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADH								
100	1.000						1.000	1.000
−20				1.000	1.000			
−50						1.000		
−60			1.000					
−100		1.000						
mAAT								
−105	1.000							
−100		1.000	1.000	1.000	1.000	1.000		
−95							1.000	1.000
sAAT								
110	1.000							
100							1.000	
95		0.063		1.000	1.000	1.000		1.000
90		0.937	0.937					
85			0.063					
CK-A								
103							1.000	
100		1.000	1.000	1.000	1.000	1.000		1.000
95	1.000							
EST								
110						1.000		
105								1.000
100	1.000			1.000			1.000	
90			1.000		1.000			
85		0.571						
80		0.429						
GAPDH								
110	1.000							
100		1.000	1.000	1.000	1.000	1.000	1.000	1.000
G3PDH								
110					1.000			
105		1.000					1.000	
100			1.000	1.000		1.000		1.000
90	1.000							
GPI-A								
105								1.000
100	1.000	1.000		1.000				
95			0.937		1.000	1.000		
92							1.000	
90			0.063					
GPI-B								
110						1.000		
100		1.000	0.750		1.000		1.000	1.000
90			0.250	1.000				
LDH-A								
105							1.000	
100	1.000							
95		1.000	1.000	1.000	1.000	1.000		1.000
LDH-B								
105			1.000				1.000	1.000
100		1.000		1.000	1.000	1.000		
90	1.000							
sMDH								
120							0.714	
110	0.909							
100	0.091	1.000	1.000	1.000	1.000	1.000	0.286	1.000

TABLE 3. —*CONTINUED*

Locus allele	Species 1	2	3	4	5	6	7	8
sMEP								
102		1.000						
100	1.000							
95				1.000	1.000	1.000	1.000	1.000
92			1.000					
MPI								
105	1.000		0.056					
102		1.000	0.944		1.000	1.000		0.125
100				0.500				0.312
95				0.500			1.000	0.500
90								0.063
PEPA								
105	1.000							
100		1.000				1.000		
95			1.000	1.000	1.000		1.000	1.000
PEPB								
105							1.000	
100		1.000	1.000	1.000	1.000	1.000		1.000
95	1.000							
PEP-LT1								
100		1.000	1.000				1.000	
90				1.000	1.000	1.000		1.000
PEP-LT2								
100	1.000	1.000						
97						1.000		
95			0.250	0.250	0.250			
90			0.750	0.750	0.750		1.000	1.000
PEP-LT3								
110		1.000						
100	1.000							
95			1.000	1.000	1.000	1.000	1.000	1.000
PER								
120	1.000							
115						1.000		
100		1.000	1.000	1.000	1.000		1.000	1.000
PGM								
105			1.000	1.000		1.000		0.500
100		1.000					1.000	0.500
95	1.000							
90					1.000			
PROT-1								
105							1.000	1.000
100				1.000				
90	1.000	1.000	1.000		1.000	1.000		
PROT-2								
105				1.000				
100		1.000	1.000			1.000	1.000	
98								1.000
95					1.000			
PROT-3								
103							1.000	
100		1.000	1.000	1.000	1.000	1.000		1.000
90	1.000							
sSOD								
120		1.000	0.750					
110		0.250			1.000	1.000	1.000	
105				1.000				
90	1.000		1.000					

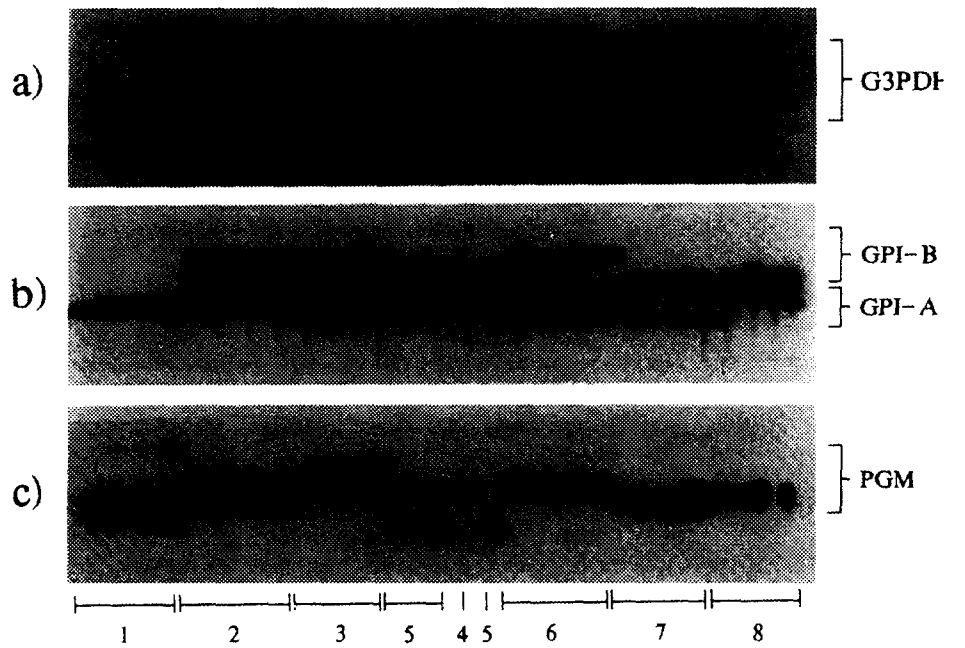

FIG. 2. ZYMOGRAMS EXHIBITING MOBILITY DIFFERENCES OF ALLELES AT THE FOLLOWING LOCI: a) GLYCEROL-3-PHOSPHATE DEHYDROGENASE (**G3PDH**), b) GLUCOSE-6-PHOSPHATE ISOMERASE (**GPI-A**, **-B**), AND c) PHOSPHOGLUCO-MUTASE (**PGM**). Four individuals each of the hitherto recognised mormyriform species studied are compared. Species are designated numerically (Table 1).

TABLE 4. MATRIX OF GENETIC DISTANCE COEFFICIENTS BETWEEN EIGHT MORMYRIFORM SPECIES: CAVALLI-SFORZA AND EDWARDS' (1967) CHORD DISTANCE (BELOW LEFT), NEI'S (1972) GENETIC DISTANCE (ABOVE RIGHT) AND NEI'S (1978) GENETIC DISTANCE FOR SOUTHERN AFRICAN MORMYRIDS (BOLD)

Species	1	2	3	4	5	6	7	8
1 *G. niloticus*	—	1.794	2.422	1.789	2.470	2.477	2.100	2.455
2 *M. lacerda*	0.822	—	0.488	0.826	0.669	0.677	1.124	0.953
			0.485	**0.824**	**0.668**	**0.676**	**1.123**	**0.950**
3 *H. discorhynchus*	0.855	0.568	—	0.582	0.433	0.512	0.915	0.542
				0.579	**0.431**	**0.510**	**0.912**	**0.537**
4 *H. ansorgii*	0.823	0.678	0.599	—	0.423	0.478	1.159	0.529
					0.421	**0.478**	**1.158**	**0.526**
5 *H.* sp.	0.860	0.630	0.535	0.530	—	0.533	1.089	0.468
						0.532	**1.087**	**0.464**
6 *M. macrolepidotus*	0.860	0.630	0.574	0.558	0.586	—	1.412	0.624
							1.411	**0.621**
7 *P. catostoma*	0.844	0.738	0.689	0.741	0.726	0.779	—	0.569
								0.565
8 *P. castelnaui*	0.860	0.699	0.578	0.580	0.545	0.610	0.587	—

coding loci (Table 3). The values of **D** and $\mathbf{D_{78}}$ are essentially the same. However, it is not possible to calculate $\mathbf{D_{78}}$ values for species where isozymes were not detected (e.g. the outgroup). **D** values are discussed in order to compare results obtained by other authors for conspecific populations, congeneric species and confamilial genera. These values ranged from 0.423–2.470 and from 0.530–0.860 for $\mathbf{D_C}$ between species (Table 4). The smallest values for both indices were

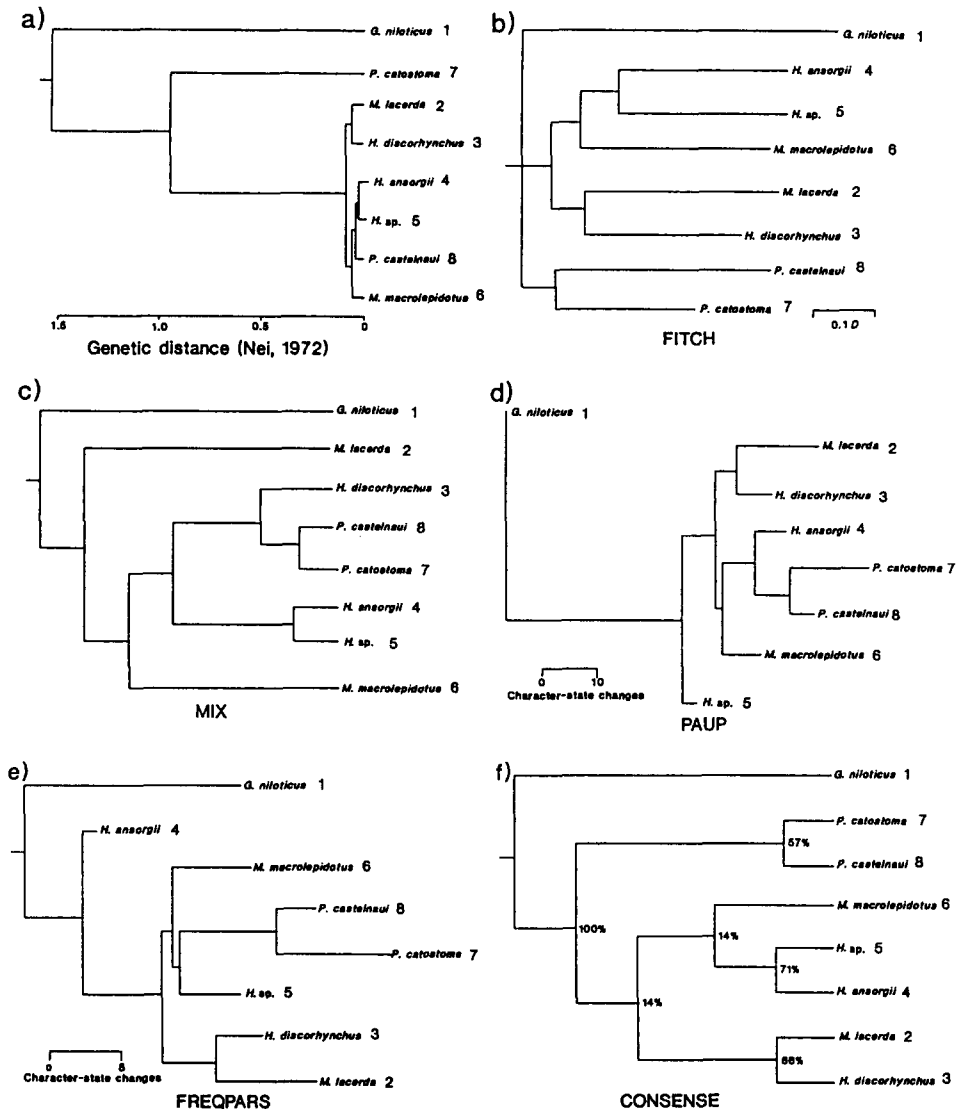

FIG. 3. DENDROGRAMS USING ALLELE FREQUENCY DATA FOR EIGHT MORMYRIFORM SPECIES (DESIGNATED NUMERICALLY IN TABLE 1) PRODUCED BY: a) **UPGMA** AND D VALUES; b) **FITCH**; c) **MIX**; d) **PAUP**; e) **FREQPARS**; AND f) **CONSENSE** (THE VALUES AT EACH FORK INDICATE THE PERCENTAGE RESOLUTION OF THE GROUP, WHICH CONSISTS OF THE SPECIES TO THE RIGHT FROM THE FORK).

between *H. ansorgii* and *H.* sp., and the largest values were obtained between the outgroup (*G. niloticus*) and the other species from southern Africa (Table 4).

The phenogram constructed using **D** values (Table 4) is shown in Fig. 3a. Similar phenetic relationships were produced with $\mathbf{D_{78}}$, $\mathbf{D_R}$ and $\mathbf{D_C}$ compared to those presented in Fig. 3a using **UPGMA**. Figures 3b–e depict cladistic relationships among the species studied using **FITCH, MIX, PAUP** and **FREQPARS**, respectively. A consistency index of 69% was obtained when character-states (Table 5) were used to produce Fig. 3d. A cladogram (Fig. 3f) produced from the data generated in Figs 3a–e, $\mathbf{D_R}$ and $\mathbf{D_C}$ values grouped: *H. ansorgii* with *H.* sp., *P. catostoma* with *P. castelnaui*, and *M. lacerda* with *H. dis-*

TABLE 5. DATA MATRIX OF ALLOZYME CHARACTER-STATES FOR EIGHT MORMYRIFORM SPECIES, DERIVED FROM ISOZYME LOCI AS INDICATED IN TABLE 3. Species are designated numerically (Table 1)

	SPECIES							
LOCUS	1	2	3	4	5	6	7	8
ADH	0	4	3	1	1	2	0	0
mAAT	0	1	1	1	1	1	2	2
sAAT	0	3	4	2	2	2	1	2
CK-A	0	1	1	1	1	1	2	1
EST	0	0	0	0	0	2	0	1
	0	2	1	0	1	0	0	0
GAPDH	0	1	1	1	1	1	1	1
G3PDH	0	2	1	1	3	1	2	1
GPI-A	0	0	0	0	0	0	0	1
	0	0	2	0	1	1	3	0
GPI-B	0	3	2	1	3	4	3	3
LDH-A	0	0	0	0	0	0	1	0
	0	1	1	1	1	1	0	1
LDH-B	0	1	2	1	1	1	2	2
sMDH	0	1	1	1	1	1	2	1
sMEP	0	1	0	0	0	0	0	0
	0	0	2	1	1	1	1	1
MPI	0	2	1	3	2	2	3	3
	0	0	0	2	0	0	3	1
PEPA	0	1	2	2	2	1	2	2
PEPB	0	1	1	1	1	1	2	1
PEP-LT1	0	2	2	1	1	1	2	1
PEP-LT2	0	0	2	2	2	1	3	3
PEP-LT3	0	1	0	0	0	0	0	0
	0	0	1	1	1	1	1	1
PER	0	2	2	2	2	1	2	2
PGM	0	1	3	3	0	3	1	2
	0	0	0	0	1	0	0	0
PROT-1	0	0	0	1	0	0	2	2
PROT-2	0	3	3	4	1	3	3	2
PROT-3	0	1	1	1	1	1	2	1
sSOD	0	4	3	0	1	2	2	2

corhynchus. Weak affinities occurred between the former group and *M. macrolepidotus*, and between this group (Fig. 3f) and the group containing *M. lacerda* and *H. discorhynchus* (14%). *Hippopotamyrus discorhynchus* was constantly separated from the other two *H.* species (Figs 3a–f). Figure 4 presents a summary of relationships based on characters obtained from this study, and additional data from other sources (Table 6).

Discussion

The waveforms of electric organ discharge are species-specific, and the different **EOD**s (Fig. 1) observed can show relatedness. For example, the bipolar waveform obtained for *H. discorhynchus* is in contrast with the monopolar **EOD**s obtained for the other two *H.* species. This suggests that they do not belong to the same genus. It is also evident that a sympatric species of *H. ansorgii* exists. Other **EOD** differences (to identify groups and/or individual species) are summarised in Table 6. The number of pericaudal scales in *H. ansorgii* is 16 (Bell-Cross and Minshull, 1988; confirmed by P. Skelton in type material, pers. comm.) and 20 in *H.* sp. (Kramer *et al.*, in prep.; $N=16$), and the **EOD** of the former species is shorter than 0.5 ms at 10% of the peak amplitude, compared to longer than 1.0 ms in *H.* sp. (Fig. 1). Other distinct differences between species are allele products that migrated faster at the **EST, PROT-1, -2, GPI-A** and **PGM**, and slower at the

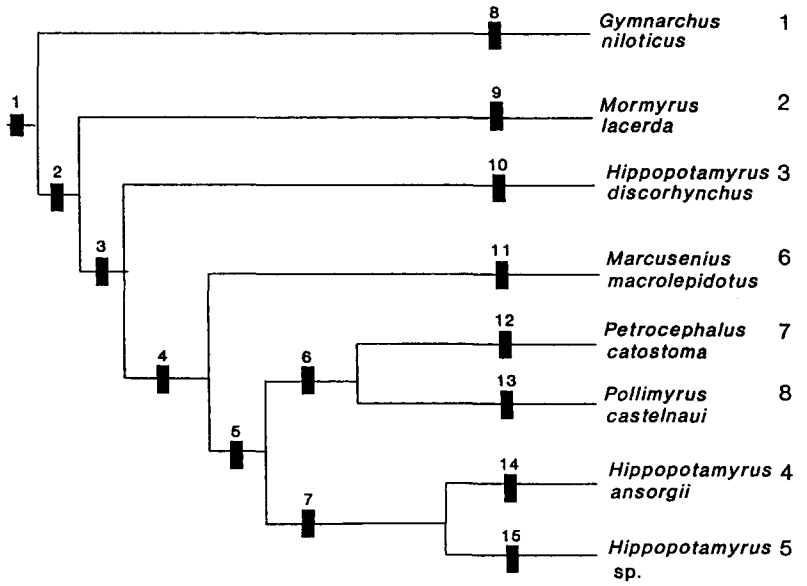

FIG. 4. CLADOGRAM PRODUCED FROM GENETIC, BEHAVIOURAL AND MORPHOLOGICAL DATA (NUMBERS NEXT TO SPECIES REFERS TO TABLE 1, AND PLOTTED ON THE TOPOLOGY REFER TO CHARACTERS LISTED IN TABLE 6).

G3PDH, GPI-B, MPI and **sSOD** protein-coding loci in *H. ansorgii* samples (Fig. 2, Table 3) compared to those of *H.* sp. It is interesting to note that the species that was more abundant in our samples was the undescribed species.

Habitat and diet preferences are similar for the species studied. The only exclusive habitat character was the preference of *Hippopotamyrus* for the bottom substrate of larger flowing rivers, and Decapoda as one of its principal food categories (Marrero and Winemiller, 1993). However, we found the habitat preference of this genus to extend to other genera (Kramer *et al.*, in prep.), and that *H. discorhynchus* prefers river channels with soft bottom and fringing vegetation whereas both *H. ansorgii* and *H.* sp. prefer rocky habitats in currents. In addition, *H. discorhynchus* is a nocturnal shoaling species (Skelton, 1993), and shows normal aggression for mormyrids in aquaria, whereas the other *H.* species are extremely intolerant of each other (A. Scheffel, personal observations). Shared morphological, behavioural and allelic characteristics (Table 6) were also useful to establish affinities.

Our results (Fig. 2, Table 3) are similar to those of Agnèse and Bigorne (1992), who studied enzyme variability at 16 presumptive loci in West African mormyrids. We also obtained one locus each for **AK, CK, MEP** and **PGM**, two loci for **GPI** and **LDH**, three for **PROT**, and polymorphism at **AAT, GPI, MDH** and **PGM** (Table 3). Agnèse and Bigorne (1992) report **D** values of 1.07–1.99 between *Pollimyrus* and *Petrocephalus*, 0.39–0.40 between *Pollimyrus* and *Marcusenius*, 0.06 between *Pollimyrus* and *Hippopotamyrus*, 0.63–1.35 between *Petrocephalus* and *Marcusenius*, 0.27–0.47 between *Marcusenius* and *Hippopotamyrus*, and 0.98–2.06 between *Petrocephalus* and *Hippopotamyrus*. These values are not congruent with our values (0.569, 0.624, 0.468–0.542, 1.412, 0.478–0.533, 0.915–1.159) for corresponding genera (Table 4). This is not unexpected since we studied more loci and different species. It is also conceivable that their limited sample size (e.g. one individual each from the genera *Hippopotamyrus* and *Pollimyrus*) contributed to produce different **D** values compared to our estimates. Nevertheless, we obtained similar results when a phenetic approach (Fig. 3a) was employed (i.e.

TABLE 6. a) BIOCHEMICAL, b) BEHAVIOURAL, AND c) MORPHOLOGICAL, PHYSIOLOGICAL AND ECOLOGICAL DATA TO DEFINE CLADES AND SPECIES ANALYSED: CHARACTERS 1–7 ARE SHARED AND 8–15 ARE UNIQUE FOR THE SPECIES STUDIED (FIG. 4)*

Characters	Description
1	a) Allozyme products of identical mobility: AK[25]; D value average 1.062[26] (typical for confamilial genera)[22]. b) No or little net current associated with bipolar EOD[2,8,11a]. c) Entirely restricted to fresh waters from the African continent[5,14]; nearly exclusive occupation of a nocturnal foraging/electrogeneration-reception niche[8,11a,12]; weak electric organs[2,19]; ampullary electroreceptors sensitive for weak, electric AC-fields of low-frequency[3,20]; two types of high-frequency tuberous electroreceptors[3,21,23]; enormous brain, especially cerebellum[8,23].
2	a) Allozyme: GAPDH[25]; isozymes present at all loci studied[25]; group always separated from *G. niloticus*[29]. b) EOD of the pulse- rather than the wave-type[27]. c) Mormyridae: dorsal, anal, tail and pelvic fins present[10]; feeding on small invertebrates; good eye-sight despite nocturnal habits[17,24]; eggs small diameter[16]; number of dorsal fin rays less than 76 and pericaudal scales less than 26[16] (for southern African mormyrids)[9,16]; no invaginations of electroreceptor cell membrane[21]; electric organ: both faces of electrocytes usually excitable[2]; mormyrid skin of exceptionally high resistance (layer of hexagonally arranged, flattened cells on top of stratum spinosum)[2,21]; tuberous electroreceptors in the form of Knollenorgane and mormyromasts[3,21,23]; extremely compact electric organ restricted to caudal peduncle[2,19]; electric organ: four columns of electrocytes[2,19,21]; valvula cerebelli (electroreceptive region) covers the whole brain[11b].
3	a) Allozyme: PEP-LT3*95[25]. b) EODs of short duration[6,8]. c) Anal fin rays more than 21, lateral line scales less than 76; pericaudal scales less than 21[16]; number of dorsal fin rays less than 37[1,7,9,16].
4	a) Allozyme: sMEP*95[25]. c) Dorsal fin posterior to, and shorter than anal fin, number of dorsal fin rays less than 27[1,7,9,10,16].
5	b) Short triphasic or monophasic rather than biphasic EOD.
6	a) Allozymes: mAAT*-95; PROT-1*95[25]. b) Triphasic EOD with head-negative main phase[27]. c) Morphologically similar[1,7,16], head rounded[1,7,16]; food different from other southern African mormyrids: microcrustacea (Cladocera, Copepoda, Ostracoda)[12].
7	a) Allozyme: ADH*-20[25]; D value between species of 0.423[26] (typical for congeneric species)[22]. b) EODs monopolar; head-positive[27]. c) Morphologically similar[9]; habitat: the bottom substrate and rocky habitats of larger flowing rivers[12]; food preference different from other mormyrids: prawns (Decapoda)[12]; high intraspecific aggression[15].
8	a) Isozymes not detected: GPI-B; PEP-LT1; PROT-2; allozymes: fixed allele differences at mAAT; sAAT; CK-A; GAPDH; G3PDH; LDH-A, -B; sMEP; PEPA, PEPB, PEP-LT3; PER; PGM; PROT-3[25,28]. b) EOD is wave-type[27]. c) Gymnarchidae: anal, tail and pelvic fins absent[10,13]; number of dorsal fin rays greater than 182 and scales on the lateral line more than 90[10]; teeth absent from parasphenoid and tongue[13]; four (rather than 6–8) branchiostegal rays[13]; electric organ: uninnervated face of electrocytes is unexcitable[2]; tuberous electroreceptors unique (Gymnarchomast types I and II)[21]; electric organ in tail in four pairs of columns up to 1/3 of the length of the body[2,18]; electroreceptor cells with deep, microvilli-covered invaginations of the apical surface[21]; valvula cerebelli covers approximately 1/3 of the brain[11b]; builds floating nests[4]; huge eggs of 1 cm diameter[4]; very poor vision (almost blind)[11b,30]; unique prey-catching behaviour (piscivorous ambush predator)[11a,30]; obligatory air-breather with modified swim bladder for this purpose[11a,15]; modified circulatory system[13].
9	a) Allozymes: fixed allele differences at ADH; EST; sMEP; PEP-LT3[25]. b) EOD distinct: long duration, strong amplitude[27] (felt by wet fingers on caudal peduncle). c) Dorsal fin greater than three times (3.7) the length of anal fin[9]; number of dorsal fin rays greater than 61, up to 68[1,7,9,16]; its origin near snout; mouth bottle-like; A 18–21, scales on lateral line 80–90, pericaudal scales 22–26[1,7,16].
10	a) Allozymes: fixed allele differences at ADH; sMEP[25]. c) Origin of dorsal fin anterior to anal fin; dorsal fin up to 1.8 times length of anal fin[9]; number of dorsal fin rays greater than 29, up to 36[1,7,9,16].
11	a) Allozymes: fixed allele differences at ADH; EST; GPI-B; PEP-LT2; PER[25,28]. b) EOD sexually dimorphic[27]. c) Chin well-developed[1,7,9,16].
12	a) Allozymes: fixed allele differences at sAAT; CK-A; GPI-A; LDH-A; PEPB; PROT-3[25,28]. c) Electroreceptor organ rosettes around the eye[11a]; mouth inferior; no chin; nares close together; scales on the lateral line less than 41[1,7,9,16].
13	a) Allozymes: fixed allele differences at EST; GPI-A; PROT-2[25,28]. c) Mouth subterminal; no chin; nares widely spaced[1,7,9,16]; unique hiding behaviour during the day in surface-floating vegetation, or among rocks[15].
14	a) Allozymes: fixed allele differences at PROT-1, -2[25]. b) EOD distinct: monopolar, short duration[27]. c) Regarded as rare[1].
15	a) Allozymes: fixed allele differences at G3PDH; PGM; PROT-2; sSOD[25,28]. b) EOD distinct: monopolar, long duration[27].

*See References on facing page.

Pollimyrus is closely related to the two *Hippopotamyrus* sibling species, but not *H. discorhynchus*, and these genera are closer to *Marcusenius* than to *Petrocephalus*). Likewise, Agnèse and Bigorne (1992) found that species of *Petrocephalus* and *Marcusenius* were genetically distinct whereas species of *Hippopotamyrus* and *Pollimyrus* were closely related.

For fish, Shaklee *et al.* (1982) found that **D** between pairs of species from the same genus ranged from 0.03–0.61 (average = 0.3), and **D** between genera in the same family ranged between 0.58–1.21. Thorpe (1982) and Thorpe and Solé-Cava (1994) report **D** values of more than 0.55 for confamilial genera and less than 0.3 for conspecific populations. The **D** value we obtained between *H. ansorgii* and *H.* sp. (0.423) is thus above average and close to the upper margin given by Shaklee *et al.* (1982) for congeneric species, and much larger than the average value (0.05) predicted for conspecific populations. This result, together with the distinct allozyme differences (Fig. 2, Table 3), clearly demonstrates the presence of a new species of *Hippopotamyrus* which we are describing (Kramer *et al.*, in prep.). From genetic distance values (Table 4), morphological differences (Table 6) and **EOD** waveforms (Fig. 1), it is also evident that *H. discorhynchus* should not be included with the other species of *Hippopotamyrus*. *Cyphomyrus, Marcusenius, Mormyrus* and *Petrocephalus* are genus names previously assigned to *H. discorhynchus*; in addition, there were several synonymies (Gosse, 1984). The dorsal length of the fin is smaller, and its origin posterior to that of the anal fin in representatives from these genera, with the exception of *Cyphomyrus* (and *H. discorhynchus*). Due to this important morphological difference, and because of sufficient genetic differences (Tables 3 and 4) between the latter species and the other genera studied, we propose the reinstatement of *Cyphomyrus discorhynchus* (Peters, 1852) as described by Myers (1960).

The cladistic trees (Figs 3b–e), in contrast to the phenetic methods, consistently clustered *P. catostoma* with *P. castelnaui*, and these relationships are reflected in the consensus tree (Fig. 3f). The grouping of these two species is congruent with morphological similarities, which previously motivated the grouping of these two species in the same genus (Jubb, 1967). Phenetic and cladistic methodologies produced different trees (Figs 3a, b–e), and the cladistic trees are more consistent with relationships between species previously established on morphological grounds. This is concordant with results obtained by other researchers who accept cladistics as the more rigorous approach to the study of phylogenetic relationships (e.g., Davis and Nixon, 1992). Figure 3f produced partial resolution of relationships, with *H. discorhynchus* and *M. lacerda* (branch separated: 14% majority-rule) not delineated as expected, based on morphological grounds. It is therefore essential not to adopt a single analytical method, but rather attempt to understand the biological assumptions and statistical behaviour of each method.

Figure 4 is a tree based on genetic data and morphology, and it presents a summary of relationships based on data obtained from the present study, and additional data from other sources (Table 6). The cladogram produced from morphological data (see results), is concordant with relationships shown in Fig. 3c,

REFERENCES FOR TABLE 6, OPPOSITE.

References: Bell-Cross and Minshull (1981)[1]; Bennett (1971a[2], b[3]); Budgett (1901)[4]; Gosse (1984)[5]; Hopkins (1986)[6]; Jubb (1967)[7]; Kramer (1990)[8]; Kramer *et al.* (in prep.)[9]; Lévêque *et al.* (1990)[10]; Lissmann (1958[11a], 1963[11b]); Marrero and Winemiller (1993)[12]; Nelson (1984)[13]; Roberts (1975)[14]; A. Scheffel (personal observations)[15]; Skelton (1993)[16]; Somiya (1989)[17]; Srivastava and Szabo (1973)[18]; Szabo (1961[19], 1967[20], 1974[21]); Shaklee *et al.* (1982)[22]; Szabo and Fessard (1974)[23]; Teyssèdre and Moller (1982)[24]; Present study: Table 3[25]; Table 4[26]; Fig. 1[27]; Fig. 2[28]; Fig. 3[29]; personal observations[30].

except for the position of *H. discorhynchus*. This information, behaviour (Table 6) and relationships determined by genetic analysis (Fig. 3) was used to construct the tree in Fig. 4. The summary of shared and distinct characters (Table 6), can also be used to construct various keys (i.e., biochemical, behavioural and/or morphological) to identify members of the species studied. The results not only showed that a cryptic species is involved and that *H. discorhynchus* is a monophyletic genus in southern Africa, but also indicated clearly that multilocus protein electrophoresis and **EOD** waveform comparison could contribute significantly in understanding the affinities amongst mormyrid species. This study therefore not only reflects the evolution of the mormyrids studied, but also achieved congruence between different data sets (Fig. 4). Such consensus is desired (Shaklee and Whitt, 1981) to provide a truly comprehensive view of biotic evolution.

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