Inter- and intraspecific allozyme comparisons of mormyrids (Pisces, Mormyridae) from South Africa and Namibia, with reference to an undescribed species

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Abstract

Allozyme comparisons of allopatric populations of *Marcusenius macrolepidotus* and *Petrocephalus catostoma* (from the eastern Caprivi, Namibia, and the Kruger National Park, South Africa) showed little differentiation between the populations of the former species mentioned, but distinct differences between the two populations of *P. catostoma* studied. Three continuous and two discontinuous buffer systems were used, and gene products of 26 protein coding loci were examined by horizontal starch gel-electrophoresis. Fixed allele differences between *M. macrolepidotus* and *P. catostoma* were obtained at 13 of these loci. Allele frequency differences were found between allopatric populations of the former species, whereas distinct allozyme differences were found at seven of the loci studied in the latter species. This, together with the mean genetic distance value of 0.311, suggests the existence of an undescribed *P. catostoma* species from the Sabie River system. The unbiased genetic distance value among the *M. macrolepidotus* populations studied was 0.023, and it averaged 0.927 between the confamilial genera *Marcusenius* and *Petrocephalus*.

Introduction

There are 18 genera and approximately 200 species in the family Mormyridae in Africa (Skelton, 1993). These fishes have large brains, relative to body mass, comparable to those of humans. They use their electric sense for location and communication, they are popular with aquarists and they are a favourite bait among anglers for catching tigerfish. These fishes can also be trained by rewarding them with treats for executing the appropriate action upon receiving a previously tape-recorded electric organ discharge, and some mormyrids have been utilised to monitor changes in water quality (Van der Bank and Kramer, 1996).

Van der Bank and Van der Bank (1995) recommended that representatives of certain mormyrid genera should be analysed electrophoretically and compared, especially populations which show differences in electric organ discharge waveforms. Examples of populations that should be studied are *Marcusenius macrolepidotus* (Peters, 1852) from the Sabie and Zambezi River systems, *Pollimyrus castelnaui* (Boulenger, 1911) from the Zambezi and Kwando River systems, and *Hippopotamyrus ansorgii* (Boulenger, 1905) from the Zambezi River. It is possible that different races or species are involved because Kramer and Skelton (1995) observed distinct differences in electric organ discharge (EOD) waveforms between *M. macrolepidotus* from the Sabie River (South Africa) and from the Zambezi River (Namibia). More species than previously recognised might exist because **EOD**s are species-specific (Van der Bank and Kramer, 1996). Kramer (1996) indicated sexual dimorphism in *M. macrolepidotus* (i.e. two distinct forms of **EOD** were present), and a statistically significant difference exists in **EOD** waveforms, correlating with age and sex in *Petrocephalus catostoma* (Günther, 1866) from Namibia. The **EOD**s of *P. catostoma* from the Sabie River have not been studied before.

An electrophoretical analysis of such populations should provide a better understanding of the genetic divergence and biogeography of the snoutfishes. The purpose of this study is to use allozyme comparisons, of allopatric populations of *M. macrolepidotus* and *P. catostoma*, as an aid to taxonomy and systematics.

Materials and methods

Electrophoretic data for five *M. macrolepidotus* and four *P. catostoma* individuals from the Upper Zambezi River (17°29'S, 24°26'E) were compared with those of 15 and 8 individuals, respectively, from the Sabie River in the Kruger National Park (25°07'S, 31°53'E). The fish were sampled within a 10 km stretch of the rivers in the area indicated by the co-ordinates. Tissue extracts were prepared and analysed by starch gel electrophoresis (12% gels) using buffers, standard electrophoretic procedures, method of interpretation of gel-banding patterns and locus nomenclature as referred to by Van der Bank and Van der Bank (1995) and Van der Bank and Kramer (1996). Statistical analysis of allozyme data was executed using BIOSYS-1 (Swofford and Selander, 1981).

Results

Locus abbreviations, enzyme commission numbers, and monomorphic loci are listed in Table 1. Allele products at the following loci were monomorphic: **AK, CK-A, PEPA-1, PER, PROT-2** and **sSOD**. Allele frequencies for polymorphic loci are presented in Table 2. Allozyme phenotypes of putative heterozygotes were congruent with those expected on the basis of the quaternary structure of the enzyme (Ward, 1977). Thus heterozygotes at **GAPDH** and **LDH** were five-banded, triple-banded at **ADH, G3PDH, GPI** and **MDH**, as expected for dimeric enzymes, and heterozygotes at the monomeric enzymes **AAT, CK** and **EST** were double-banded. Zymograms of **GPI, LDH** and **MDH**,

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Received 6 December 1995; accepted in revised form 13 March 1996.

showing the quaternary structure of heterozygotes, as well as distinct differences between species in allele product mobilities are presented in Fig. 1. Fixed allele mobility differences between genera occurred at **sAAT, ADH-1, -2, CK-B, EST, G3PDH, GPI-B, LDH-A, -B, MPI, PEPA-2, PEP-LT** and **PROT-3**, and genetic markers to identify allopatric species of *P. catostoma* were observed at **GPI-A, -B, LDH-B, PEPB, PEB-LT, PGM** and **PROT-1** (Table 2).

The value of Wright's (1978) fixation index of individuals relative to the total population, F_{IS}, is 0.182; 0.883 for the total population and its subpopulations (F_{IT}) and F_{ST}=0.857 for the amount of differentiation among subpopulations relative to the limiting amount under complete fixation (Table 3). The loci that contributed least to inter- and intraspecific differences are **mAAT*** (0.220) and **sMDH*** (0.146) because all populations shared the most common allele at these loci (Table 3).

Nei's standard (1972) and unbiased (1978) genetic distance values between populations and taxa are presented in Table 4. The mean genetic distance (Nei, 1978) value between the two genera studied was 0.927, 0.311 between the *P. catostoma* populations, and 0.023 between the *M. macrolepidotus* populations studied. These values were 0.050, 0.30 and 0.58-1.21 respectively for Nei's (1972) genetic distance (Table 4). The latter values are included for comparisons with values listed in the literature. Phylogenetic relationships based on Nei's (1978) genetic distance values (Table 4) are depicted in Fig. 2. The cophenetic correlation value for the result in Fig. 2 is 97.1%.

Discussion

Genetic data, produced by electrophoresis, can be used by systematisists to determine if samples are from different gene pools, representing different species (Thorpe and Sòl-Cava, 1994). The taxonomic uses of allozyme electrophoretic data (in both alpha and beta systematics) were reviewed by Avise (1974), Thorpe (1982), Thorpe and Sòl-Cava (1994), and many other authors. These reviews give details of methods for distinguishing and identifying cryptic and sibling species. The distinction is due to genetic differentiation, which is to be expected for populations that are geographically separated so that little or no gene flow can occur between them. The criterion which must be applied if electrophoretic data are to be used, is to assess the level of differentiation found between populations (a test of whether or not they are from the same gene pool). A test to determine the statistical probability that two samples are from the same gene pool is discussed by Thorpe and Sòl-Cava (1994). The method to estimate probabilities for fixed allelic differences in samples N_1 and N_2 is: $P < (1/2N_1)^{2N_2}$. Fixed allele mobility differences occurred at 13 loci for the two genera studied, and at seven of the loci between the *P. catostoma* populations sampled (Table 2, Fig. 1). Therefore, the probability that the genera are from the same gene pool is extremely small (P<1.9 x 10^{-12}) and that for the latter populations is P<0.001. Since no fixed allele mobility differences (only allele frequency differences) were found between the two *M. macrolepidotus* populations

TABLE 1
LOCUS ABBREVIATIONS AND ENZYME COMMISSION NUMBERS (E.C. NO.) ARE LISTED AFTER EACH PROTEIN

Protein	Locus	E.C. No.
Aspartate aminotransferase	**mAAT, sMDH**	2.6.1.1
Adenylate kinase	***AK**	2.7.4.3
Alcohol dehydrogenase	**ADH-1,-2**	1.1.1.1
Creatine kinase	***CK-A, CK-B**	2.7.3.2
Esterase	**EST**	3.1.1.-
General protein	**PROT-1,-3, *PROT-2**	
Glyceraldehyde-3-phosphate dehydrogenase	**GAPDH**	1.2.1.12
Glycerol-3-phosphate dehydrogenase	**G3PDH**	1.1.1.8
Glucose-6-phosphate isomerase	**GPI-A,-B**	3.5.1.9
L-lactate dehydrogenase	**LDH-A,-B**	1.1.1.27
Malate dehydrogenase	**sMDH**	1.1.1.37
Mannose-6-phosphate isomerase	**MPI**	5.3.1.8
Peptidase:		3.4.-.-
Substrate: Glycyl-L-leucine	***PEPA-1, PEPA-2**	
Leucyl-glycyl-glycine	**PEPB**	
Leucyl-tyrosine	**PEP-LT**	
Peroxidase	***PER**	1.11.1.7
Phosphoglucomutase	**PGM**	5.4.2.2
Superoxide dismutase	***sSOD**	1.15.1.1

* = Monomorphic loci

TABLE 2
ALLELE FREQUENCIES FOR POLYMORPHIC LOCI IN POPULATIONS OF *M. MACROLEPIDOTUS* AND *P. CATOSTOMA*

		M. macrolepidotus		*P. catostoma*	
Locus	Allele	Sabie	Zambezi	Sabie	Zambezi
mAAT	A	0.727	1.000	1.000	1.000
	B	0.273			
sAAT	A	1.000	0.800		
	B		0.200		
	C			1.000	1.000
ADH-1	A	0.955	1.000		
	B	0.045			
	C			1.000	0.875
	D				0.125
ADH-2	A	0.417			
	B	0.583	1.000		
	C			1.000	1.000
CK-B	A	0.833	1.000		
	B	0.167			
	C			1.000	1.000
EST	A	0.625	0.500		
	B	0.375	0.500		
	C				0.375
	D			1.000	0.625
PROT-1	A				1.000
	B	1.000	1.000	1.000	
PROT-3	A			1.000	1.000
	B	1.000	1.000		
GAPDH	A	0.278		1.000	1.000
	B	0.722	1.000		
G3PDH	A			1.000	1.000
	B	0.500	1.000		
	C	0.500			
GPI-A	A	1.000	1.000		0.875
	B				0.125
	C			0.938	
	D			0.062	
GPI-B	A	1.000	1.000		
	B				1.000
	C			0.813	
	D			0.187	
LDH-A	A			1.000	1.000
	B	1.000	1.000		
LDH-B	A				0.125
	B			1.000	
	C				0.875
	D	1.000	1.000		
sMDH	A				0.250
	B	0.864	1.000	1.000	0.750
	C	0.136			
MPI	A	1.000	1.000		
	B			1.000	1.000
PEPA-2	A	1.000	1.000		
	B			1.000	1.000
PEPB	A				1.000
	B	1.000	1.000	1.000	
PEP-LT	A				1.000
	B			1.000	
	C	1.000	1.000		
PGM	A	1.000	1.000	1.000	
	B				1.000

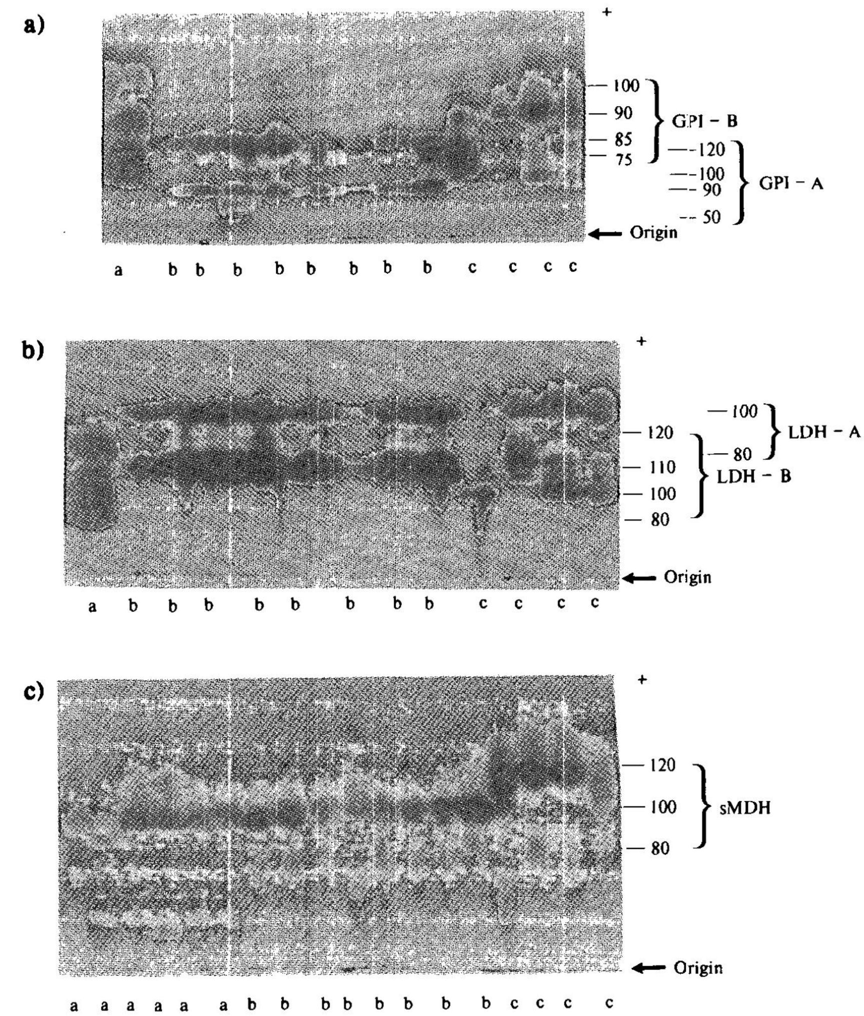

Figure 1
Zymograms showing allele product mobility differences between M. macrolepidotus *(a),* P. catostoma *from the Sabie (b), and* P. catostoma *from the Zambezi Rivers (c) at the glucose-6-phosphate isomerase, lactate dehydrogenase and malate dehydrogenase enzyme coding loci respectively.*

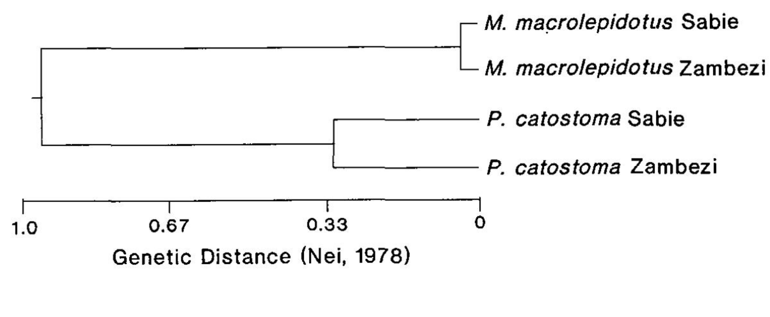

Figure 2
Dendrogram showing phylogenetic relationships, based on Nei's (1978) genetic distance values, between the taxa studied.

studied, these populations may represent conspecific populations.

Wright's (1978) fixation index is another measure to describe differentiation between populations. The mean F_{ST} value (0.857) for polymorphic loci (Table 3) in the mormyrids studied is an indication of large genetic differentiation between the populations resulting from genetic drift. The extent of allelic fixation of individuals relative to its sub-populations (F_{IS}=0.883) also reflects the above phenomenon. Values of F_{IS} are close to zero in most natural populations where random mating within subpopulations occurs (Nei, 1986). The F_{IT} value of 0.182 (which quantifies inbreeding due to population subdivision), is indicative of effective barriers to gene flow between the populations studied. This is in agreement with geographical data (no gene flow is possible between the allopatric populations studied).

Several statistically based measures of genetic distance are also available to reduce genetic differentiation between populations over a range of enzyme loci to a single figure level, but Nei's (1978) measure is now used predominantly (Thorpe and Sòl-Cava (1994). Allopatric conspecific populations tend to have relatively small allele frequency differences at a few loci, whereas congeneric species are often completely different at some loci (i.e. fixed for different alleles). Shaklee et al. (1982), Thorpe (1982) and Thorpe and Sòl-Cava (1994) showed the relationship between taxonomic divergence and genetic distance, and concluded that the genetic distance (Nei, 1972) average 0.05 (range: 0.002 to 0.07) for conspecific populations; 0.30 (range: 0.03 to 0.61) for congeneric species; and it ranged from 0.58 to 1.21 between genera in the same family. The genetic distance values obtained in the present study (Table 4) between the congeneric species (average: 0.927) fall within the upper limit for confamilial genera estimated by Shaklee et al. (1982), and it was 0.023 between the two *M. macrolepidotus* populations. The latter value also corresponds to the values obtained by Shaklee et al. (1982) for populations from the same species. The genetic distance value (0.311) calculated between the *P. catostoma* populations shows a relatively large degree of differentiation, and together with the fixed allele mobility differences (Table 2, Fig. 1), it suggests that they represent separate species rather than allopatric populations of the same species.

Figure 2 shows the phylogenetic relationships between the taxa studied. The *M. macrolepidotus* populations are grouped together, as are the *P. catostoma* populations. This is expected for conspecific populations in the former instance, as well as for congeneric species (in the latter instance). It is also

TABLE 3
SUMMARY OF *F*-STATISTICS AT ALL LOCI

Locus	F_{IS}	F_{IT}	F_{ST}
mAAT	0.542	0.642	0.220
sAAT	1.000	1.000	0.853
ADH-1	-0.116	0.842	0.859
ADH-2	0.314	0.857	0.791
CK-B	-0.200	0.845	0.871
EST	0.061	0.517	0.486
PPOT-1		1.000	1.000
PROT-3		1.000	1.000
GAPDH	0.723	0.943	0.795
G3PDH	0.333	0.860	0.789
GPI-A	-0.116	0.781	0.803
GPI-B	-0.231	0.854	0.822
LDH-A		1.000	1.000
LDH-B	-0.143	0.902	0.914
sMDH	0.032	0.174	0.146
PEPA-2		1.000	1.000
PEPB		1.000	1.000
PEP-LT		1.000	1.000
MPI		1.000	1.000
PGM		1.000	1.000
Mean	0.182	0.883	0.857

TABLE 4
NEI'S (1972) STANDARD GENETIC DISTANCE VALUES ABOVE DIAGONAL AND NEI'S (1978) UNBIASED GENETIC DISTANCE VALUES BELOW DIAGONAL

Population	*M. macrolepidotus*		*P. catostoma*	
	Sabie	Zambezi	Sabie	Zambezi
M. macrolepidotus				
Sabie	-----	0.028	0.806	1.027
Zambezi	0.023	-----	0.836	1.058
P. catostoma				
Sabie	0.802	0.834	-----	0.316
Zambezi	1.019	1.051	0.311	-----

evident that more differentiation occurred in the *P. catostoma* populations (Fig. 2), to support the hypothesis that the amount of differentiation reflects the existence of congeneric species rather than populations of the same species.

From the above information, it is evident that an undescribed *Petrocephalus* sp. exists, and that allozyme data were useful to distinguish between species and populations. The results obtained in the present study show that the two *M. macrolepidotus* populations may represent conspecific populations. This is in contrast to the results on **EOD** waveforms by Kramer (1996), who found distinct differences between *M. macrolepidotus* from the same two river systems sampled also in this study. It should be noted that allozyme data cannot prove that two populations are conspecific (but only that no significant differences could be found). It is possible that small but genuine differences could have been concealed by sampling error or that differentiation may be present at loci which have not been examined. However, Van der Bank and Kramer (1996) were also able to identify a cryptic mormyrid species using corresponding allozyme data, indicating that the former (sampling error) might be an unlikely explanation. Nevertheless, it would be interesting to extend the present study, by analysing more enzyme systems, in order to determine if genetic markers can be found to differentiate between the allopatric species of *M. macrolepidotus*.

The type locality of *M. macrolepidotus* is the Lower Zambezi River in Moçambique, and that of *P. catostoma* is the Ruvuma River on the Tanzanian/Moçambique border (Bell-Cross and Minshull, 1988). Kramer (1996) presents results for 17 anatomical characters measured or counted for the above-mentioned species. The fish studied by Kramer (1996) are a super-sample of the present sample (same individuals). The general habitat preferences of the species (see Bell-Cross and Minshull (1988) and Skelton (1993)) at the two localities sampled were similar. A small difference between Kramer's (1996) results, and that reported by Bell-Cross and Minshull (1988) and Skelton (1993) was mentioned for *P. catostoma* from the Upper Zambezi River. In contrast, some meristic counts for *M. macrolepidotus* were consistently below the ranges given by the latter authors (Kramer, 1996). These differences were attributed to genetical isolation (by the Victoria Falls). In addition, Kramer (1996) obtained distinct morphological differences between the *M. macrolepidotus* population from the Zambezi River, and those from the Sabie River. It is also possible that *P. catostoma* specimens from the type locality may differ from that of the Sabie and from the Upper Zambezi Rivers, suggesting three *Petrocephalus* species. Kramer, Skelton and Van der Bank plan to extend this study to include **EOD** waveform data for *P. catostoma* as well as morphological data to formally describe the new species.

Acknowledgements

I am grateful for the logistical support given by Andrew Deacon from the Kruger National Park, who assisted in collecting mormyrids, and I wish to express my sincere gratitude to Prof. B Kramer for reading and criticising the manuscript.

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