The identification of hybrids of *Barbus aeneus X B. kimberleyensis* and *Labeo capensis X L. umbratus* in Hardap Dam, SWA/Namibia

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ABSTRACT

A morphometric and biochemical genetic comparison of *Barbus aeneus* and *B. kimberleyensis* with *B. cf. kimberleyensis* from Hardap Dam, SWA/Namibia, revealed that the latter population showed distinct evidence of hybridization between *B. aeneus* and *B. kimberleyensis*. A similar comparison of the three *Labeo*-populations from Hardap Dam also indicated hybridization between *L. capensis* and *L. umbratus*. It was also possible to identify *L. capensis* x *L. umbratus* individuals, according to electrophoresis, that have been identified as "pure" specimens with the aid of morphometric and meristic characteristics.

INTRODUCTION

Large cyprinids of the genera *Barbus* and *Labeo* are among the most valuable freshwater fishes all over Africa (Jackson & Coetzee 1982). According to these authors, labeos are greatly esteemed as food in most parts of tropical Africa, while in South Africa the yellowfishes *Barbus aeneus* and *B. kimberleyensis* are among of the most highly regarded of our indigenous freshwater angling species. Despite the economic importance of these species, their continued existance as pure species are being threatened by hybridization.

In Hardap Dam, SWA/Namibia, the genus *Labeo* is represented by *L. capensis* and *L. umbratus* as well as a range of intermediate forms due to certain morphological characteristicts. The genus *Barbus* however is represented by a single population described by Gaigher (1976) as *B. cf. kimberleyensis*, but recently it has been referred to as *B. aeneus* (Schrader 1986). To prevent confusion, this population will be referred to as *B. cf. kimberleyensis* in this paper. In an attempt to determine the taxonomic status of the Hardap Dam populations (*Labeo* and *Barbus*), morphometric, meristic and electrophoretic comparisons were performed between the intermediate forms and the pure populations of each species. Due to the fact that only one *Barbus* population exists in the Hardap Dam, pure populations of *B. aeneus* and *B. kimberleyensis* were collected from the Vaal River for comparison. It was also possible to determine the genetic distance between the two intermediate forms and the pure species, using electrophoresis as it has proven itself a useful complement to traditional systematic methods (Grant & Leslie 1983; Stratil *et al.* 1983).

MATERIALS AND METHODS

Specimens were collected at three locations in southern Africa. Both intermediate forms as well as the *Labeo* species were collected in the Hardap Dam (17°57'E, 24°37'S) using gill and seine nets. *B. aeneus* was collected in the Vaal River near the Barrage, while *B. kimberleyensis* was collected in the Vaal Dam. Samples of skeletal muscle and blood serum were collected as described by Mulder (1986) and Van Vuuren (1986) and stored at −20°C for later electrophoretic analysis.

Morphometric and meristic characteristics which were determined are the following:

Scale counts on the lateral line and around the caudal peduncle.
Number of spines and rays in the dorsal and anal fins.
Gillraker counts
Vertebrae counts
Standard lenght/Body depth (SL/BD)
Head length/Eye diameter (HL/ED)
Head length/Head width (HL/HW)
Standard lenght/Head length (SL/HL)
* Snouth lenght/Opercular groove (SN/OG)
* = Performed on *Barbus* only.

Electrophoretic analysis:

Polyacrylamide gel electrophoresis:
Serum transferrin and serum esterase phenotypes were determined by using a 6% vertical, tris-citrate buffer system (Table 1) as decribed by Avtalion and Wojdani (1971) and Van der Bank (1984).

Starch gel electrophoresis:
Horizontal starch gel electrophoresis were used to detect the banding patterns of nine muscle proteins (Table 1). The gels consisted of a 13% hydrolyzed potato starch (Sigma, S-4501). The gel constitution was applied as described by Smith (1976) and tissue preparation by May *et al.* (1979).

Three buffer systems were used to achieve maximum resolution of the protein bands on the gels:

RW: gel: TRIS 0,03M, citric acid 0,005M (pH 8,5).
tray: Lithium hydroxide 0,06M, boric acid 0,3M (pH 8,1). (Rideway *et al.* 1970).

TC: gel: 1:15 dilution of tray solution.
tray: Citric acid 0,05M, Tris 0,15 (pH 6,9). (Whitt 1970).

MF: gel: 1:4 dilution of tray solution.
tray: TRIS 0,18M, boric acid 0,1M, EDTA 0,004M (pH 8,7). (Markert & Faulhaber 1965).

TABLE 1: Summary table of proteins screened in *Labeo umbratus, L. capensis* and their intermediate form as well as *Barbus aeneus, B. kimberleyensis* and their intermediate form. In each case the buffer system which gave best resolution, the number of loci coding for each protein and the genus which were examined using the specific protein, are given.

Protein	Abbr.	E.C. No.	Buffer	Loci scored	Genus
Adenylate kinase	AK	2.7.4.3	TC	1	B
Esterase (serum)	EST	3.1.1.1	Tris/citrate	1	L,B
Glyceraldehydephosphate dehydrogenase	GAP	1.2.1.12	RW	2	L
Glycerol-3-phosphate dehydrogenase	GPD	1.1.1.8	RW	2	L
Isocitrate dehydrogenase	IDH	1.1.1.42	MF	2	L
Lactate dehydrogenae	LDH	1.1.1.27	RW/TC	2	L,B
Malic enzyme	ME	1.1.1.40	MF	2	B
Purine nucleoside phosphorylase	NP	2.4.2.1	RW	1	B
Superoxide dismutase	SOD	1.15.1.1	MF	1	B
Transferrin (serum)	TF	–	Tris/citrate	1	L,B

L: *Labeo* – B: *Barbus*

Allelic nomenclature was applied as described by Allendorf and Utter (1979).

Statistical procedures

Morphometry and meristics

The mean and standard deviation were determined for all characteristics. These data were also tested for normal distribution (95% probability) by using the *Chi*-square test.

Electrophoresis

Numbers of observed phenotypes at each locus were tested for deviations from Castle-Hardy-Weinberg proportions using the log-likelihood-statistic G, for goodness of fit (Sokal and Rohlf 1969). The genetic distance, D, (Nei 1972) and it's standard deviation (Nei & Roychoudhury 1974), were determined for each population using all loci. Finally the average population heterozygosity was determined as described by Grant (1985).

RESULTS AND DISCUSSION

Morphometric and meristic analysis

The results obtained for the *Barbus* population are presented in Table 2 and 4 and those for the *Labeo* populations in Table 3 and 5 and Figure 1.

Comparing the lateral line and peduncle scale counts of the *Barbus* populations with that found in the literature (Barnard 1943; Groenewald 1958), shows the same degree of variation within species from different localities.

As it is known that these differences can be induced by environmental conditions (Wallace 1973), it was not possible to determine the taxonomic status of the Hardap Dam *Barbus* population using the characteristics.

The same applies to the dorsal and anal rays and spines (Table 2). Of *B. kimberleyensis* 88% had nine rays in the dorsal fin, while 94% and 90% of individuals of respectively *B. aeneus* and *B. cf. kimberleyensis* had eight rays in the dorsal fin. Although it seems that there is a greater similarity between the last two populations, much variation within a species at different locations were recorded. Barnard (1943) found that two out of every three individuals of *B. aeneus,* which were collected from the Fish River near Gibeon, had nine rays in the dorsal fin. From a total of 103 individuals of the same species collected from the Oranje River near Goodhouse, only six had eight rays, while the rest had nine rays in the dorsal fin.

Variation of morphological characteristics among localities were again emphasized by the gill raker count. The Hardap Dam population, which might be a pure population of either *B. aenus* or *B. kimberleyensis* or an intermediate form, had a mode lower than the first mentioned two populations.

Vertebral counts included the Weber-apparatus as one element. Although it seem that *B. cf. kimberleyensis* has greater similarity with *B. aeneus* concerning this characteristic, it cannot be taken for granted, as Van der Bank (1984) found that a higher water temperature produced individuals with less vertebrae.

Considering that all three *Barbus* populations differ in locality, the possibility of environmentally induced morphological differences between the three populations cannot be excluded. No meaningful conclusion can thus be made before more populations of each species has been studied at different locations. The fact that *B. aeneus* and *B. kimberleyensis* are quite closely related makes useful conclusions all the more difficult.

The standard length/head length, determined for *B. cf. kimberleyensis* (Table 4), had a range overlapping with the ranges of both *B. aeneus* and *B. kimberleyensis.* This indicates intermediate characteristics which is an indication of hybridization.

The standard lenght/body depth, head length/eye diameter, as well as the head length/head width of all three populations stretches over a very narrow range (Table 4), with *B. cf. kimberleyensis* having either the highest of lowest mean. This again illustrates the influence of the environment on external characteristics but it is also an indication of the high degree of similarity between *B. aeneus* and *B. kimberleyensis.*

TABLE 2: Meristic data of the three *Barbus* populations with sample size, range and mode.

	B. aeneus			*B. cf. kimberleyensis*			*B. kimberleyensis*		
	N	Range	Mode	N	Range	Mode	N	Range	Mode
Lateral line count	51	38 – 46	42	61	39 – 47	42	33	38 – 45	42
Cauldal peduncle aount	51	15 – 17	16	61	15 – 17	16	33	15 – 18	16
Gill raker count	51	14 – 18	16	61	10 – 7	13,5	33	11 – 18	14
Dorsal spines, rays	51	iv7 – 9	8	61	iv8 – 9	8	33	iv8 – 9	9
Anal spines, rays	51	iii5	5	51	iii5	5	33	iii4 – 5	5
Vertebral count	10	39 – 41	50	10	38 – 41	40	19	38 – 40	39

TABLE 3: Meristic data of the three *Labeo* populations with sample size, range and mode.

	L. capensis			*L. capensis* x *L. umbratus*			*L. umbratus*		
	N	Range	Mode	N	Range	Mode	N	Range	Mode
Lateral line count	33	33 – 48	45	51	44 – 54	47	30	49 – 60	55
Cauldal peduncle count	33	20 – 25	22	51	21 – 32	26	30	28 – 36	32
Gill raker count	33	42 – 53	48	51	43 – 57	49	30	43 – 53	47
Dorsal spines, rays	33	iii10 – 11	10	51	iii9 – 10	9	30	iv9 – 10	9
Anal spines, rays	33	iii5	5	51	iii5	5	30	iii5	5
Vertebral count	33	39 – 41	40	51	39 – 41	40	30	39 – 40	39

TABLE 4: Morphometric data of the three *Barbus* populations with sample size, mean and standard deviation.

	N	*B. aeneus*	N	*B. cf. kimberleyensis*	N	*B. kimberleyensis*
SL/BD	51	4,233 + 0,205	61	4,362 + 0,616	33	4,209 + 0,207
HL/ED	51	6,641 + 0,677	61	5,774 + 0,863	33	8,259 + 1,375
HL/HW	51	1,515 + 0,099	61	1,950 + 0,280	33	1,757 + 0,099
SL/HL	51	4,641 + 0,239	61	4,419 + 0,633	33	3,930 + 0,197
SL/GL	51	3,28 + 0,767	61	2,35 + 0,671	33	1,63 + 0,332
SN/OG	51	1,422 + 0,135	61	1,294 + 0,127	33	0,916 + 0,103

TABLE 5: Morphometric data of the three *Labeo* populations with sample size, mean and standard deviation.

	N	*L. capensis*	N	*L. capensis* x *L. umbratus*	N	*L. umbratus*
SL/BD	33	4,159 + 0,323	51	4,040 + 0,260	30	3,955 + 0,196
HL/ED	33	7,397 + 1,428	51	9,633 + 0,916	30	10,325 + 1,101
HL/HW	33	1,579 + 0,145	51	1,600 + 0,077	30	1,508 + −,103
SL/HL	33	4,507 + 0,352	51	4,349 + 0,237	30	4,022 + 0,208

B. aeneus and *B. kimberleyensis* can be distinguished from each other by using the ratio of snout length/distance from the eye to the opercular groove. The same applies to the ratio of gut length/standard length. The results obtained for these ratios again indicate possible hybridization in Hardap Dam, because *B. cf. kimberleyensis* had intermediate ranges.

Figure 1a shows the external morphology of *L. umbratus*, the typical intermediate forms and *L. capensis*. The external morphology of the intermediate forms varies, but the head generally resembles that of *L. umbratus* whilst the body is more *L. capensis*-like. The mouth varies from terminal to sub-inferior when opened, but never inferior as in *L. capensis* (Figure 1b). The same tendency was observed by Gaigher and Bloemhof (1975) for the hybrids that they have described from Hardap Dam.

The results presented in Table 3 show that there is no significant difference between the three populations in terms of the number of gill-rakers on the anterior gill-arch, the number of vertebrae as well as the number of spines and rays in the anal fin. But when comparing the scale counts on the lateral line and around the caudal peduncle, it is possible to distinguish between a population with a lower scale count *(L. capensis)* and one with a higher scale count *(L. umbratus)* as well as

a third population which overlaps with the scale counts of both the other two populations. When a further comparison is made of these scale counts, it is clear that the mode of the number of scales on the lateral line of the intermediate population, is closer to that of *L. capensis,* while the mode of the caudal peduncle is intermediate to that of *L. capensis* and *L. umbratus.*

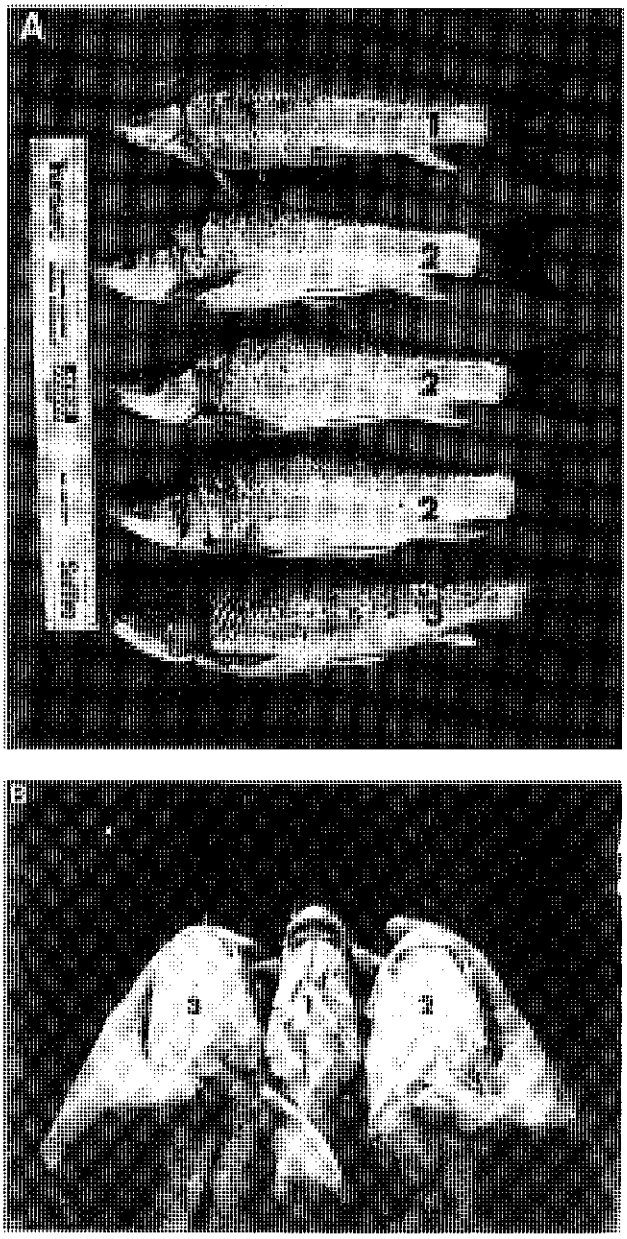

FIGURE 1: A. *Labio capensis* (1), *L. umbratus* (3) and the intermediate forms (2) collected from the Hardap Dam. B. The mouth postion of *L. capensis* (1), *L. umbratus* (3) and the intermediate form (2).

The same tendency as above, is observed when the meristic data in Table 5 are compared. It is therefore of interest to note that the intermediate forms are closer to *L. umbratus* in some features (eye diameter, head width, head form), intermediate in other features (position of the mouth, number of scales around the caudal peduncle, head length) and closer to *L. capensis* in still other features (number of scales on latera line and spines in the dorsal fin). Thus, according to this morphometric and meristic comparison, it seems that these intermediate forms could indeed be hybrids between *L. capensis* and *L. umbratus.* But it is also clear that none of these characteristics could be used alone to identify a single hybrid unambiguously. For this reason these populations have been analised electrophoretically, as this technique has proved itself to be useful in distinguishing between hybrids and closely related species (Valenta 1978; Allendorf & Utter 1979).

Electrophoretic analysis

The products of the different protein coding loci were examined for Mendelian variation. In the *Barbus* populations eight of the loci were monomorphic (LHD-1; LHD-2; ME-1; ME-2; AK-1; NP-1; NP-2 SOD), while the other two were polymorphic (TF EST) (Table 6). In the *Labeo* populations four of these loci were monomorphic (LDH-1; LDH-2; GAP-1 and GPD-1), while the other six were controlled by one or more polymorphic loci (TF; EST; IDH-1; IDH-2 GAP-2 and GPD-2).

Banding patterns in the *Barbus* populations

Serum transferrins (TF)

Banding patterns of *B. kimberleyensis* and *B. cf. kimberleyensis* are quite simple in contrast to that of *B. aeneus.* The fact that both former populations possess only the 90 and 100 allele, suggest a marked similarity between these two populations concerning the transferrin locus. *B. cf. kimberleyensis* had a small excess of heterozygotes. This strongly suggest hybridization in Hardap Dam as it is known that hybridization leads to an increase in heterozygotes in a population (Stratil *et al.* 1983). The excess heterozygotes is especially significant when taking into account that this is an isolated population with a relatively small gene pool which may have led to inbreeding with a resulting decrease in heterozygotes.

Serum esterase (EST)

The three *Barbus* populations all had a single polymorphic locus with double banded heterozygotes and single banded homozygotes. This suggests a monomeric enzyme structure. A total of four bands were visible on the gel, but the 96 band were absent from the *B. aeneus* and the 95 band from the *B. kimberleyensis* populations.

The *B. cf. kimberleyensis* population had the full complement of bands. This again is an indication of hybridization as bands unique for *B. aeneus* and *B. kimberleyensis* were found in the *B. cf. kimberleyensis* population.

Lactate dehydrogenase (LDH)

The three *Barbus* populations all had five bands reflecting the gene products of two loci where the three

TABLE 6: Allelic frequencies of electrophoretic variants of *B. aeneus, B. cf. kimberleyensis* and *B. kimberleyensis*. Alleles are designated by their mobilities relative to the common allele.

Locus	Allele	*B. aeneus*	*B. cf. kimberleyensis*	*B. kimberleyensis*
TF	90	–	0,365	0,365
	100	0,394	0,635	0,635
	105	0,154	–	–
	113	0,144	–	–
	120	0,154	–	–
	128	0,154	–	–
	N	52	60	33
EST	88	0,212	0,217	0,442
	95	0,442	0,017	–
	96	–	0,442	0,212
	100	0,346	0,325	0,346
	N	52	60	33
LDH-1	100	1,0	1,0	1,0
	N	52	60	33
LDH-2	100	1,0	1,0	1,0
	N	52	60	33
ME-1	100	0,5	0,5	0,5
	189	0,5	0,5	0,5
	N	52	60	33
ME-2	100	0,5	0,5	0,5
	139	0,5	0,5	0,5
	N	52	60	33
AK	100	1,0	1,0	1,0
	N	52	60	33
NP-1	100	1,0	1,0	1,0
	N	52	60	33
NP-2	100	1,0	1,0	1,0
	N	52	60	33
SOD	92	–	–	1,0
	100	1,0	1,0	–
	N	52	60	33

central bands represented the heterotetrameric product between the two loci. These zones of activity were identical in migration speed as well as banding patterns for all three populations.

Malic enzyme (ME)
Two zones of activity were observed for all three *Barbus* populations. ME-1 consisted of only five-banded phenotypes. The second zone of lesser activity had three bands. The low intensity of the bands might indicate that this locus is of mitochondrial origin.

Again the three populations were identical in migration rates as well as banding patterns.

Adenylate kinase (AK)
A monomorphic locus with single-banded homozygotes was expressed in the three populations investigated. Although five bands were observed for all samples, four were identified as satellite bands on the basis of band intensities.

Purine nucleoside phosphorylase (NP)
Two monomorphic loci with two heterotrimeric bands were observed for all *Barbus* populations investigated.

Superoxide dismutase (SOD)
A single zone of banding, with only homozygotes appeared in the gel. The enzymes of *B. aeneus* and *B. cf. kimberleyensis,* however, had a higher migration speed than that of *B. kimberleyensis.* This implies a definite similarity between the former two populations.

Banding patterns in the *Labeo* populations

Serum transferrins (TF)
A single zone of activity was observed for all three populations. In this zone several different single- and double banded phenotypes reflected the products of four alleles (Table 7). No significant departures from the Castle-Hardy-Weinberg proportions were detected for the two "pure" populations, but the intermediate population shared an excess of heterozygotes. Such an

excess of heterozygotes was also observed for hybrids of *Barbus barbus* and *Barbus meridionalis petenyi* by Stratil *et al.* (1983).

Serum esterases (EST)

A single zone of activity, having single banded homozygotes and double banded heterozygotes, was observed. "Ghost bands" were observed for all the variants and care must therefore be taken not to attribute any genetic value to these bands, as it will give an erroneous impression of the genetic structure of a specific population (Kirpichnikov 1981).

The fastest band (A) showed the highest frequency in the *L. capensis* population, while the slowest band (D) showed the highest frequency in the *L. umbratus* population. It is of interest to note, however, that these two bands showed almost the same frequency in the intermediate population. This phenomenon is surely evidence for hybridization between these two populations. This hybridization is further emphasized by the low frequency of D-bands that were observed in the *L. capensis* population and A-bands in the *L. umbratus* population, probably because of individuals that were wrongly identified as pure, while being hybrids. This is supported by the fact that no D-bands and A-bands were observed respectively in pure populations of *L. capensis* and *L. umbratus* (Van Vuuren *et al.* in press). Hybridization between these two populations is still further emphasized by the excess of heterozygotes that were observed in the intermediate population.

Lactate dehydrogenase (LDH)

Two zones of activity that appeared to reflect the products of two loci, were observed for all three populations. Both loci showed only the one homozygote but heterotetrameric bands were visible between these two loci. No variance in the mobility of the homozygotes, of the different populations, was visible.

Isocitrate dehydrogenase (IDH)

The products of two loci appeared in all three popula

TABLE 7: Allelic frequencies of electrophoretic variants of the three *Labeo* populations in Hardap Dam. Alleles are dessignated by their mobilities relative to the common allele.

Locus	Allele	*L. capensis* x *L. umbratus*	*L. capensis*	*L. umbratus*
TF	84	–	–	–
	93	–	–	–
	100	0,636	0,500	0,983
	109	0,061	0,333	–
	113	0,030	–	–
	116	0,273	0,167	0,017
	122	–	–	–
	N	33	51	30
EST	100	0,076	0,529	0,967
	103	0,045	–	–
	108	0,879	0,471	0,033
	113	–	–	–
	N	33	51	30
LDH-1	100	1,0	1,0	1,0
	N	33	51	30
LDH-2	100	1,0	1,0	1,0
	N	33	51	30
IDH-1	77	0,121	0,520	0,917
	100	0,879	0,480	0,083
	N	33	51	30
IDH-2	84	–	0,5	0,933
	100	1,0	0,5	0,067
	N	33	51	30
GAP-1	100	1,0	1,0	1,0
	N	33	51	30
GAP-2	100	0,045	0,049	0,050
	115	0,955	0,951	0,950
	N	33	51	30
GPD-1	100	1,0	1,0	1,0
	N	33	51	30
GPD-2	100	0,091	0,108	0,900
	120	0,909	0,892	0,100
	N	33	51	30

tions (Table 7), where heterodimeric bands did not form between the loci. IDH-1 stained more intense than IDH-2 in the skeletal muscle, but according to Grant and Leslie (1983), IDH-2 will stain more intense in liver. A two banded heterozygous phenotype was consistent, which is unexpected for dimeric enzyme. But these two banded heterozygotes for IDH were also observed by Grant and Leslie (1983) for *Lophius upsicephalus.*

It is not surprising that the intermediate population shows again an excess of heterozygotes. It is however conspicuous that the fast migrating allele (A) shows the highest frequency in the *L. capensis* population, while the frequency for these two alleles are about the same intermediate population (Table 7). IDH confirms thus the possibility of hybridization between *L. capensis* and *L. umbratus* in Hardap Dam.

Glyceraldehyde-phosphate dehydrogenase (GAP)
Two zones of activity were observed, where heterotetrameric bands did form between the loci. GAP-1 was monomorphic for all three populations as only one homozygote was observed for all the individuals. GAP-2 was polymorphic for three alleles that produced one single and one triple-banded phenotype. GAP-2 did not show the same intensity as GAP-1 in skeletal muscle, but will probably show a higher activity in liver (Philip *et al.* 1983; Grant 1985)

Glycerol-3-phosphate dehydrogenase (GPD)
Two zones of banding were observed. The first, GAP-1, appeared as a single band and was located anodally to the origin. GAP-2 was polymorphic for three alleles where the fast migrating homozygote was not apparent in the gel. The triple-banded heterozygous phenotypes observed for GAP-2, are typical of a dimetric enzyme.

Average Population Heterozygosity

There was a tendency for average heterozygosities to differ according to the geographic complexity of the system from which the different populations were collected. More complex systems had a higher average heterozygosity. The river population of *B aeneus,* for instance, had an average heterozygosity of 0,2659.

The effect of a less complex system on the average heterozygosity was most obvious in the population of *B. cf. kimberleyensis* with 0,2232. This population was isolated after the completion of the Hardap Dam in 1963. No new genetic material could therefore enter the dam by way of yearly spawning run. On the other hand, no spawning run takes place from the dam (Gaigher 1976), and this leads to a relatively small effective population size. The possibility of a genetic bottleneck in this population, that would result in a reduction of the gene pool, is therefore larger than the same happening for *B. aeneus.*

The average heterozygosity based on the electrophoretic data are 0,1200; 0,2395 for *L. capensis, L. umbratus* and the hybrid respectively. Comparing these estimates with those of the Barrage namely, 0,1892 for *L capensis* and 0,1698 for *L. umbratus* (Van Vuuren *et al.* in press), the same tendency that occured in the *Barbus* populations was found here. The dam populations have a lower average population heterozygosity than those of the river populations, because of the isolation factor by the dam wall and the resulting smaller effective population size. As may be expected, the intermediate population of Hardap Dam has the highest average population heterozygosity (0,2395) of all five these populations. This high value for the average population heterozygosity, of this dam population, confirms thus the conjecture that this is in fact a hybrid between *L. capensis* and *L. umbratus.*

Genetic distance

The genetic distance between the samples for the *Barbus* species from the Barrage, is 0,1027 and between the "pure" *Labeo* samples from Hardap Dam 0.1317. However, it is of interest to note that the genetic distance between *B. cf. kimberleyensis* as well as *L. capensis* x *L. umbratus* are again intermediate to that of the respective pure populations (Table 8 and 9). This is a definite quantitative indication of hybridization between these species.

TABLE 8: Standard genetic distance (below diagonal) and standard deviations (above diagonal) between the respective *Barbus* populations from the Barrage and Hardap Dam.

	B. aeneus	*B. cf. kimberleyensis*	*B. kimberleyensis*
B. aeneus	–	0,0328	0,0752
B. cf. kimberleyensis	0,0408	–	0,0668
B. kimberleyensis	0,1027	0,0694	–

TABLE 9: Standard genetic distance (below diagonal) and standard deviations (above diagonal) between the respective *Labeo* populations from Hardap Dam.

	L. capensis	*L. capensis* x *L. umbratus*	*L. umbratus*
L. capensis	–	0,0194	0,0754
L. capensis x *L. umbratus*	0,0347	–	0,0188
L. umbratus	0,1317	0,0386	–

CONCLUSION

The results of this study prove unequivocally that morphometrical and meristical data alone are insufficient to distinguish between closely related species and their hybrids. This was clearly illustrated by the fact that some of the *Labeo* individuals, identified as either *L. umbratus* or *L. capensis* were indicated by electrophoretic results to hybrids.

The results also show that hybridization between fish species has occured in Hardap Dam because of the disruption of biological cycles in fish populations and the construction of obstructions in rivers which prevent their natural movement. The *Barbus* population in this dam consists clearly of a hybrid population. *B. aeneus* and *B. kimberleyensis* are, however, quite closely related and more enzyme systems need to be analyzed before the taxonomic status of the Hardap Dam population can be proven beyond doubt. The third *Labeo* population is also a hybrid population and it is therefore of importance to identify natural, genetically pure populations as soon as possible, which can be conserved as breeding populations in the future.

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REFERENCES

ALLENDORF, F.W. & UTTER, F.M. 1979. Population Genetics. In: HOAR, W.S., RANDALL, D.J. & BRETT, J.R., (eds.). Fish Physiology. New York: Academic Press: 407–454.

AVTALION, R.R. & WOJDANI, A. 1971. Electrophoresis and immuno-electrophoresis of sera from some known D^1-hybrids of Tilapia. *Bamidegh* 23: 117–124.

BARNARD, K.H. 1943. Revision of the indigenous freshwater fishes of the S.W. Cape region. *Ann. S. Afr. Mus.* 36(2): 101–262.

GAIGHER, I.G. 1976. The reproduction of *Barbus cf. kimberleyensis* (Pisces, Cyprinidae) in the Hardap Dam, South West Africa. *Zool. Afr.* 11(1): 97–110.

GAIGHER, I.G. & BLOEMHOF, H.J. 1975. Hybrid *Labeo umbratus* x *L. capensis* (Pisces, Cyprinidae) from the Hardap Dam, South West Africa. *Madoqua* 9(1): 53–55.

GRANT, W.S. 1985. Biochemical genetic variation in the Cape Anchovy *Engraulis capensis. Gilchrist. S. Afr. J. Mar. Sci.* 3: 23–31.

GRANT, W.S. & LESLIE, R.W. 1983. Morphological and biochemical genetic comparison of two morphs of the southern African anglerfish (*Lophius upsicephalus,* Smith). *Colln. Scient. Pap. int. Commn. 5e. Atl Fish.* 77–85.

GROENEWALD, A.A.V.J. 1958. A revision of the genera *Barbus* and *Varicorhinus* (Pisces: Cyprinidae) in Transvaal. *Ann. Trans. Mus.* 23(3): 263–330.

JACKSON, P.B.N. & COETZEE, P.W. 1982. Spawning behaviour of *Labeo umbratus* (Smith) (Pisces, Cyprinidae). *S. Afr. J. Sci.* 78: 293–295.

KIRPICHNIKOV, V.S. 1981. Genetic Bases of Fish Selection. New York: Springer-Verlag pp. 40.

MARKERT, C.L. & FAULHABER, I. 1965. Lactate dehydrogenase isozyme patterns of fish. *J. Exp. Zool.* 159: 319–332.

MAY, B., WRIGHT, J.E. & STONEKING, M. 1979. Joint segregation of biochemical loci in salmonidae: Results from experiments with *Salvelinus* and review of the literature on other species. *J. Fish. Res. Board Can.* 36: 1114–1128.

MULDER, P.F.S. 1986. 'n Vergelykende morfometriese en elektroforetiese ondersoek na *Barbus aeneus* en *Barbus kimberleyensis.* M.Sc.-thesis. Rand Afrikaans University. pp. 195.

NEI, M. 1972. Genetic distance between populations. *Amer. Natur.* 106: 283–292.

NEI, M. & ROYCHOUDHURY, A.K. 1974. Sampling variances of estimated phylogenetic trees from molecular data. *J. Mol. Evol.* 19: 153–170.

PHILIP, D.P., CHILDERS, W.F. & WHITT, G.S. 1983. A biochemical genetic evaluation of the Northern and Florida subspecies of largemouth bass. *Trans Am. Fish. Soc.* 112: 1–20.

RIDGWAY, G.J., KLONTZ, G.W. & LEWIS, R.D. 1970. Polymorphism in the esterases of Atlantic herring. *Trans. Amer. Fish. Soc.* 99: 147–151.

SCHRADER, H.J. 1986. 'n Ondersoek na die potensiële maksimale volgehoue visopbrengs van Hardapdam en die moontlike invloed van kommersiële ontginning op hengel en visvretende watervoëls. M.Sc.-thesis. University of the Orange Free State. pp. 134.

SMITH, I. 1976. Chromatographic and Electrophoretic Techniques. Vol. 2. London: Heinman. pp. 485.

SOKAL, R.R. & ROHLF, F.J. 1969. Biometry. San Francisco: Freeman. pp. 776.

STRATIL, A., BOBAK, P., TOMASEK, V. & VALENTA, M. 1983. Transferrins of *Barbus barbus, Barbus meridionalis petenyi* and their hybrids: Genetic polymorphism, heterogeneity and partial characterization. *Comp. Biochem. Physiol.* 76B(4): 845–850.

VALENTA, M. 1978. Protein polymorphism in European fish species of the Cyprinidae family and utilization of polymorphic proteins for breeding in fish. In: MÜLLER, F. (ed.) Increasing productivity of fishes by selection and hybridization. Szarvas: 37–38.

VAN DER BANK, F.H. 1984. Die gebruik van morfometriese en elektroforetiese metodes in die bepaling van genetiese lyne by *Oreochromis mossambicus* (Peters) uit drie lokaliteite in Transvaal. M.Sc.-thesis. Rand Afrikaans University, Johannesburg. pp. 164.

VAN VUUREN, N.G. 1986. Die nuttigheidswaarde van morfometriese kenmerke en biochemiese merkers in die tipering van *Labeo*-spesies. M.Sc.-thesis. Rand Afrikaans University, Johannesburg. pp. 164.

VAN VUUREN, N.G., MULDER, P.F.S., FERREIRA, J.T. & VAN DER BANK, F.H. – in press. A morphometric and electrophoretic analysis of *Labeo capensis* and *Labeo umbratus* from two localities in southern Africa. *S. Afr. J. Wildl. Res.*

WALLACE, C.R. 1973. Effects of temperature on developing meristic structures of smallmouth bass, *Micropterus dolomieui* (Lacepede). *Trans. Am. Fish. Soc.* 102: 142–144.

WHITT, G.S. 1970. Developmental genetics of the lactate dehydrogenase isozymes of fish. *J. Exp. Zool.* 175 (1): 1–35.