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THE KARYOLOGY AND TAXONOMY OF THE SOUTHERN AFRICAN YELLOWFISH (PISCES: CYPRINIDAE)

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ABSTRACT

The southern African yellowfish (Barbus aeneus, B. capensis, B. kimberleyensis, B. natalensis and B. polylepis) are very similar, which limits the utility of traditional taxonomic methods. For this reason yellowfish similarities were explored using multivariate analysis and karyology. Meristic, morphometric and Truss (body shape) data were examined using multiple discriminant, principal component and cluster analyses. The morphological study disclosed that although the species were very similar two distinct groups occurred; B. aeneus-B. kimberleyensis and B. capensis-B. polylepis-B. natalensis. Karyology showed that the yellowfish were hexaploid, B. aeneus and B. kimberleyensis having 148 chromosomes while the other three species had 150 chromosomes. Because the karyotypes of the species were variable the fundamental number for each species was taken as the median value for ten spreads. Median fundamental numbers were B. aeneus = 196, B. natalensis = 200, B. kimberleyensis = 204, B. polylepis = 206 and B. capensis = 208. The lower chromosome number and higher fundamental number was considered the more apomorphic state for these species. Silver-staining of nucleoli showed that the yellowfish are probably undergoing the process of diploidization. Southern African Barbus and closely related species used for outgroup comparisons showed three levels of ploidy. The diploid species karyotyped were B. anoplus (2N=48), B. argenteus (2N=52), B. trimaculatus (2N=42-48), Labeo capensis (2N=48) and L. umbratus (2N=48); the tetraploid species were B. serra (2N=102), B. trevelyani (2N=±96), Pseudobarbus afer (2N=96) and P. burgi (2N=96); and the hexaploid species were B. marequensis (2N=130-150) and Varicorhinus nelspruitensis (2N=130-148). The taxonomic implications of polyploidy for the African cyprinids were considered, and its effect on species was discussed.

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This work is dedicated to my parents, Keith and Dawn Oellermann.

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A. INTRODUCTION

Taxonomists have reached the secondary stage of review and refinement in African fish taxonomy. Major problems still exist in groups such as the Cyprinidae, which are difficult to resolve using the traditional methods of taxonomy. The yellowfish are such a group of species in southern Africa. The group has been incorporated into the *Barbus* genus, and divided into five species; *Barbus aeneus* (Burchell 1822), *B. capensis* Smith 1841, *B. natalensis* Castelnau 1861, *B. polylepis* Boulenger 1907 and *B. kimberleyensis* Gilchrist and Thompson 1913.

The yellowfish are characterized by their size (adults attain lengths greater than 200 mm standard length) and their longitudinally striated scales, as opposed to the radially striated scales of the other *Barbus* species (Jubb, 1967). Species identification within the yellowfish group is based on scale size, the strength of the fourth dorsal spine, the position of the dorsal fin origin in relation to the origin of the pelvic fin, and the distance from the opercular/preopercular groove to the eye (Jubb, 1967).

The taxonomic status of the five yellowfish species is in question. The adults show strong phenotypic similarity, and it is often extremely difficult to distinguish between juveniles of the species. Eccles (1986) has found that the only reliable method of separating *B. aeneus* and *B. kimberleyensis* juveniles less than 60 mm is by the differences in length and shape of the gut. Crass (1960) observed that the length and thickness of *B. natalensis* spines increased with the increase in calcium content found toward the lower reaches of the Natal rivers. Butler (unpublished) found a cline in the number of precaudal vertebrae in *B. aeneus* along the Orange River.

The form of the mouth is variable in the yellowfish. *Barbus aeneus* and *B. capensis* sometimes have thickened lips which

facilitate feeding off a stony or pebbly substrate. Barbus natalensis has a range of mouth shapes; from the thickened "rubberlip" type to a form where the broad lower jaw has a sharp cutting edge ("Varicorhinus" type). These fish probably feed by scraping algae and other food particles off submerged rocks (Gaigher, 1975). In the Elands River a population of B. polylepis has been found which also contains these extreme mouth forms (Gaigher, 1975). Barbus kimberleyensis is the only yellowfish which does not show mouth shape variations, possibly due to the predatory nature of the species. The mouth forms are probably not genetically determined, but develop as epigenetic adaptations to differences in grazing actions on different substrata (Jubb, 1967).

Each species, except B. aeneus and B. kimberleyensis occurs over a unique, adjacent range (fig. 1). The strong interspecific similarities and the plastic phenotypes of the group suggests that the taxa are geographic variants rather than different species. This study attempts to provide further taxonomic and systematic information on the yellowfish by applying more recent taxonomic methods to the group.

Traditional measurements for taxonomy involves simple statistical comparisons of single character measurements. The advent of computer-assisted multivariate analyses allows many character measurements to be compared simultaneously. Instead of using morphometric characters based on body features, body shape measurements could also be used as characters. Strauss and Bookstein (1982) describe their method of body shape characterization as the Truss system.

Cytotaxonomic methods are becoming more popular in fish taxonomy as the techniques improve and the reports of successful studies increase. This is especially so in karyology, where recently developed techniques to expose Nucleolar Organizer Regions (NORs) and chromosomal band-staining have greatly enhanced chromosome detail.

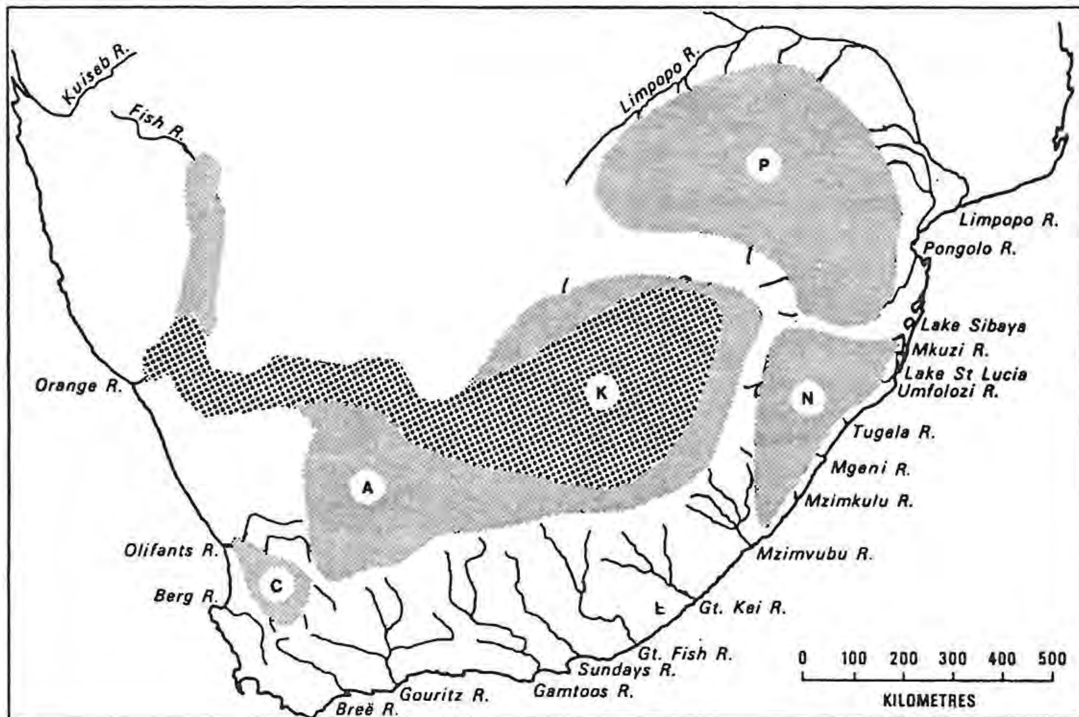


Figure 1. The distribution of the southern African yellowfish species. A= B. aeneus, C= B. capensis, K= B. kimberleyensis, N= B. natalensis and P= B. polylepis.

This study approaches the taxonomy of the yellowfish from two directions. Firstly, the traditional morphometrics, Truss morphometrics (body shape characterization) and meristics of the species are compared using multivariate methods. These methods include analysis of variance, principal component analysis, discriminant analysis and cluster analysis. The second approach involves a karyological study in which chromosome numbers, karyotypes and silver-staining studies of the five species are compared.

The chromosomes of African cyprinids are poorly known and there are no published data on southern African species. Thus it was necessary to study the chromosome numbers of as many other southern African cyprinids as possible, to have a data base from which to compare the karyotypes of the yellowfish species.

B. INTRODUCTION TO THE YELLOWFISH

Barbus aeneus (Burchell, 1822)

Taxonomic History

The smallmouth yellowfish was originally described as Cyprinus aeneus by Burchell (1822). Steindachner described the same species as Barbus holubi in 1894. Barnard (1943) reviewed this synonymy and recommended that the valid name be Barbus aeneus. Groenewald (1958) disagreed as he felt Burchell's description was inadequate to distinguish the species from Barbus kimberleyensis, and the drawing was of no diagnostic value. In a further consideration of the synonymy Hocutt and Skelton (1983) accredit the species to Burchell. Barbus mentalis Gilchrist & Thompson, 1913 and Barbus gilchristi Boulenger, 1911 were synonyms of Barbus aeneus, and merely described the rubberlip varieties of the species (Groenewald, 1958). Weber (1897) mistakenly identified a population of juvenile Barbus aeneus from the Orange River as Barbus capensis, which was later corrected by Barnard (1938).

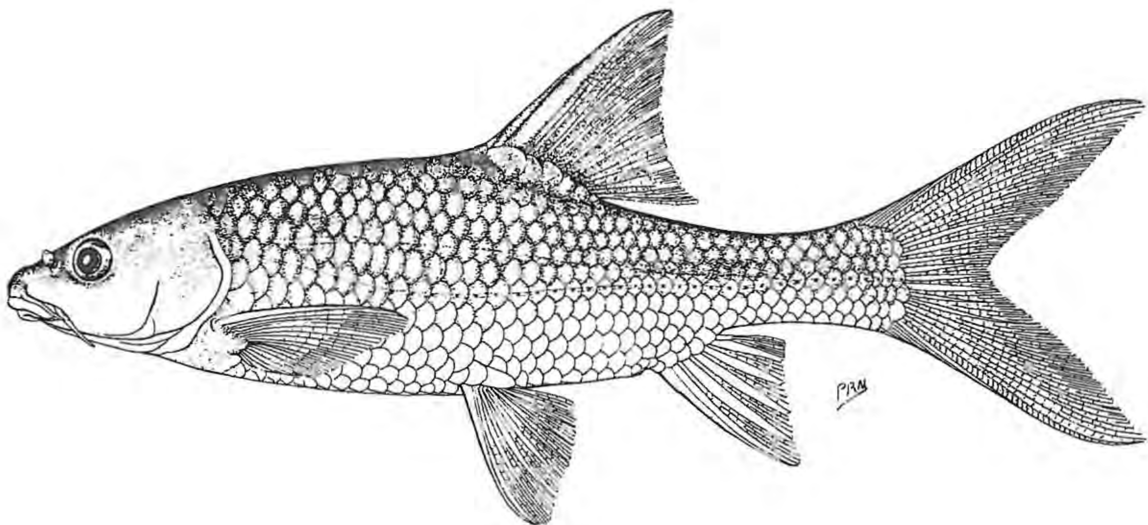


Figure 2. Barbus aeneus

Life History

The species is endemic to the Vaal-Orange River system, but has been introduced into the Calitzdorp dam on the Gamka River tributary (Gouritz River system) in 1953, the Olifants River of the Limpopo River system, the Tsomo River (1963), Kibusie River and Klipplaats River of the Great Kei River system, Settlers dam on the Kariega River in 1964 as well as Lake Kyle in Zimbabwe (Jubb, 1968). The Orange-Fish River tunnel was the means in which B. aeneus translocated to the Great Fish River system (Cambray and Jubb, 1977; Laurenson and Hocutt, 1986).

Barbus aeneus prefers clear, fast running water (even rapids) with a sandy, gravel or rocky substrate (Mulder, 1973; Skelton and Cambray, 1981). However its presence in the Sak River (Orange River system) which dries up and leaves a series of pools which are subject to extremes in temperature and salinity, and are often eutrophic and anaerobic, suggests that the species can tolerate extreme conditions (Hocutt and Skelton, 1983). The species in this genus can tolerate low temperatures (de Moor and Bruton, 1988).

Barbus aeneus is a long-lived species, obtaining ages of at least 12 years and reaching a size and mass of at least 67cm and 3.5 kg (Tomasson, 1983). Mulder (1973) reported a sex ratio of 1.8 females to males due the greater longevity of the females. The males reach sexual maturity at 4 years and the females at 5 years, although during adverse conditions the fishes may reach sexual maturity at a smaller size (Tomasson, 1983). Their gonads start developing in June and spawning takes place in early October. Mulder (1973) recorded a second spawning in January. Photoperiod and temperature are important factors regulating gonadal development in the cyprinids (de Vlaming, 1972) and it is likely that water temperature changes during flooding affects the development of yellowfish gonads. Both males and females develop tubercles during the spawning

season, but only males have tubercles on the branched rays of the anal fin (Groenewald, 1958).

The fishes migrate upstream during the first floods in spring or summer to spawn in the well oxygenated sections of the river (de Moor and Bruton, 1988). Courtship was observed during daylight (de Moor and Bruton, 1988), but spawning probably takes place at night, in gravel nests constructed by the fish.

The fertilized eggs of B. aeneus incubate for three to eight days at a temperature of 18 to 21,5°C. The larvae hide in the substrate and absorb their yolk sacs, only becoming motile four to six days after hatching (le Roux, 1968). The fry remain in calm, shallow sections of the river (de Moor and Bruton, 1988) until they reach a size of about 50 to 70 mm (Tomasson, 1983).

Barbus aeneus is an opportunistic omnivore; the juveniles eat zooplankton, insects and insect larvae while the adults eat mainly algae and aquatic vegetation. The ability to digest plant material is acquired as the fish grows larger; the gut length to fish length ratio increases with increasing fish size (Kruger and Mulder, 1973). The size at which a fish switches from a zooplankton dominated diet to a benthos dominated diet is governed by water turbidity (Tomasson, 1983) as it visually selects its prey. The species also feeds on large numbers of the freshwater mussel Corbicula africana (Mulder, 1973; Skelton and Cambray, 1981).

The juvenile specimens of the Orange River tend to be silvery in colour with salmon orange fins (Skelton and Cambray, 1981) while those of the Toise River are of a silvery-gold hue. The large specimens of the Vaal-Orange River system are golden-yellow with olive-green tints on the dorsal surfaces and sides of the head, but the colour varies in turbid water (Jubb, 1962). These large specimens sometimes also have salmon-orange

fins (Skelton and Cambray, 1981).

Barbus capensis Smith, 1841

Taxonomic History

This species was described by Smith in 1841, but the range given was incorrect. Barnard (1937) found that the species was limited to the Olifants River system, and the species occurring in the Breede River system was a different species which he recommended be called *Barbus andrewi*. He also found that the specimens described as *B. seeberi* by Gilchrist and Thompson (1913) from the Olifants River were in fact *B. capensis*.

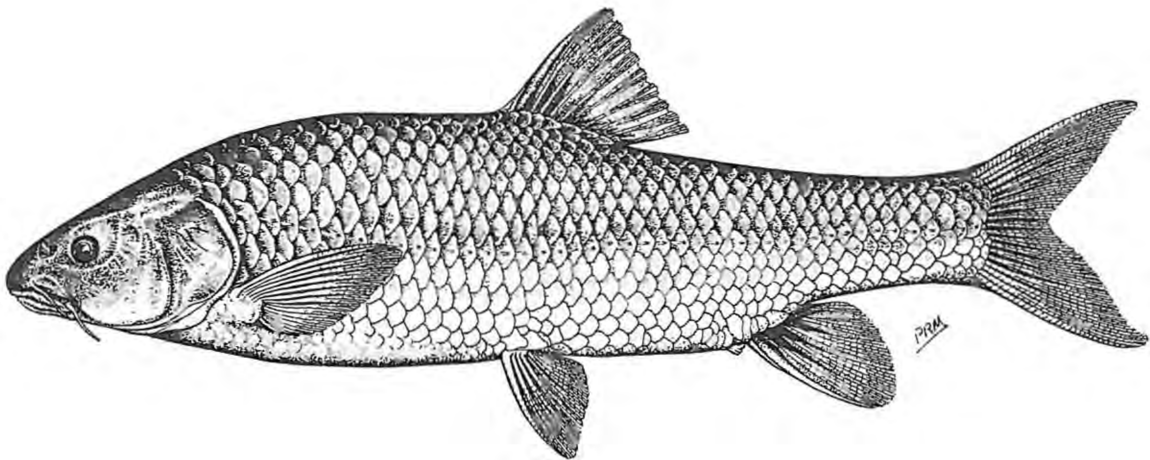


Figure 3. *Barbus capensis*

Life History

Barbus capensis is listed as rare in the South African Red Data Book (Skelton, 1987) and is endemic to the Clanwilliam Olifants River system (Jubb, 1967). The fish prefer clear rocky pools and deeper river stretches, and are also found in

impounded river stretches (Skelton, 1977). The juveniles inhabit pools and sheltered backwaters and marginal areas of the mainstreams (Skelton, 1987).

Mass breeding migrations upstream and into the smaller tributaries occur during September to December. The fish breed from October to January and young fish were found near the head of the mainstream in November and December (van Rensburg, 1966).

Barbus capensis has an omnivorous diet, eating algae and aquatic invertebrates including gastropods, insect larvae and crabs. Large specimens tend to become predatory, taking small fish and frogs readily (Jubb, 1967). The species varies in colour from olive-yellow to a brilliant golden-yellow during the breeding season (Skelton, 1977). The juveniles are silver to silvery-gold in colour.

Barbus kimberleyensis Gilchrist and Thompson, 1913

Taxonomic History

The species was originally described by Gilchrist and Thompson in 1913. Barnard (1943) rejected B. kimberleyensis as a valid species, considering B. aeneus as the only large species of Barbus occurring in the Orange River system. Fitzsimons (1949) described a species, Barbus pienaari, from the Vaal River. Groenewald (1958) examined the type specimens and found B. pienaari to be synonymous with B. kimberleyensis, and resurrected the B. kimberleyensis species.

Life History

Barbus kimberleyensis occurs in the Vaal-Orange River system and extends into the Orange River to below the Augrabies falls (Groenewald, 1958; Skelton and Cambray, 1981). The species

prefers clear, fast flowing water with a sandy or rocky substrate (Jubb, 1962; Mulder, 1973).

Mulder (1973) reported a sex ratio of 1.8 females to males (females reach greater ages), and sexual maturity is reached at 8 years (46 cm) for the females and 6 years (35 cm) for the males). The gonads start developing in June and are well developed by late October (males) and November (females); spawning is expected to occur later than that of B. aeneus (Mulder, 1973). Spawning sites are probably very similar to those of Barbus aeneus. Tubercles are present in both sexes during the spawning period.

The incubation period for artificially bred B. kimberleyensis is two to three days, and the larvae become motile in three to four days at 23 to 25°C (van der Merwe, 1981).

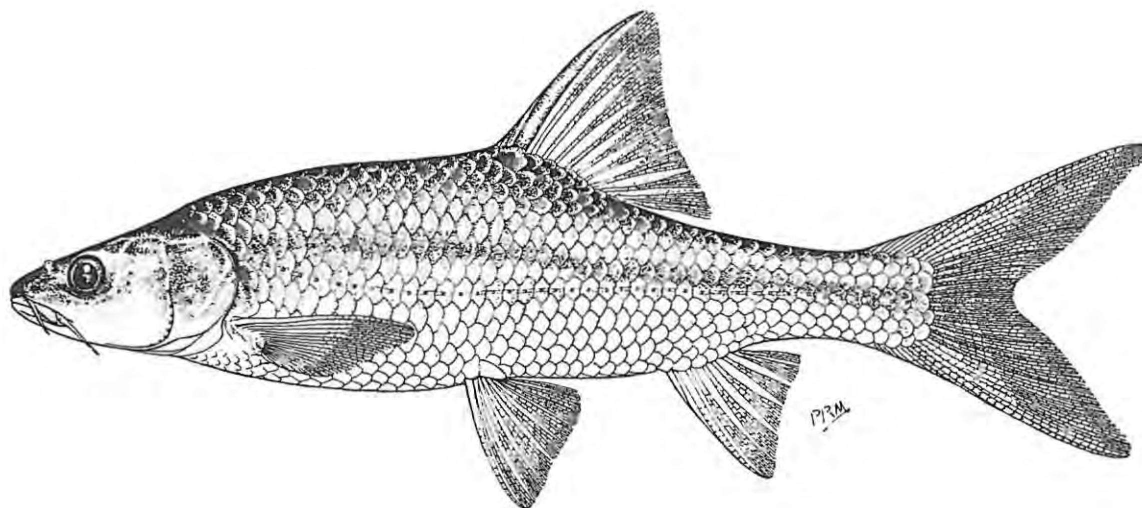


Figure 4. Barbus kimberleyensis

Barbus kimberleyensis is a predator from its juvenile stages, initially having a similar diet to B. aeneus juveniles, but develops increasingly piscivorous tendencies with age (Mulder, 1973; Tomasson, 1983). This is probably why the species is the

only yellowfish not to develop variable mouth-forms, which appear to be linked to feeding substrate variations. The gut is shorter than that of B. aeneus, as one would expect for a carnivorous species (Eccles, 1986).

The juvenile specimens are silvery-gold in colour, and the adults are silvery-white with silver grey on the dorsal surface in muddy waters, while the clear water specimens are a silvery-yellow colour (Jubb, 1967; Mulder, 1973).

Barbus natalensis Castelnau, 1861

Taxonomic History

Castelnau's (1861) description of Barbus natalensis was considered inadequate by Crass (1960), there was no drawing of the type specimen and the type specimen was lost. Natal yellowfish were described as Labeobarbus aureus Cope, 1869 until 1907 when Boulenger redescribed the species as Barbus aureus. Crass (1960) pointed out that Castelnau's description gave enough information to show he had described a large Barbus specimen occurring in Natal, and thus the species should be called B. natalensis. Jubb (1963) listed the following synonyms of B. natalensis: B. bowkeri Boulenger, 1902; B. lobochilus Boulenger, 1911; B. mfongosi Gilchrist and Thompson, 1913; B. robinsoni Gilchrist and Thompson, 1913; B. zuluensis Gilchrist and Thompson, 1913; B. dendrotrachelus Fowler, 1934a; B. grouti Fowler, 1934a; B. stigmaticus Fowler, 1934a; B. tugelensis Fowler, 1934a and B. marleyi Fowler, 1934b.

Life History

Barbus natalensis occurs in all major Natal rivers south of the Phongolo River, from the Mkuzi River to the Umtamvuna River, up to altitudes of 1500 meters (Jubb, 1967; de Moor and

Bruton, 1988). The species has been found in waters ranging from just above freezing point to over 30°C (Crass, 1960). Waterfalls have prevented the species from entering the upper reaches of some rivers such as the Umzimkulu and Ingwangwana Rivers (Crass, 1964).

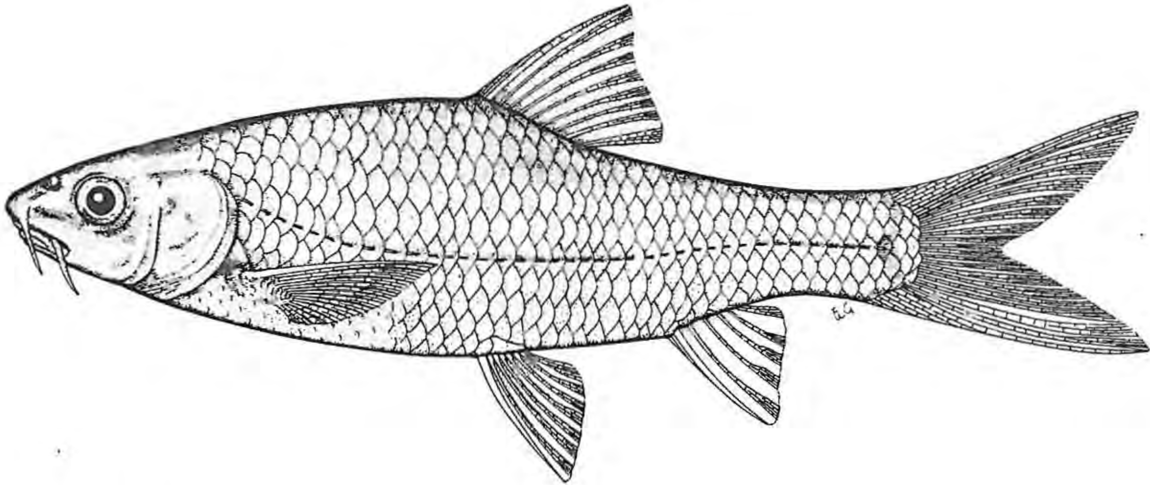


Figure 5. Barbus natalensis

The species migrates upstream after the first Spring rains in large shoals (de Moor and Bruton, 1988). Spawning probably takes place in tributaries where silt levels are lower, as the eggs and larvae are susceptible to high silt loads (Wright and Coke, 1975b). These fishes are unable to breed in still water (Crass, 1964). Wright and Coke (1975a) found that the species spawned over clean, algae-free, well circulated gravel in fast flowing water at a temperature exceeding 19°C. The larvae hatched in six to eight days and burrowed into the gravel, showing a negative phototrophic response (Wright and Coke, 1975b).

The different mouth-forms present in the populations of the species point to substrate feeding on algae and other aquatic plants. The fishes will take flies, lures, earthworms and crabs as bait, thus B. natalensis is omnivorous (Jubb, 1967).

The juvenile specimens of B. natalensis are silver to silvery-gold in colour, with irregular dark markings or spots on the sides. The adults are a yellowish-gold, with a greenish tint dorsally. Barbus natalensis adults can weigh as much as 4,6 kilograms (de Moor and Bruton, 1988).

Barbus polylepis Boulenger, 1907

Taxonomic History

Barbus polylepis was described by Boulenger in 1907. He reported the species as occurring in the Limpopo River system. In 1913 Gilchrist and Thompson described a species Barbus lineolatus which occurred in the Magalies River. Groenewald (1958) considered these two species to be synonymous with Barbus marequensis. Jubb (1963) reviewed this synonymy and resurrected B. polylepis, but considered B. lineolatus to be synonymous with this species.

Life History

The species occurs in the southern tributaries of the Limpopo River system and tributaries of the Incomati and Phongolo River systems (Jubb, 1968). It is a highveld species, generally occurring higher than 610 meters above sea level (Gaigher, 1969). Specimens were caught in deepish pools below rapids.

Gaigher (1969) found that more males than females were caught in his samples, but the females grew faster and to a greater size. The females are sexually mature at 30 cm while the males are mature at 17 cm. Spawning takes place from September or October to later than February. The eggs ripen from the distal to the proximal ends of the ovary, over the entire breeding season (Gaigher, 1969). Natural spawning

probably occurs at night over gravel in fast flowing areas of the rivers.

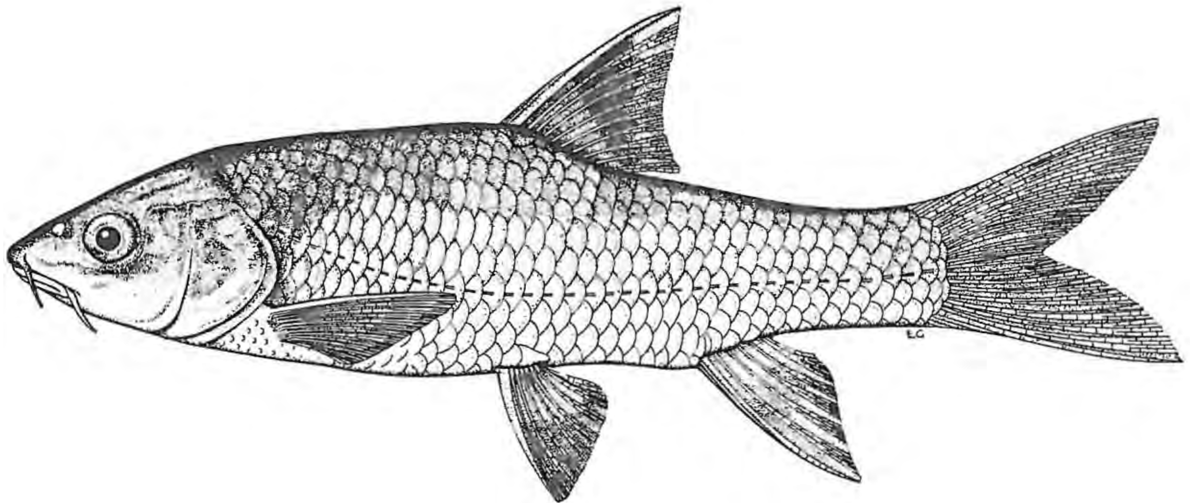


Figure 6. Barbus polylepis

The species shows similar variable mouth-forms to B. natalensis when not in the presence of B. marequensis (Gaigher, 1975). It is probably a facultative omnivore and takes a lure, small frog or crab as bait (Jubb, 1967). During winter and spring B. polylepis feeds off the algae blooms (mostly Chlorophyceae) which build up in still pools, but during the flood months it feeds on benthic insect larvae and nymphs, terrestrial insects, crabs, freshwater mussels, gastropods, amphibian larvae, fish and detritus washed down with the floods (Gaigher, 1969).

Juvenile specimens are silvery in colour with a pale, olive-green dorsal surface Jubb, 1967). The adults are silvery gold with a greenish tinge dorsally, and have dark fins.

C. MORPHOMETRICS AND MERISTICS

INTRODUCTION

Traditionally taxonomy is based on comparisons and descriptions of characteristic morphological features of species. Hubbs and Lagler (1958) described a set of morphometric measurements and meristic counts suitable for fishes. These measurements have proved to be very useful, but with the increasingly sophisticated means available are now limited in a number of ways (Strauss and Bookstein, 1982). The measurements tend to be aligned along a very few axes, usually laterally. Information on variation in oblique directions is rare. Coverage of body features is dense in some regions (e.g. the head) while sparse in others. Two types of features are measured; anatomical (e.g. orbital diameter) and dimensional (e.g. body depth). Because the dimensional features are defined in terms of maximum and minimum distances, their placement may not be homologous from specimen to specimen.

Morphometrics can be approached in another way. Instead of measuring linear body features, body shape could be measured. Strauss and Bookstein (1982) have described the Truss system, which uses a series of arbitrary but easily recognized points on the midsaggital plane of the body. Distances are measured from point to point in such a way that each point represents the corner of a quadrilateral, and is connected to its opposite corner by a diagonal (fig. 8). This system takes into account shape changes in most directions. The Truss system measures distances from a single point to three other points. The geometric nature of this system allows for measurement error corrections, as well as for archiving the geometric shape of the specimens. For a detailed account of the technique, error corrections, data transformation and further analyses see Bookstein *et al*. (1984).

Sometimes it is impossible to discriminate between species on

the basis of one character. In these cases it is necessary to analyze the simultaneous relationships among many characters (Dillon and Goldstein, 1984). Analysis of variance, principal component analysis, discriminant analysis and cluster analysis were used to explore the simularities between the five yellowfish species. The multivariate methods were used on the traditional and Truss morphometric data, as well as the meristic data of the yellowfish.

Principal components analysis does not presume multiple groups (species) within the data set, and therefore allows for their discovery (Humphries et al., 1981). The discriminant analysis uses species-labeled data, and attempts to discriminate between the data as much as possible. The cluster analysis operates on the assumption that five species exist in the data, and attempts to partition the data into five groups on the basis of similarity.

The combination of analysis of variance and three unique multivariate approaches to three distinct data sets were used to provide further insight into the taxonomic relationships of the the southern African yellowfish, and to test the hypothesis that they consist of less than five species.

MATERIALS AND METHODS

Traditional Morphometrics

Traditional morphometric measurements of the species Barbus aeneus, B. capensis, B. kimberleyensis, B. natalensis and B. polylepis were taken following Hubbs and Lagler (1958), as modified by Skelton (1980) (fig. 7). Dial calipers were used, and measurements were taken to 0.1 millimeters. The measurements were standard length (SL), head length (HL), snout length (SNL), orbital diameter (OD), post orbital (PO), post orbital to preoperculum (POPO), inter orbital (IO), predorsal length (PRD), dorsal fin length (DFL), body depth (BD), body width (BW), caudal peduncle length (CPL), caudal peduncle depth (CPD), anterior barb (AB), posterior barb (PB), pectoral to pelvic fin (PFPF) and pelvic to anal fin (PFAF).

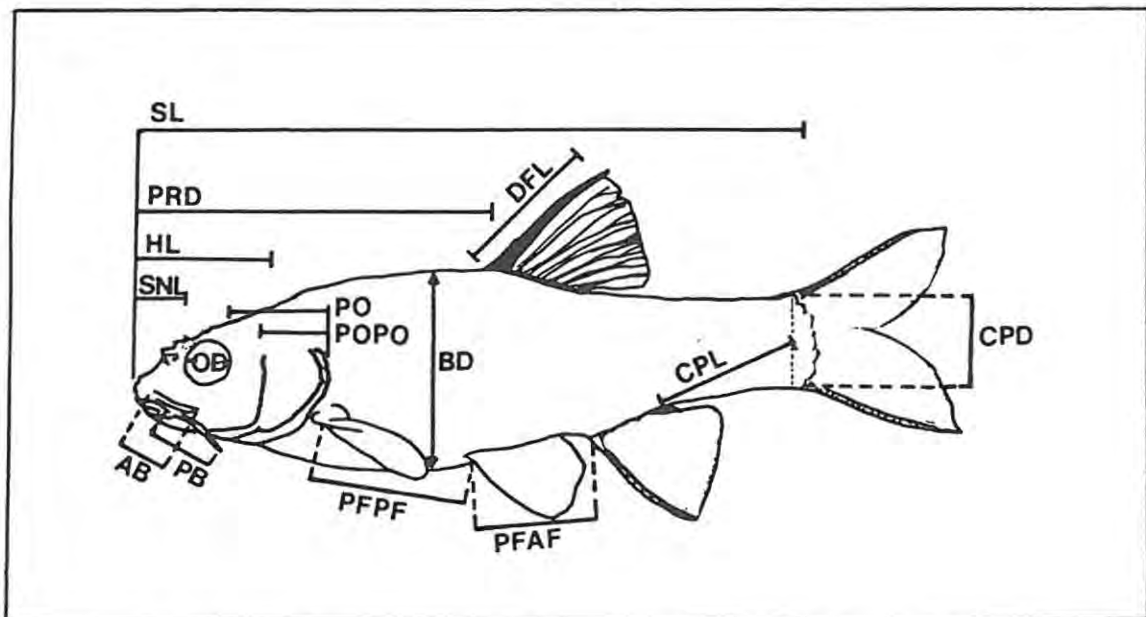


Figure 7. Traditional morphometric measures used in the yellowfish (See text for abbreviations).

Thirty specimens were measured for each species. The specimens had been preserved in formalin and stored in propanol, and came from the collections of the JLB Smith Institute of Ichthyology (RUSI) and the Albany Museum (AMSA). Table two lists the material examined. The morphometric data were

standardized as a percentage ratio of standard length.

TABLE 1. Collection numbers and sampling localities of specimens used in traditional morphometric and meristic analyses.

SPECIES	COLL. No.	LOCALITY
B. aeneus	AMSA/P2676	Fish R., at Ai-Ais
B. capensis	AMSA/P7492	Olifants R., Cape Province
	AMSA/P7709	Citrusdal road bridge, Olifants R.S.
	AMSA/P7548	Citrusdal road bridge, Olifants R.S.
	AMSA/PF1381	Noordhoek stream, Olifants R.S.
	AMSA/PF1370	Noordhoek stream, Olifants R.S.
	AMSA/P8948	Ceres causeway, Olifants R.S.
	AMSA/P7674	Noedhoek R., Olifants R.S.
	AMSA/PF1172	Jonkershoek stock, from Olifants R.
B. kimberlyen-sis	AMSA/P3922	Serfontein bridge, Orange R.
	AMSA/P7914	Groblershoop bridge, Orange R.
	AMSA/P7893	Kakamas bridge, Kakamas R.
	AMSA/P8296	P.K. LeRoux, Seekoei R.
	AMSA/P4807	Verwoed Dam, Orange R.S.
	AMSA/P3922	---------
	AMSA/P4815	---------
	AMSA/P7914	---------
B. natalensis	AMSA/P7315	Oribi Gorge Nat. Res., Umzimkulu R.
	AMSA/PF40	Tugela R.
	AMSA/PF163	Lowsburg, Mkuzu R. tributary
	AMSA/PF998	Pietermaritzburg, Mgeni R.
	AMSA/PF243	Umzimkulwana R.
B. polylepis	AMSA/P919	Sabie R., Incomati R.S.
	AMSA/P6880	---------
	AMSA/P4708	Nooitgedacht Dam, Komati-Inkomati RS
	AMSA/P6196	DeLagersdrift, Middelburg district, Steelpoort-Olifants-Limpopo R.S.
	AMSA/P6320	Little Usutu R., Maputo R.S.
	AMSA/P5240	Vryheid, Phongola-Maputo R.S.
	AMSA/P4648	Goedverwacht, Buffelspruit R.

Truss Morphometrics

Thirty specimens were measured according to the Truss system shown in figure eight, from each of the five species. Eighteen

distances were measured between nine landmarks on the body of the fish specimens. The landmarks chosen were the tip of the snout (ST), posterior margin of the supraoccipital (SO), dorsal fin origin (DFO), base of the last dorsal ray (DFB), upper caudal peduncle origin (UCP), lower caudal peduncle origin (LCP), anal fin origin (AFO), pelvic fin origin (PFO) and the branchiostegal junction (BJ). The distances were measured to an accuracy of 0.1 millimeters between these landmarks, using dial calipers.

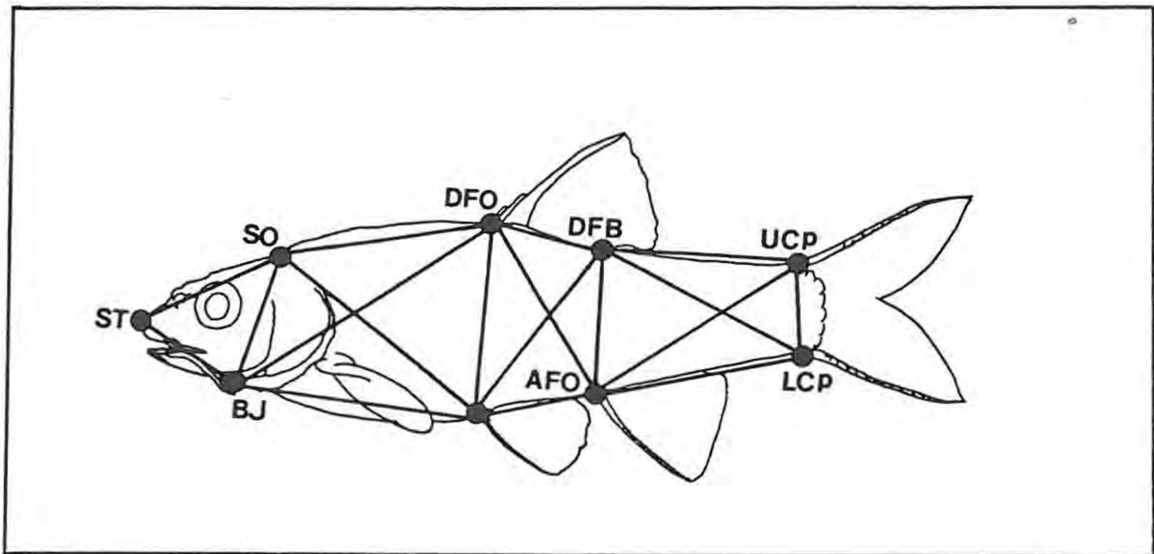


Figure 8. Truss morphometric measurements used in the yellowfish (Abbreviations in text).

A catalogue of the specimens measured has been provided in table two. The data were not corrected for measurement error (Strauss and Bookstein, 1982), as this caused greater intraspecific variance. The distances between points were standardized for growth differences by converting the data into percentage ratios of standard length.

TABLE 2. Collection numbers and sampling localities of specimens used in Truss morphometric analyses.

SPECIES	COLL. No.	LOCALITY
B. aeneus	AMSA/P7858	Pella Drift, Orange R.S.
	AMSA/P9202	Raubenheimer Dam, Gouritz R.S.
	AMSA/P10579	Krugers Drift Dam, Modder R., Orange River System
	AMSA/P7837	Blouput, Orange R.S.
	AMSA/P2677	Fish R., Orange R.S.
	AMSA/P7588	Riflespruit R. Orange R.S.
	AMSA/P2072	Augrabies Falls, Orange R.S.
	RUSI 23794	Teebus R.
	RUSI 23800	Great Brak R.
B. capensis	AMSA/PF1400	Matjies R., Doorn-Olifants R.S.
	AMSA/PF1381	Noordhoek stream, Olifants R.S.
	AMSA/P9231	Kobee trib., Doring R., Olifants RS
	AMSA/P7674	Noedhoek R., Olifants R.S.
	AMSA/P9980	Kobee trib., Doring R., Olifants RS
	AMSA/PF1370	Noordhoek stream, Olifants R.S.
	AMSA/PF1172	Jonkerhoek stock, from Olifants R.
B. kimberley-ensis	AMSA/P7893	Kakamans Bridge, Orange R.
	AMSA/J7810	Orange R.
	AMSA/B6923	
	AMSA/P4807	Verwoed Dam, Orange R.S.
	AMSA/P7914	Groblershoop bridge, Orange R.S.
B. natalensis	AMSA/PF40	Tugela R.
	AMSA/PF163	Louwsburg, Mkuze R.
	AMSA/P7315	Umzumkulwana R., Umzumkulu R.S.
B. polylepis	RUSI 21771	Phongolo-Margot R. confluence
	RUSI 21763	Yarrow R., Natal
	RUSI 25672	Usutu R.
	AMSA/P4655	Sabie-Incomati R.S.
	AMSA/P10120	Pivaan-Phongolo-Maputo R.S, Uitrecht
	AMSA/M67185	Meritz ?

Meristics

Counts of meristic characters were taken from the same specimens used for traditional morphometrics (table 1). These counts were taken as described by Skelton (1980) and are: Lateral line scales (LLS), caudal peduncle scales (CPS), lateral line to dorsal fin scales (LLD), lateral line to

pelvic scales (LLP) and predorsal scale rows (PDS) were counted. Dorsal fin ray (DFR) and pelvic fin ray (PFR) counts were also made. Figure 9 shows the meristic counts measured.

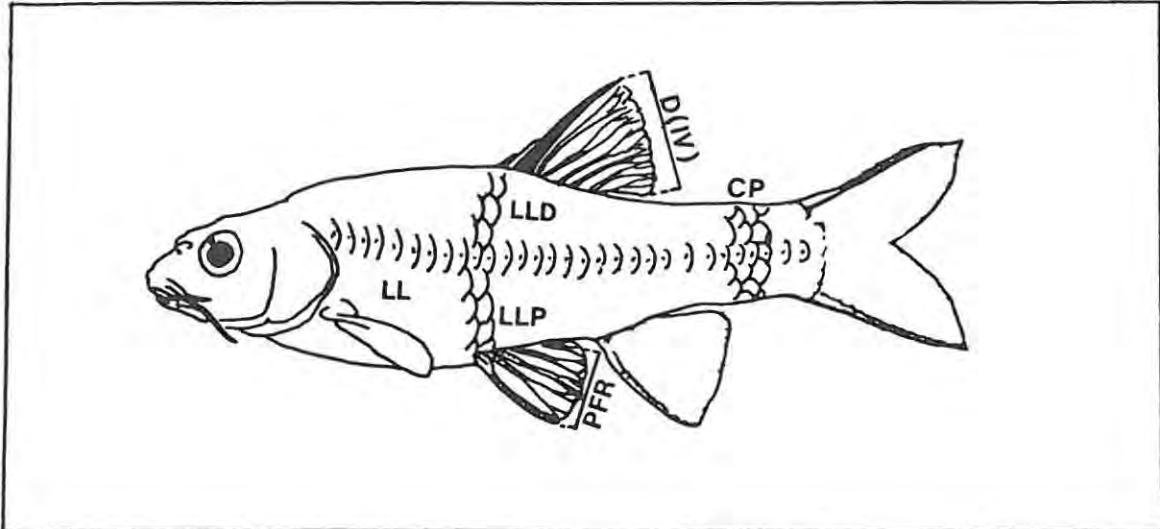


Figure 9. Meristic counts used in the yellowfish (Abbreviations in text).

Sexual Dimorphism

Barbus aeneus and B. kimberleyensis were tested for sexual dimorphism. Thirty male and 30 female specimens of B. aeneus and 12 males and 12 females of B. kimberleyensis were measured using the traditional morphometric method (see table 3 for specimen catalogue). The morphometric data were converted to a percentage ratio of standard length. Regression analysis was then carried out on graphs of the mean character ratios of males versus females for each species.

TABLE 3. Collection numbers and sampling localities of specimens used in a traditional morphometric analysis of sexual dimorphism in B. aeneus and B. kimberleyensis.

SPECIES	COLL. No.	LOCALITY
B. aeneus	AMSA/P2676	Fish R., at Ai-Ais
	AMSA/P7846	Onseepkans, Orange R.
B. kimberleyensis	AMSA/P3922	Serfontein Road Bridge, Orange R.
	AMSA/P8296	P.K. LeRoux Dam, Seekoei R.
	AMSA/P7847	Onseepkans, Orange R.
	AMSA/P4807	Verwoed Dam, Orange R.
	AMSA/P7914	Groblershoop Bridge, Orange R.

Analysis of Variance

Analysis Of Variance (ANOVA) is used to determine if the means of a group of more than two samples differ significantly. ANOVA first finds the total sum of squares (TSS) of the data set, a measure of the dispersion of all the values about the grand mean of the values. The between means sum of squares or sample sum of squares (SSS) is then calculated, which is a measure of the dispersion of the sample means about the grand mean. The dispersion of the values within the samples about their respective sample means is measured by the within samples sum of squares, or sum of squares for error (SSE). This is a measure of experimental error of the data. Working from the assumption that the samples are the same, then two best estimates of variance (Z^2 and S^2) can be found:

$$Z^2 = \frac{SSE}{k(n-1)} \quad \text{or} \quad S^2 = \frac{SSS}{k-1}$$

(where k= samples, n= values).

If the samples are the same, ratio $F = S^2/Z^2$ should be equal to one. If the means of the sample are different, the estimated variance S^2 will be greater than Z^2. Once the calculated F value exceeds the expected F value on an F-distribution curve (at the 5% level of significance) at least one pair of sample means within the data set is considered significantly different.

A oneway analysis of variance was applied to the ratios (percent standard length) of the traditional morphometric measurements of the five species.

There are various methods used to find out which pairs of sample means are significantly different (Alder and Roessler, 1972). Of these the Scheffe test is considered the most conservative method. The Scheffe multiple range test was used

to find significant differences between the five species for each of the 16 characters. The ANOVA was also used to analyze the sexual dimorphism existing within Barbus aeneus and B. kimberleyensis, using the same standardized ratios as in the regression analysis.

Principal Component Analysis

Principal component analysis (PCA) is a mathematical method of restating data and is used primarily as a method of reducing the data set. The principal components can be more easily described geometrically. Given m dimensions (characters), the first component is that direction axis from which the sum of the squared distances is the smallest.

The second component is that axis from which the sum of the squared distances is smallest, but is perpendicular to the first component. The third component is a similar distance axis, but perpendicular to the first and second components. There can be as many principal components as there are characters in the data set. A principal component is thus an uncorrelated function of the original characters (Kendall, 1980).

The goal of the PCA is to account for most of the variance in a data set using as few principal components as possible (Dillon and Goldstein, 1984). The PCA can be used diagnostically to find character relationships within principal components. In the following analysis however the PCA is used primarily to discover distances between the specimens as data points on the axes of the first and second components.

The standardized ratios from traditional and Truss morphometric measurements and the meristic counts were used in PCAs. These PCAs were used to find the first and second components of variation in the data sets, and scattergraphs of

their values were plotted. The component weights of the characters were also plotted for the first and second components.

Many workers have warned against removing size variation from data by using standardizing ratios (Achley _et al._, 1976; Humphries _et al._, 1982; Libosvarsky, 1982). For this reason unchanged data from traditional and Truss morphometrics were used in further PCAs to test the effects of data standardization.

Multiple Discriminant Analysis

Discriminant analysis is based on the desire to statistically distinguish between two or more groups of individuals. The mathematical objective is to weight and linearly combine the independent discriminating characters of the individuals in some fashion so the groups are as statistically distinct as possible (Klecka, 1975). The data set must consist of independent characters and a dependent variable which contains labels for the different groups of individuals.

The basis of discriminant analysis is the assumption that the independent character values follow multivariate normal distributions for each group, and the variances are the same per group (Jackson, 1983). This assumption allows a probability distribution curve to be fitted to each known group of values of a character. The probability curves are models of the groups in each character. Thus an unknown individual can be assigned to a known group if its character values fall within the probability curves of that group. Classification of unknown individuals and groups is one of the primary features of discriminant analysis.

Multiple Discriminant Analysis (MDA) tests the predicting power of the probability curves by reassigning the original known individual values to groups on the basis of the

probability curves. The character values are then expressed as discriminant scores. These scores are the distances from the values within a group's probability curve to the point where the curve intersects another group's probability curve. The discriminant function is the equation which describes the discriminant scores of the individuals for all the characters.

This is the simplest form of the multiple discriminant analysis. Other modifiers such as prior probabilities and costs can be added to the mathematics of the method (Jackson, 1983).

Multiple discriminant analysis can be used as a data reduction technique in much the same way as principal component analysis. The first discriminant function maximizes the ratio of between-groups to within-groups variability; the second discriminant function maximizes the ratio of residual between-groups to within-groups variability, but must be uncorrelated to the first discriminant function. The remaining discriminant functions follow this pattern, in order of decreasing statistical importance (Dillon and Goldstein, 1984).

Discriminant analysis was used on the standardized ratios of traditional and Truss morphometrics and meristic counts of the yellowfish to maximally discriminate between the five species of individuals. The discriminant scores of each individual was plotted on a graph of the first and second discriminant functions. The accuracy of the discriminating power of the MDA was disclosed in a matrix of predicted against actual species for each specimen.

Cluster Analysis

The objective of Cluster analysis is to group an initially undifferentiated set of data (e.g. specimens) into subgroups that differ in some meaningful way (e.g. species). Cluster analysis is really a collection of many methods which attempt

to reach this goal (Kendall, 1980). One must be very careful to use the method best suited to the data set. The procedure is based on a set of data containing **P** measurements taken from **N** individuals. This **N** x **P** matrix is transformed into a **N** x **N** matrix of similarity (or dissimilarity) between individuals. The similarities are computed between pairs of individuals across the **P** measurements (characters). The measure of similarity used in the procedure must be chosen by the researcher (see Jackson, 1983; Dillon and Goldstein, 1984). The researcher then selects a clustering algorithm which best defines the rules of how to cluster the individuals into subgroups, based on the inter-individual similarity values (Dillon and Goldstein, 1984). Cluster analysis can be conducted on either individuals or their characters.

Cluster analysis can be misleading if the algorithm chosen does not suit the data. The similarity index used is also vulnerable to non-linearity. Outliers (or sample error) can induce false cluster decisions (Pimentel and Smith, 1985).

In the following cluster analysis Euclidean distances were used as the distance (or similarity) measure (Sneath and Sokal, 1973). An agglomerative hierarchical technique, the Furthest Neighbour algorithm (FNA) was used as the clustering mechanism. The FNA merges the closest pair of individuals to form the first cluster. The next step can be to either include another individual into the sub-cluster or to form a second sub-cluster of two individuals, depending on the shortest distance between the individuals. The distances between clusters is measured as that pairwise distance between two clusters' furthest members (Dillon and Goldstein, 1984). Jackson (1983) describes the furthest neighbour method as being particularly suited to finding naturally separated, homogeneous clusters (e.g. species, genera etc.). Furthest neighbour clustering is also less affected by outliers than other methods.

The cluster analysis was performed on 50 specimens. Ten specimens considered closest to the mean for each species sample were chosen. Although the sample set decreased, this approach enhanced the differences between the species and similarities within the species, and decreased the presence of outliers. The cluster analysis attempted to cluster the individuals of the data set into five groups (representing the five species).

Standardized ratio data from traditional and Truss morphometrics were used in two cluster analyses. A cluster analysis was also carried out on the meristic counts of the five yellowfish species. The means of the characters from both morphometric data sets were then used in further cluster analyses. One could consider these mean values as belonging to the "average specimen" of each species sample. By decreasing the number of clusters from five (where each specimen occurs in its own cluster) to four, three and two clusters I followed the linkage pattern for the yellowfish, and thus the most similar means for the five species samples.

RESULTS

Traditional Morphometrics and Meristics

The means, standard deviations, standard errors and ranges of the traditional morphometric character ratios (% SL) of the five species are shown in figures 10-12. Appendix one contains the means and standard deviations of the traditional and Truss morphometric ratios. Further statistical analyses of these data are dealt with under the relevant headings. The meristic characters are compared for the five yellowfish species in tables four and five.

TABLE 4. Meristic scale counts of the five yellowfish species.

SPECIES	N	LATERAL LINE SCALES													
		32	33	34	35	36	37	38	39	40	41	42	43	44	45
B.aeneus	26						1	3	11	9	2	3	2		
B.capensis	24								1	6	11	3	2	1	
B.kimberleyensis	24								1	6	7	6	4		
B.natalensis	24	4	3	5	5	4	3								
B.polylepis	23				1	7	3	7	5						

SPECIES	N	CAUDAL PEDUNCLE Sc.							LAT.LINE-DORSAL Sc.		
		12	13	14	15	16	17	18	6	7	8
B.aeneus	30					30				30	
B.capensis	24			7	17					4	20
B.kimberleyensis	30					28		2		30	
B.natalensis	25		3	13	7	2			3	22	
B.polylepis	23	4	6	6	6	1				23	

SPECIES	N	LAT.LINE-PELVIC Sc.		PREDORSAL SC.					
		3	4	13	14	15	16	17	18
B.aeneus	30	4	26		1	7	11	7	4
B.capensis	24	6	18				9	13	2
B.kimberleyensis	30	2	28			14	12	3	1
B.natalensis	25	15	10	2	8	11	4		
B.polylepis	23		23		9	9	5		

TABLE 5. Dorsal and pelvic fin ray counts for the yellowfish species.

SPECIES	N	DORSAL RAYS				PELVIC RAYS		
		7	8	9	10	7	8	9
B.aeneus	30		20	10			30	
B.capensis	30		14	16			14	16
B.kimberleyensis	30		5	24	1	2	28	
B.natalensis	30	4	26				20	10
B.polylepis	26	12	14			6	15	5

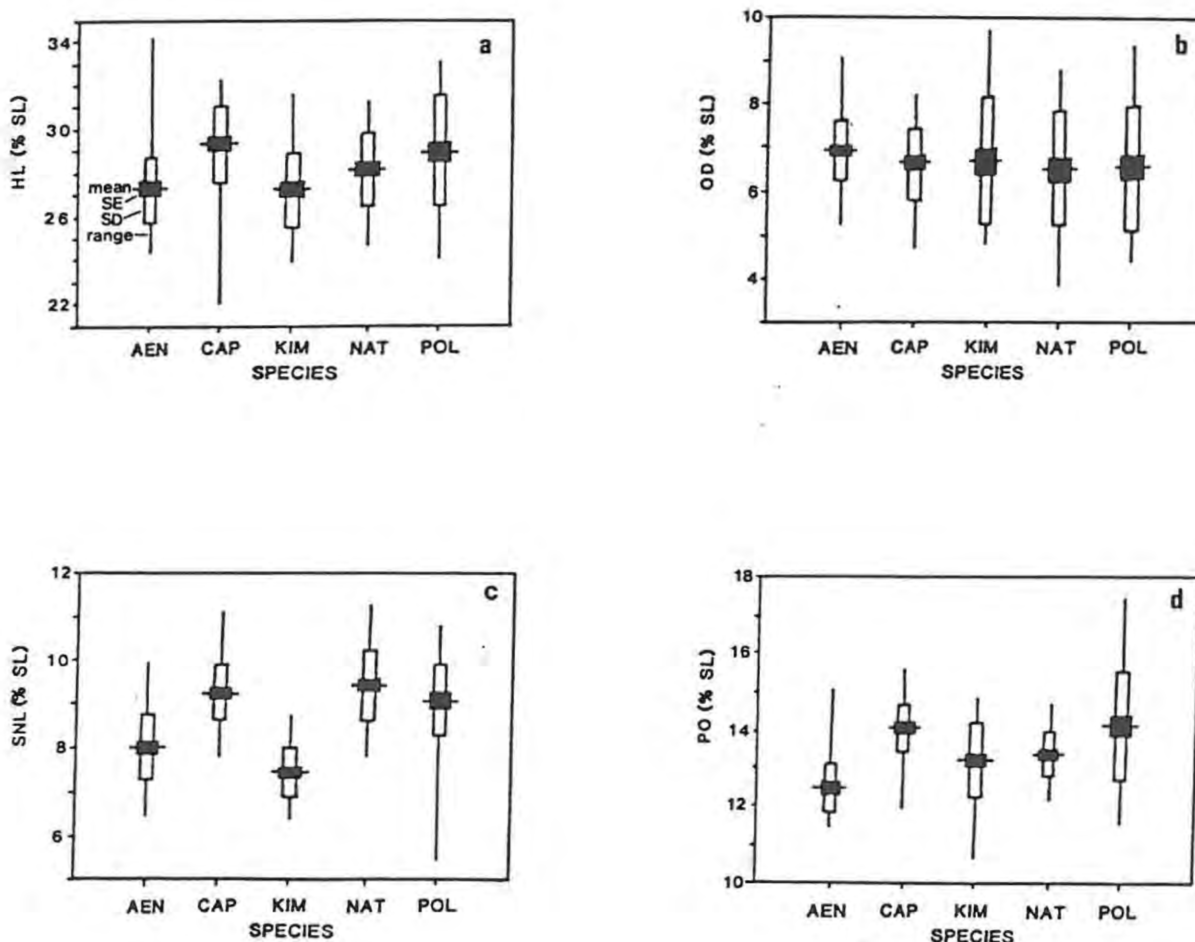
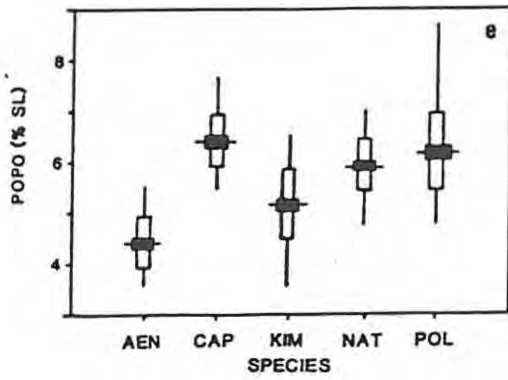
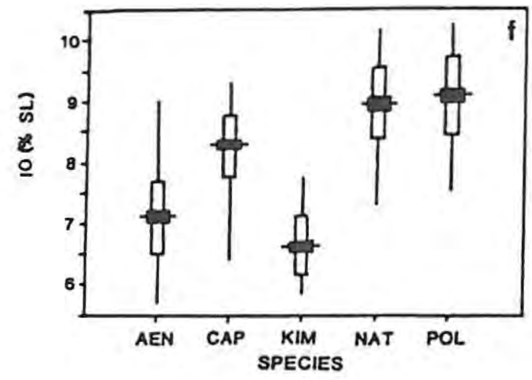
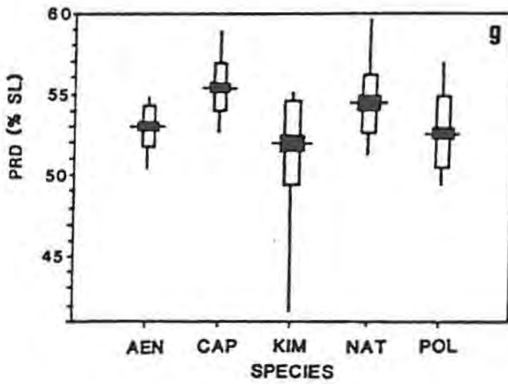
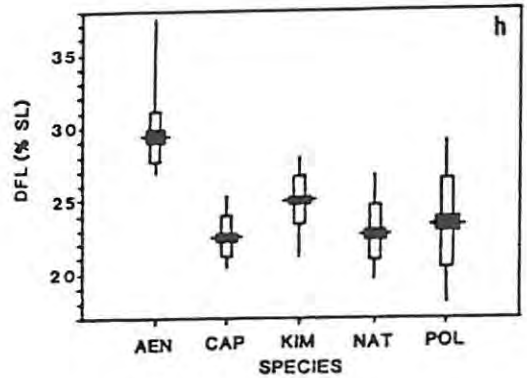


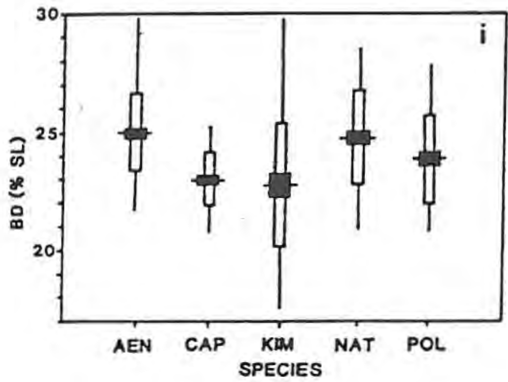
Figure 10. Head length (a), snout length (b), orbital diameter (c) and post orbital (d) percentages of standard length for the five yellowfish species.











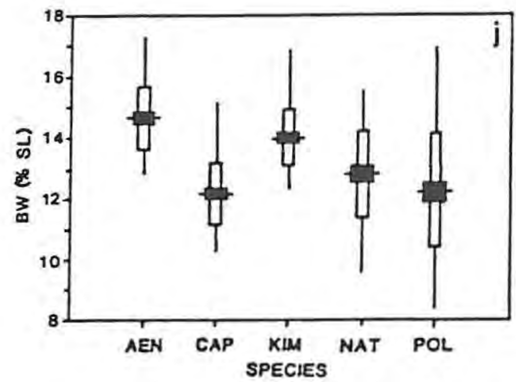


Figure 11. Post orbital to preoperculum (e), interorbital (f), predorsal length (g), dorsal fin length (h), body depth (i) and body width (j) percentages of standard length for the five yellowfish species.

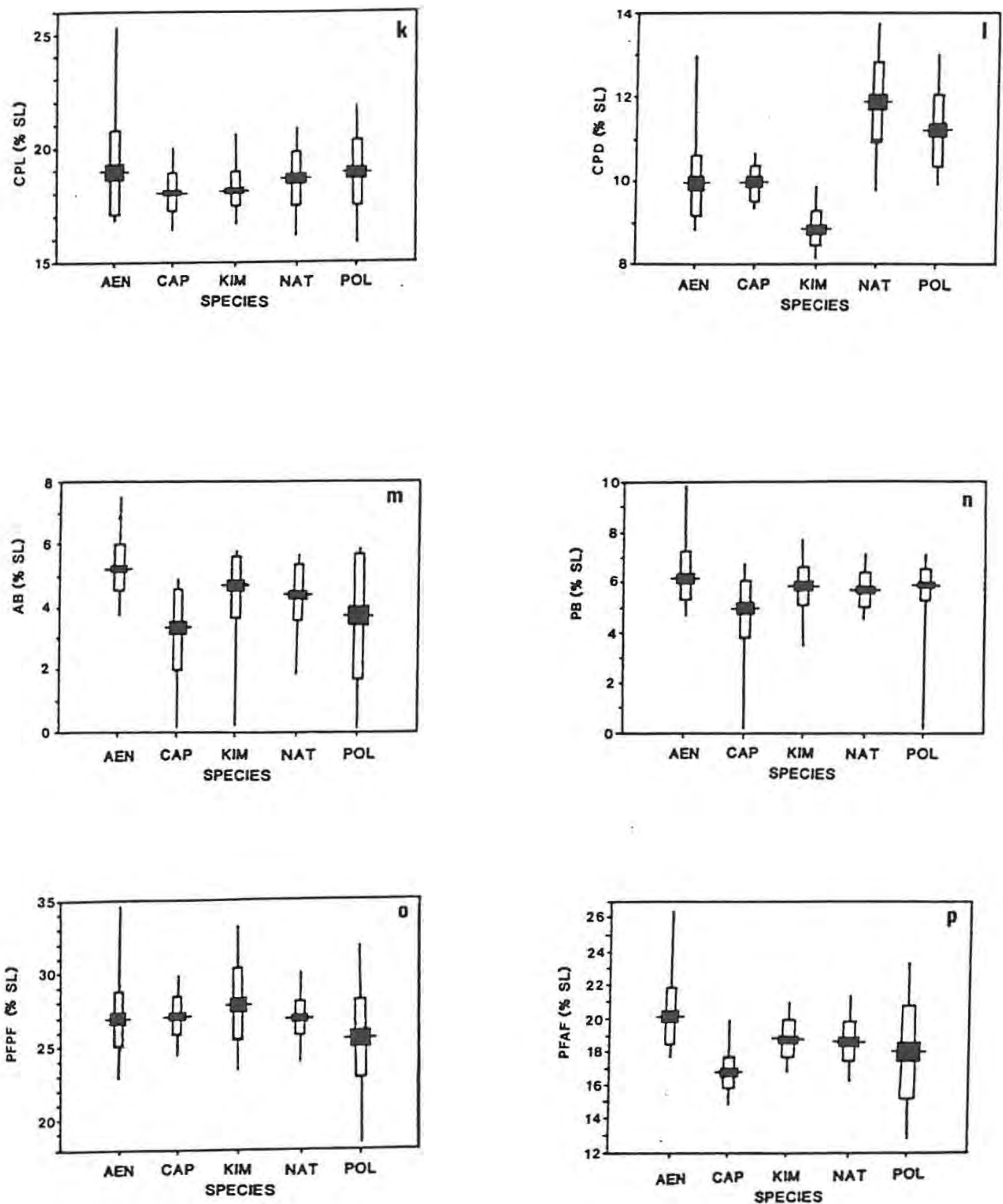


Figure 12. Caudal peduncle length (k), caudal peduncle depth (l), anterior barb (m), posterior barb (n), pectoral to pelvic fin (o) and pelvic to anal fin (p) percentage standard length for the five yellowfish species.

Sexual Dimorphism

No sexual dimorphism in Barbus aeneus and B. kimberleyensis is apparent for the morphometric characters compared (fig. 13). The regressions of males against females for B. aeneus and B. kimberleyensis characters have an r^2 coefficient of determination equal to one. The coefficient of slope (x coefficient) equaled 0.99 when the regression lines were adjusted to pass through the origin [0,0]. Thus the male and female character means are almost identical.

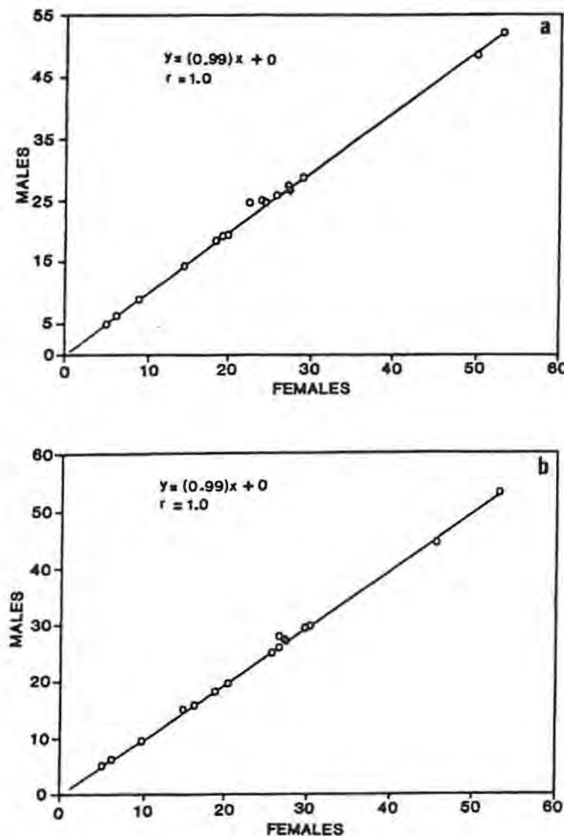


Figure 13. The regression of mean character values for males against females in B. aeneus (a) and B. kimberleyensis (b).

The results show that sexual dimorphism does not effect the morphometric characters of B. aeneus and B. kimberleyensis. Based on these results, and the fact that there is no obvious sexual dimorphism in any of the yellowfish species, sexual dimorphism was discounted as an element affecting morphological variance of the species.

Analysis of Variance

An ANOVA based on the traditional morphometric characters of the yellowfish showed that the calculated F-ratio exceeded the tabulated F-value for each of the characters except orbital diameter and interorbital distance (at the 5% level of significance). Therefore the means of one or more pairs of species differed significantly for each character. The Scheffe multiple range test was used to find out which means were significantly different. The homogeneous groups are presented in table 6.

TABLE 6. Results of the Scheffe Multiple Range ANOVA test of traditional morphometric characters for the yellowfish.

CHARACTER	HOMOGENEOUS GROUPS				
	AEN	CAP	KIM	NAT	POL
HEAD LENGTH	K	P	A		C
SNOUT LENGTH		P,N		C	C
POST ORBITAL		P	N	K	C
PO-PREOPERC.		P		P	C,N
PREDORSAL	P		P		A,K
DORSAL FIN L.		P,N		C,P	C,N
BODY DEPTH	N	K	C	A	
BODY WIDTH		P			C
PEDUNCLE L.	N,P	K	C,N	A,P	A,N
PEDUNCLE D.	C	A			
BARB (A)		P		P	C,N
BARB (P)	K	P,N	A	C,P	C,N
PECT-PELV.	C,N	A,K,N	C,N	A,C,K	
PELV-ANAL			N,P	K,P	K,N

where: A,AEN= B.aeneus C,CAP= B.capensis P,POL= B.polylepis K,KIM= B.kimberleyensis N,NAT= B.natalensis

A summary of shared characters between the 5 yellowfish species follows in table 7.

TABLE 7. **Number of shared traditional morphometric characters between the five yellowfish species.**

SPECIES	CAP	KIM	NAT	POL
AEN	2	2	3	2
CAP		3	4	8
KIM			4	2
NAT				6

where: AEN= B.aeneus CAP= B.capensis KIM= B.kimberleyensis
NAT= B.natalensis POL= B.polylepis

The ANOVA showed that a number of similar character means are found between B. capensis-B. polylepis and B. polylepis-B. natalensis. Barbus aeneus and B. kimberleyensis shared fewer similarities to each other than to the rest of the group.

The ANOVA also indicated that there was no significant difference in sexual dimorphism of Barbus aeneus and B. kimberleyensis, which substantiated the findings of the regression analysis. No significant difference was found in any of the morphometric characters tested (at the 5% level of significance).

Principal Component Analysis

a) Meristics

Principal component one (PC1) contained 38.2 % of the variance found in the meristic data, while the second component (PC2) contained 20.3%. Together they accounted for over half (58.5%) of the variation found in the meristic data of the yellowfish. Figure 14 shows the species ranges of yellowfish specimens on a graph of the first two components.

Barbus capensis was distinct from the other species groups, mainly along the second component axis for B. aeneus and B. kimberleyensis, and along the first component for B. natalensis and B. polylepis. Separation of B. natalensis and B. polylepis was poor along both axes. There was no distinct separation between specimens of B. aeneus and B. kimberleyensis, but the groups were less similar than B. natalensis-B. polylepis. There was a distinct separation between the B. natalensis-B. polylepis and B. aeneus-B. kimberleyensis groups along the first component axis.

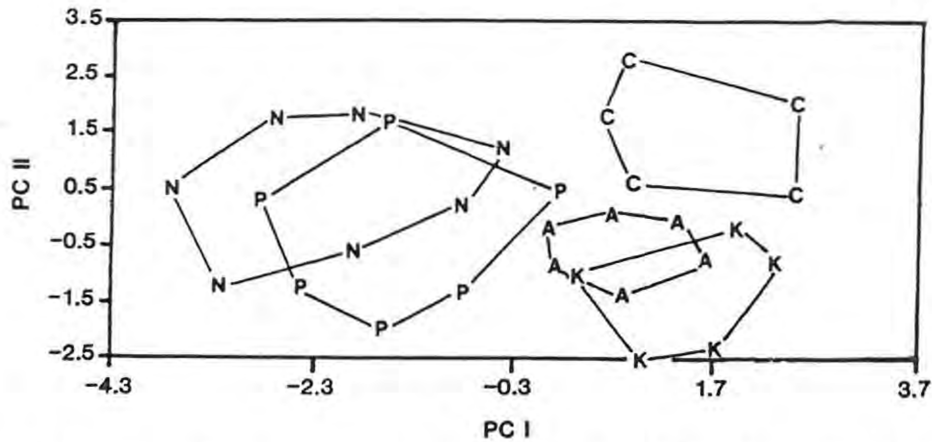


Figure 14. Species ranges of the yellowfish specimen plots for Principal Component 1 (PCI) and Principal Component 2 (PCII) of the meristic data. A= B. aeneus, C= B. capensis, K= B. kimberleyensis, N= B. natalensis and P= B. polylepis.

The graph of the character weights for the first two components (fig. 15) shows that lateral line scales and predorsal scales were the most important characters in component 1, while pelvic fin rays was an important character in component 2.

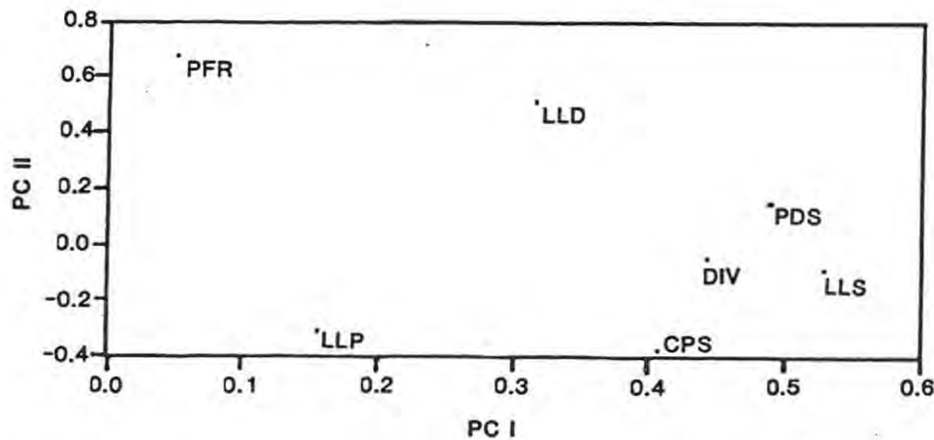


Figure 15. Character weights of the first (PCI) and second (PCII) principal components for meristic data.

b) Traditional Morphometrics

The first and second components of the traditional morphometric data accounted for 97.4% of the variation amongst the yellowfish (PC1= 94.5%, PC2= 2.9%). The first component is generally considered the component of size variation, and the second the component of shape variation (Winan, 1985; Humphries et al., 1981; Schaefer and Cavender, 1986). The first and second components of the size-

standardized ratios accounted only for 50.2% of the variance (PC1= 32.5%, PC2= 17.7%). Most of the variation in the unmodified data set must have been caused by the size differences between specimens. Figures 16 and 17 show the change in character weights from unmodified to standardized data. The graph changed from clusters of characters to a more even spread of character weights within the two principal components.

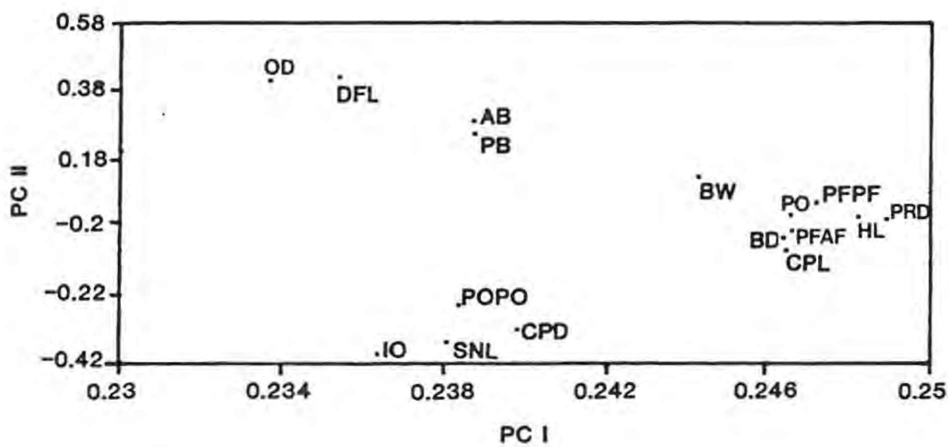


Figure 16. Character weights of the first (PCI) and second (PCII) principal components for original traditional morphometric data.

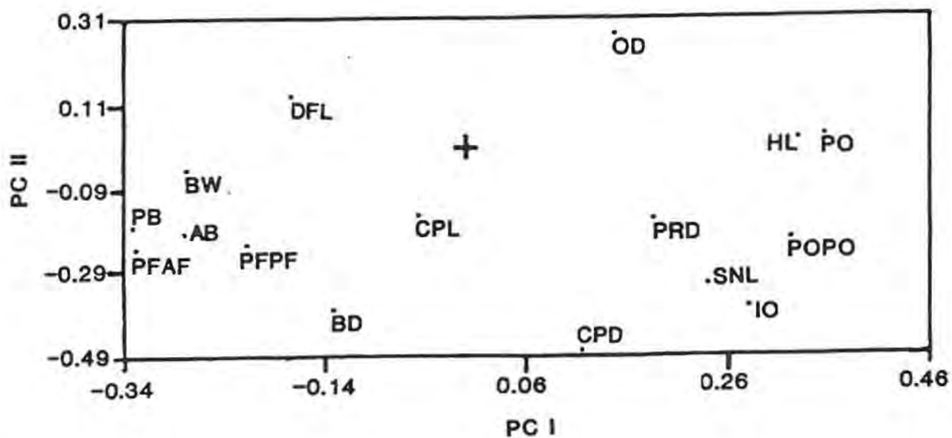


Figure 17. Character weights of the first (PCI) and second (PCII) principal components for standardized traditional morphometric data.

The scatterplots of specimens on the PC1 and PC2 axes of the original and transformed data were very different (fig. 18 and 19). The specimen having the largest value for PC1 of the unchanged data also had the largest standard length of the entire yellowfish data set. Thus it seems that PC1 of the untransformed data merely reflected the size range of the data and had no taxonomic value.

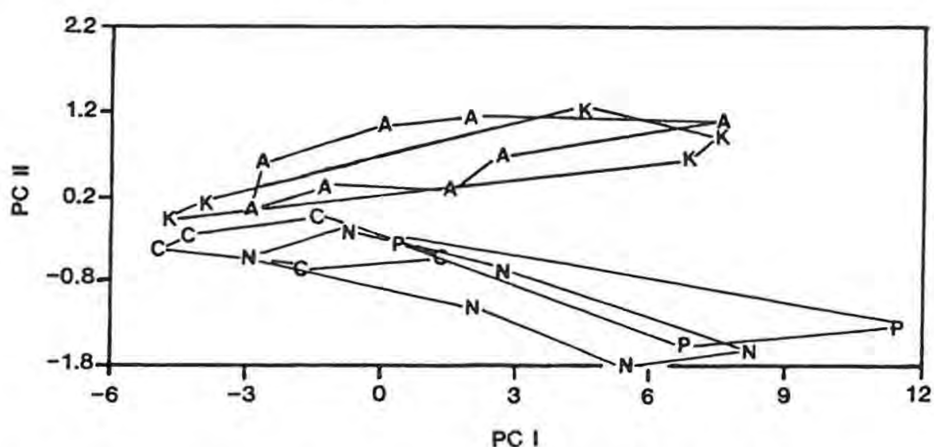


Figure 18. Species ranges of the yellowfish specimen plots for Principal Component 1 (PCI) and Principal Component 2 (PCII) of the original traditional morphometric data. A= B. aeneus, C= B. capensis, K= B. kimberleyensis, N= B. natalensis and P= B. polylepis.

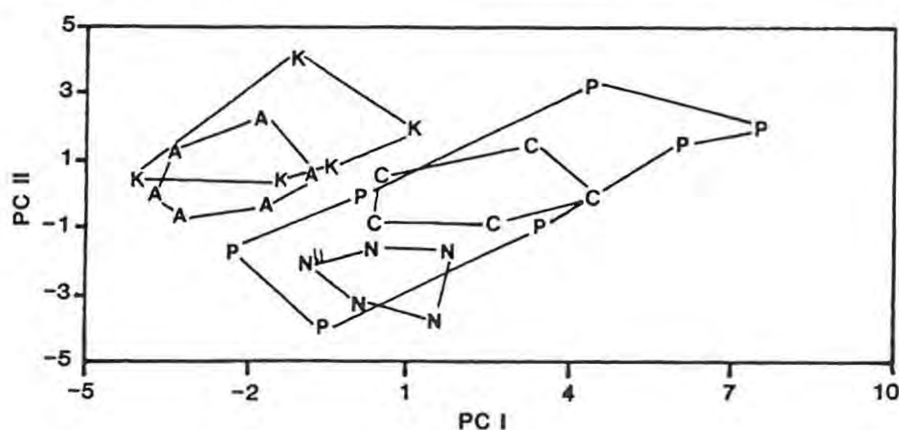


Figure 19. Species ranges of the yellowfish specimen plots for Principal Component 1 (PCI) and Principal Component 2 (PCII) of the ratio-transformed traditional morphometric data. A= B. aeneus, C= B. capensis, K= B. kimberleyensis, N= B. natalensis and P= B. polylepis.

Ratio-standardized data shows B. capensis and B. natalensis to

be inseparable from B. polylepis, but distinct from each other, while Barbus aeneus was almost inseparable from B. kimberleyensis using traditional morphometric characters.

c) Truss Morphometrics

The original Truss data PC1 and PC2 contained 97.8% and 0.8% of the variation between species respectively (98.6% in total). The PCA produced a similar scatterplot of specimens on the axes of PC1 and PC2 to that of the original traditional morphometric data. The largest specimen also proved to have the greatest PC1 value. The results of this PCA were not used in further analysis.

The Truss ratio PC1 and PC2 made up 48.4% of the variation in the data set (28.3% and 20.1% respectively) and produced the specimen scatterplot shown in figure 20. Barbus kimberleyensis was inseparable from B. aeneus along the axes of both components. Barbus natalensis and B. polylepis had many inseparable individuals. The B. aeneus-B. kimberleyensis group and the B.natalensis-B.polylepis group had specimens in common with Barbus capensis, but not with each other (along the axis of the second principal component).

It is interesting to note that the characters weighted the most in principal component two were dorsal fin base to upper caudal peduncle, dorsal fin base to lower caudal peduncle, supraoccipital to dorsal fin origin, and branchiostegal junction to dorsal fin origin (fig. 21). All these characters are related to the position of the dorsal fin on the yellowfish. The first two characters are positively weighted, the other two are negatively weighted. As the distance from the branchiostegal junction and supraoccipital to the dorsal fin origin increases so the distance between the lower caudal peduncle and upper caudal peduncle to the dorsal fin base decreases. The dorsal fin moves posteriorly on the fish body from the B. natalensis-B. polylepis group to the

B.aeneus-B. kimberleyensis group.

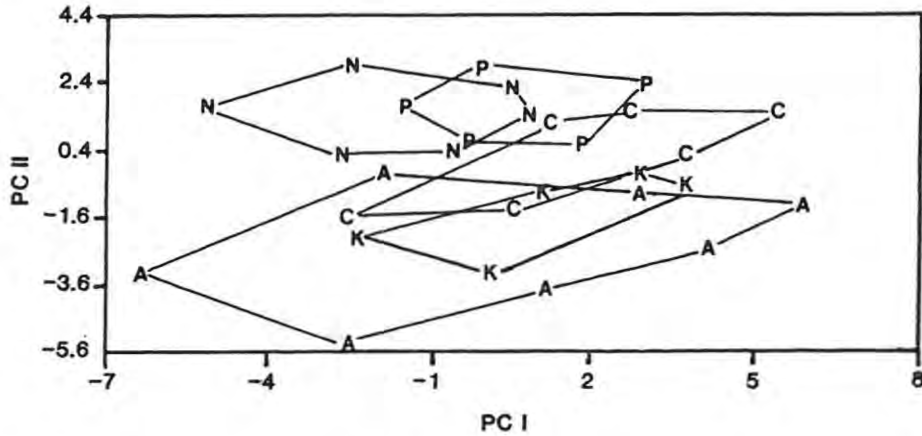


Figure 20. Species ranges of the yellowfish specimen plots for Principal Component 1 (PCI) and Principal Component 2 (PCII) of the ratio-transformed Truss morphometric data. A= B. aeneus, C= B. capensis, K= B. kimberleyensis, N= B. natalensis and P= B. polylepis.

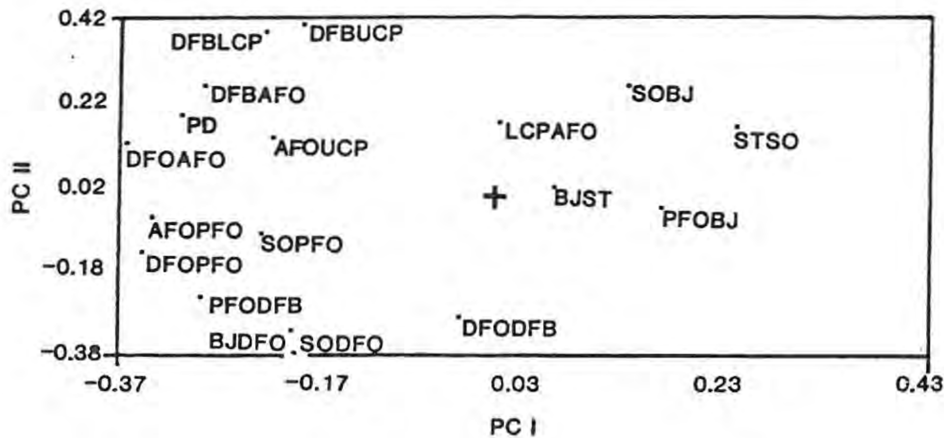


Figure 21. Character weights of the first (PCI) and second (PCII) principal components for ratio-standardized Truss data.

Discriminant Analysis

a) Meristics

The first discriminant function (DF1) contained 67.4% of the

discriminant information needed to separate the groups within the meristic data. The second discriminant function (DF2) contained 22.2% of the discriminating information and the total percentage for DF1 and DF2 was 89.6%. The lateral line scale counts held the most discriminant information in DF1, while the number of scales between the lateral line and dorsal fin origin was most important in DF2. Table 8 shows the confusion matrix (Jackson, 1983) produced when the meristic discriminant analysis attempted to predict groups for specimens, according to its discriminant models.

The confusion matrix tests the discrimination power of the functions derived. Barbus aeneus proved to be predicted correctly the least number of times, but was still 80% accurate.

TABLE 8. Predicted and actual species for the meristic discriminant analysis.

Actual Group	Predicted Group AEN	CAP	KIM	NAT	POL	TOTAL
AEN	24	0	6	0	0	30
CAP	1	28	1	0	0	30
KIM	5	0	25	0	0	30
NAT	1	0	0	27	2	30
POL	1	0	0	2	27	30

Where: AEN= B.aeneus CAP= B.capensis KIM= B.kimberleyensis NAT= B.natalensis POL= B.polylepis

A familiar pattern emerged from the scatterplot of specimens on the axes of DF1 and DF2 (fig. 22).

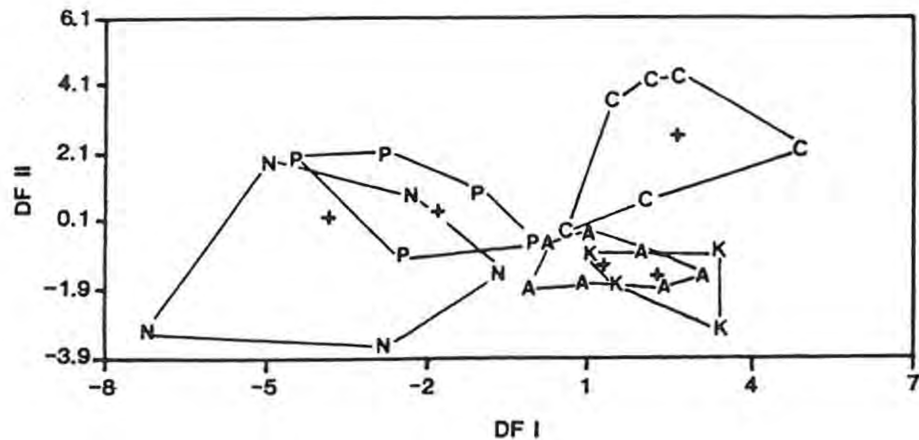


Figure 22. Species ranges of the yellowfish specimen plots for Discriminant Function 1 (DFI) and Discriminant Function 2 (DFII) of the meristic data. A= B. aeneus, C= B. capensis, K= B. kimberleyensis, N= B. natalensis and P= B. polylepis, '+'= group centroid.

For meristic data which has been transformed to discriminate maximally between species, the specimens were found in three groups. The B. aeneus-B. kimberleyensis group and the B. natalensis-B. polylepis group were separated along DF1. Barbus capensis was separated from the first group along DF2, and from the second group along DF1. The most important discriminating meristic value between B.aeneus-B. capensis-B. kimberleyensis and B. natalensis-B. polylepis was lateral line scales. Lateral line to dorsal origin scales was important in separating B. capensis from the rest of the group. Table 4 showed a similar result.

b) Traditional Morphometrics

Discriminant function one and two contained 95% of the discriminant information available in the standardized traditional morphometric data set (DF1= 86.2%, DF2= 8.8%). The success rate of specimen allocation to predicted groups was high (table 9).

TABLE 9. Predicted and actual species for the standardized traditional morphometric discriminant analysis.

Actual Group	Predicted Group AEN	CAP	KIM	NAT	POL	TOTAL
AEN	29	0	1	0	0	30
CAP	0	28	0	1	1	30
KIM	0	0	30	0	0	30
NAT	0	2	0	27	1	30
POL	1	3	0	2	25	30

where: AEN= B.aeneus CAP= B.capensis KIM= B.kimberleyensis NAT= B.natalensis POL= B.polylepis

The scatterplot of the specimens on the first and second discriminant functions show a different spatial arrangement to that of the meristic discriminant analysis (fig. 23). Three groups are present, but consist of B. aeneus, B. kimberleyensis and the B. capensis-B. natalensis-B. polylepis group. Barbus capensis and B. natalensis are distinct from each other but both share B. polylepis specimens. Barbus aeneus and B. kimberleyensis are strongly discriminated from the rest of the group along DF1.

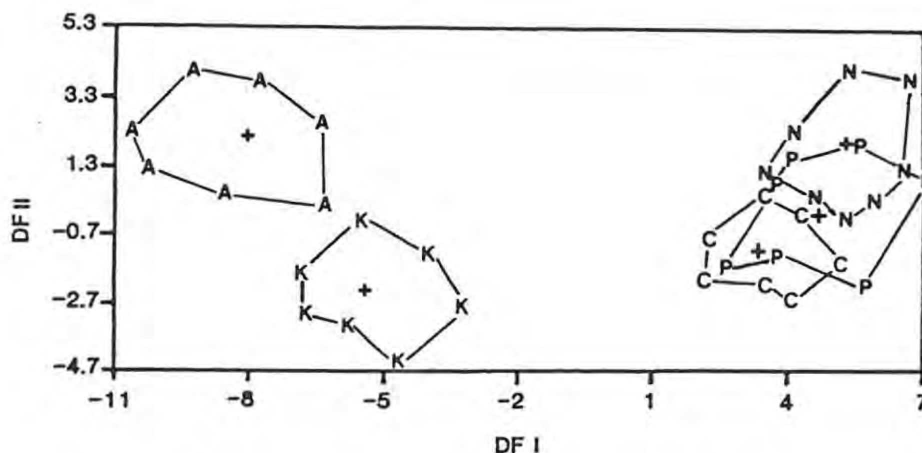


Figure 23. Species ranges of the yellowfish specimen plots for Discriminant Function 1 (DFI) and Discriminant Function 2 (DFII) of the ratio-transformed traditional morphometric data. A= B. aeneus, C= B. capensis, K= B. kimberleyensis, N= B. natalensis and P= B. polylepis, '+'= group centroid.

The most important characters in DF1 are dorsal fin length and caudal peduncle depth. Jubb (1967) used the size of the fourth dorsal spine as a means of differentiating between Barbus aeneus-B. kimberleyensis and the rest of the yellowfish in his taxonomic key.

The characters with the most discriminant information for DF2 were head length and snout length. Only the mature specimens of B. aeneus and B. kimberleyensis can be visually distinguished. The head of the B. kimberleyensis specimens changes anteriorily from convex to concave with age, lengthening relative to the head of Barbus aeneus specimens. Thus the two species were well discriminated along the DF2 axis. Relative head length also appeared to discriminate between B. capensis and B. natalensis.

c) Truss Morphometrics

The standardized Truss data was converted into discriminant functions, of which discriminant one and two contained 91% of the discriminant data (DF1= 80.4%, DF2= 10.6%). The confusion matrix in table 10 exposes the precision with which the discriminant analysis was able to predict species from the discriminant scores of the specimens.

The least accurate species prediction proved to be for B. aeneus, where 83.3% of the specimens were correctly allocated. The graph of the discriminant scores of specimens for DF1 and DF2 (Fig. 24) shows that B. aeneus and B. kimberleyensis are very similar. Their group centroids (mean discriminant scores) fall well within each others range. Barbus capensis and B. natalensis specimen ranges overlap the B. polylepis range to a small extent, but are well discriminated from each other.

TABLE 10. Predicted and actual species for the standardized Truss morphometric discriminant analysis.

Actual Group	Predicted Group AEN	CAP	KIM	NAT	POL	TOTAL
AEN	25	1	4	0	0	30
CAP	0	29	0	0	1	30
KIM	2	0	28	0	0	30
NAT	0	0	0	29	1	30
POL	0	1	0	3	26	30

where: AEN= B.aeneus CAP= B.capensis KIM= B.kimberleyensis NAT= B.natalensis POL= B.polylepis

The most important characters in DF1 are the distances from supraoccipital to dorsal fin origin, dorsal fin origin to dorsal fin base, dorsal fin base to upper caudal peduncle and dorsal fin origin to anal fin origin. The greatest discrimination information in DF1 therefore is the relative position of the dorsal fin on the yellowfish. Caudal peduncle depth was the major contributor to the discrimination between species in DF2.

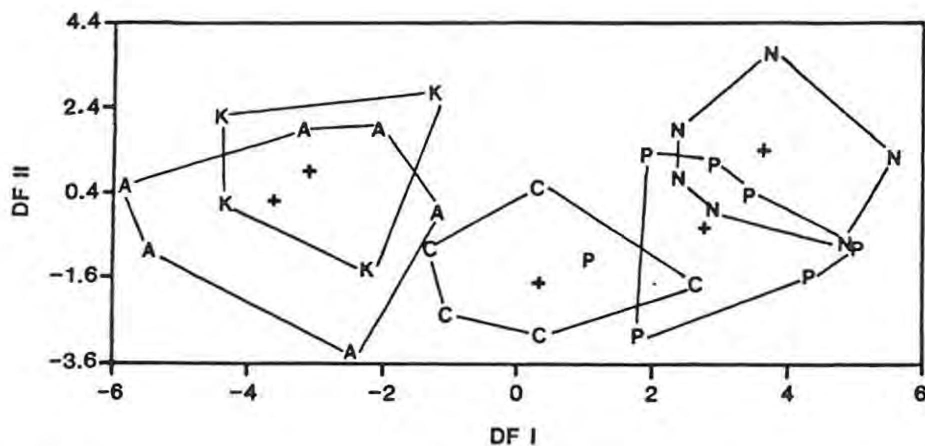


Figure 24. Species ranges of the yellowfish specimen plots for Discriminant Function 1 (DFI) and Discriminant Function 2 (DFII) of the ratio-transformed Truss morphometric data. A= B. aeneus, C= B. capensis, K= B. kimberleyensis, N= B. natalensis and P= B. polylepis, '+'= group centroid.

Cluster Analysis

The expected result was five clusters of specimens, reflecting the five species. The meristic cluster analysis however produced one cluster containing 44 specimens, two clusters containing two specimens each, and two clusters containing one specimen each (out of a total of 50 specimens). Traditional morphometrics grouped the specimens into one main cluster, consisting of 34 specimens, and four lesser clusters. The Truss cluster analysis produced one cluster of 45 specimens, one cluster of two specimens and three clusters of one specimen. The difficulty in clustering the specimens further highlights variability within the species and the close similarities between the yellowfish group.

The cluster analyses of the mean character values for the Truss and traditional morphometric data sets provided an indication of the morphological similarities between the five species. Figure 25 summarizes the clustering pattern as the number of clusters was decreased. The pattern was almost the same for both data sets.

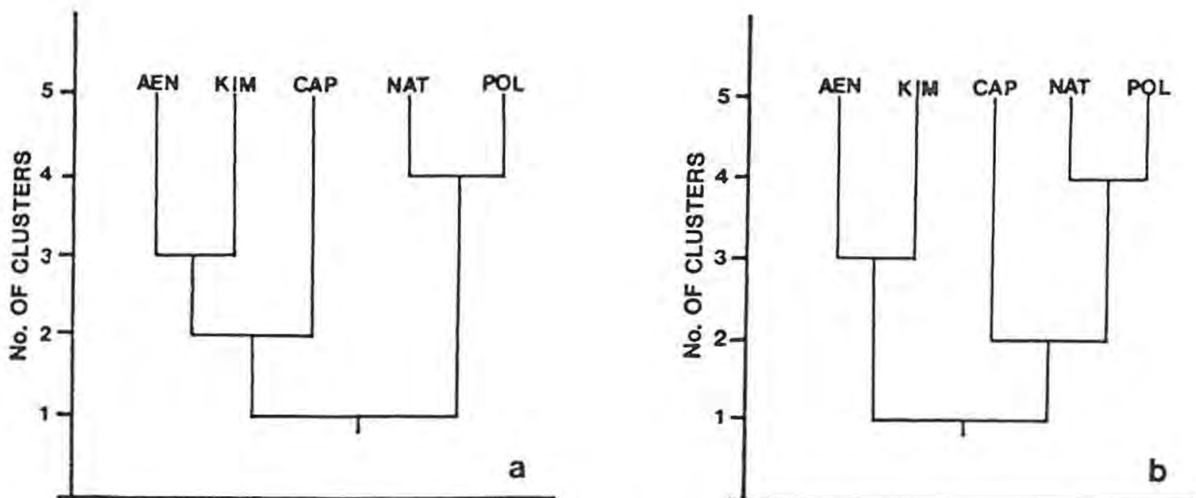


Figure 25. The linkage pattern in the cluster analysis of the mean character values of (a) Truss and (b) Traditional morphometrics.

Barbus natalensis and *B. polylepis* were linked first, indicating the greatest similarity, followed by *B. aeneus* and *B. kimberleyensis*. *Barbus capensis* was then linked to the *B. aeneus*-*kimberleyensis* cluster in the Truss morphometrics, while the species was linked to the *B. natalensis*-*polylepis* cluster in the traditional morphometric analysis. Thus while *B. capensis* has a similar shape to *B. aeneus* and *B. kimberleyensis*, it has more morphometric characters in common with *B. natalensis* and *B. polylepis*.

DISCUSSION

The objective of the multivariate analyses was to test the hypothesis that the yellowfish specimens represented different species or species-groups, using various data sets. The principal component analysis showed very similar results to the discriminant analysis. Nevertheless it could not separate the B. aeneus-kimberleyensis specimens using traditional morphometric data. Thus discriminant analysis, which reduced the data set to functions of greatest discrimination between the species, produced the most useful information.

Barbus aeneus and B. kimberleyensis specimens had overlapping ranges on all of the graphs except that of the standardized traditional morphometric discriminant analysis. The results portrayed by this graph (fig. 25) are very similar to the results of the analysis of variance of traditional yellowfish morphometrics. The ANOVA showed that Barbus polylepis shared many characteristics with B. capensis and B. natalensis, whereas B. aeneus and B. kimberleyensis shared few characteristics with each other or the rest of the group.

Such a situation may have developed due to character displacement (Brown and Wilson, 1956). Barbus aeneus and B. kimberleyensis are sympatric, both endemic to the Orange-Vaal River system. It is probable that a common ancestor to the southern African yellowfish attained southern Africa in the mid-pliocene (Skelton, 1980) and the remaining yellowfish species have developed as relict, geographically isolated populations of this once broadly distributed species. The three isolated species, B. polylepis, B. natalensis and B. capensis have diverged less from each other morphometrically than have the two sympatric species. Sympatry may have led to an enhanced rate of phenotypic divergence as the two species competed for resources within the river habitats.

Alternatively, the sympatric species may have developed from

two populations of an ancestral Orange-Vaal River species following differing life history styles. Tomasson (1983) notes two important differences between B. aeneus and B. kimberleyensis:

1. The latter appears to be adapted to a warmer climate, as it spawns four to six weeks later (into summer) than B. aeneus, and has a later resumption of growth. Time of spawning is related to the survival of eggs and larvae, and a weak year class results from a late spawning. This may be why B. kimberleyensis is far less abundant than B.aeneus.

2. Barbus kimberleyensis becomes increasingly piscivorous with size, allowing the species to reach a considerably larger ultimate size than B. aeneus.

The most noticeable physical differences between the two species is the large terminal mouth and relatively depressed head of B. kimberleyensis. These differences are linked to the differing feeding strategies. The difference in diet is also expressed in the different gut lengths of the two species (Eccles, 1986). Barbus kimberleyensis has a shorter, less convoluted gut in keeping with its carnivorous nature. The species can also generally be distinguished by differences in colour; B. aeneus has a golden hue, while B. kimberleyensis tends to be silvery-grey.

The two species hybridize under artificial conditions, producing viable young; therefore the species are only separated by prezygotic isolating mechanisms (Ayala, 1978). The difference in spawning times and choice of breeding sites probably plays a role in limiting hybridization. The species often hybridize when kept in artificial ponds, possibly because the species cannot move off to preferred breeding habitats. Accidental cross-fertilization could occur due to the breeding activities of the species taking place in close

proximity (van Loggenberg, pers comm.).

Although B. aeneus and B. kimberleyensis have a similar body shape and set of meristic counts, the morphometric and biological information available on the species supports their status as separate species.

Multivariate analysis of three distinct data sets has shown that it is difficult to separate B. natalensis and B. polylepis morphologically. Skelton (pers comm.) questions the validity of recognizing B. polylepis and B. natalensis as two distinct species on available data. The major difference between these two species is their geographical range. The ranges are questionable as well; B. polylepis occurs in the Phongolo River and northwards up to the southern tributaries of the Limpopo River, while B. natalensis occurs in the Natal rivers south of the Phongolo River. This disjunction in the ranges at the Phongolo River appears to be more a political boundary than a geographical one. An important distinguishing feature of B. polylepis was that the species did not experience changes in mouthshape. However Gaigher (1975) found that the species does show mouthshape changes when not in competition with B. marequensis.

Barbus natalensis and B. polylepis are not separated on the basis of principal component one and two and discriminant function one and two of the three data sets. However, for all three data sets the discriminant analysis confused less than 10% of the B. natalensis specimens with B. polylepis specimens (see tables 8, 9 and 10). Thus when considered as a whole, there is enough variance in the data sets of the two species for accurate allocation of specimens. This is not necessarily evidence against synonymy, because the variance may merely reflect geographic variants of the same species. Further discussion on the synonymy of these species is continued in the karyology discussion.

Barbus capensis is differentiated from the other yellowfish species by meristic counts and body shape, but cannot be separated from B. polylepis on the basis of traditional morphometric characters. Barbus capensis and B. polylepis also shared the most common characters in the ANOVA of traditional morphometrics. It is unusual that the two species geographically most widely separated should be most similar morphometrically. Barbus polylepis appears to have a general phenotype, showing characteristics of both B. capensis and B. natalensis.

Multivariate analyses are only as useful as the data provided. Thus, two species sharing the same range on a graph of principal components or discriminant functions may be very similar for that data set, but not for other characters. Using body shape measurements may produce misleading results, depending on the relative condition of the specimens. For example, Oreochromis mossambicus showed two distinct clusters on a Truss discriminant analysis graph for two populations. Although the same species, one population was undernourished, while the other was in exceptionally good condition and had a far deeper body depth (James, pers comm.). The multivariate analysis of shape data is useful in the present analysis of the yellowfish because the most important characters in the principal components and discriminant functions are not affected by condition i.e. position of the dorsal fin, head shape and to an extent peduncle depth.

It is vitally important to know as much as possible about the nature of the data being used in a statistical analysis, and therefore it was necessary to test the effects of sexual dimorphism. There was no sexual dimorphism in the traditional morphometrics of B. aeneus and B.kimberleyensis, on the basis of regression analysis and analysis of variance. The result is not surprising as it is impossible to tell apart externally with any certainty the sexes of the five yellowfish species. Both sexes develop tubercles during the spawning

season, however only B. aeneus males develop tubercles on the branched rays of the anal fin. Mulder (1973) found no length or length/mass dimorphism between the sexes of B. aeneus and B. kimberleyensis, but the females in both cases had greater longevity. In the present study it was assumed that the sexes of the other yellowfish species were equally undifferentiated.

It has been recommended that multivariate analyses be applied to untransformed data as much as possible, as transformations of data may introduce artifacts not present in the original data. For this reason untransformed and ratio-transformed data were used in the principal component analyses. The size variations of the untransformed data set proved too great, and PC1 merely consisted of size variance. Once size was standardized, useful information on character variance was forthcoming.

In conclusion, the results of the analyses of morphometrics, body shape and meristics can be summarized as follows:

1. The yellowfish can be divided into two groups; B. natalensis-B. polylepis-B. capensis and B. aeneus-B. kimberleyensis.

2. Barbus natalensis and B. polylepis are very similar, and may be synonymous.

3. Barbus capensis is very similar to B. polylepis morphometrically.

4. Barbus aeneus and B. kimberleyensis are distinct morphometrically, and are discreet species.

The above observations form the background for the next chapter; the exploration of the yellowfish karyotypes.

D. KARYOLOGY

INTRODUCTION

Ever since Tjio and Levan's (1956) revolutionary work on the human karyotype, karyology has been used to define and distinguish species successfully in many taxa. Karyology in fish has proved to be a more problematic endeavor (Denton, 1973), because the chromosomes of fishes are small and numerous, probably due to polyploidy in the ancestral fish genome (Sharma, 1972).

The first fish chromosomes were observed by Retzius (1890) in the species Myxine gluttinosa. It was not until the squash and flame-dry techniques of Roberts (1964) and Denton and Howell (1969) were developed that fish chromosome numbers and karyotypes could be determined.

Blaxhall (1975) noted that cell culture techniques produced the best chromosome results. Chen and Ebeling (1975) used cultured gill epithelium cells from hybrid killifish and platyfish. They noted that for cytotaxonomic studies short term primary culture cells were necessary to minimize the risk of altering the essential karyotype of the species. Al-Sabti (1987) used blood leukocyte culture to produce fish karyotypes. Cultured lymphocyte cells have also been used as a chromosome source in fishes (Blaxhall, 1983; Hartley and Horne, 1985).

However, the cell culture techniques tend to be complex and time consuming. Many workers prefer the simple but effective methods similar to that described by Kligerman and Bloom (1977), using tissue removed directly from the fish.

The karyology of fishes has advanced little more than the description of gross chromosome morphology. A change in a gene sequence causing a phenotypic variation in the species may not

necessarily alter the gross shape of the chromosome. Gold (1980) encountered this problem in the speciose cyprinid genus Notropis. Twenty-two of the species he studied displayed similar gross karyotypes.

Chromosomal banding has proved to be a useful refinement in the karyology of mammals. Unfortunately, less success has been had with fishes.

C-banding may prove useful in identifying different genetic stocks of fish, or homologies between species (Hartley and Horne, 1985). Giles et al. (1985) used C-banding to show that variations of karyotype in a population of Gobius paganellus occurred due to Robertsonian translocations (i.e. centromeric fusions).

G-banding has proved very useful in the human karyotype, enabling the identification of almost every chromosome, but does not have the resolution needed for accurate karyotyping in fishes (Blaxhall, 1983; Hartley and Horne, 1985). Rivilin et al. (1985) found that only the larger chromosomes of Apogon maculatus can be identified with confidence. Schmid (1978) experienced a similar problem when attempting to G-band amphibian chromosomes.

Another type of differential chromosome staining presently in use is the silver staining of nucleolar organizer regions (NORs).

The NOR site is negatively heteropycnotic, and, if present interstitially, causes a Giemsa-stained chromosome to appear to be made up of a main body and a satellite fragment. Silver staining allows the NOR site to be seen. Each species has at least one pair of homologous NOR-containing chromosomes. These sites could be used as follows:

1. The NOR satellite chromosomes can be used as chromosome markers for specific basic genomes i.e. systematic or phyletic markers (Schmid, 1978; Schulz-Schaeffer, 1980; Rivilin et al., 1985).

2. The sites could be used to provide genetic information e.g. as an indication of polymorphism (Gold, 1984; Giles et al., 1985).

The following variations in NORs between different groups have been documented (Gold, 1984):

1. Absolute number of NOR sites per genome (interspecific)

2. Position or chromosomal location of the sites (interspecific)

3. Relative size of individual NOR sites (intraspecific)

4. Number of active NOR sites per cell (intraspecific)

Gold (1984) silver-stained the NOR sites of *Notropis lutrensis*, *N. emiliae*, *N. venustus*, *Notemigonus crysoleucas*, *Pimephales vigilax* and *Campostoma anomalum*. He found that the interspecific differences occurring between the NOR sites appeared to correspond to the established taxonomy of the six species, and concluded that the sites may be useful as systematic markers in cyprinids.

Karyology has been used with varying degrees of success in fish taxonomy. Campos and Hubbs (1973) used karyotype comparisons to show that the placement of *Opsopoeodus emiliae* into *Notropis* may have been premature, as the species gross karyotype was closer to that of the genus *Notemigonus*.

Gjedrem et al. (1977) found the karyotypes of Salmonidae hybrids to be similar to those of the parent species, while

Loginova and Krasnoperova (1982) observed that the crossbreeds of *Salmo salar* (2N = 58) and *Oncorhynchus garbuscha* (2N = 52) had a karyotype varying between 52 and 58. Marian and Krasznai (1979) crossed the cyprinids *Ctenopherygodon idella* (grass carp) and *Aristichthys nobilis* (big head carp) to produce a triploid F1 hybrid. Beck *et al*. (1980) showed that the female (in this case *C. idella*) provided the diploid chromosome set, and the male (*A. nobilis*) provided the haploid set.

Loudenslager and Thorgaard (1979) analyzed the karyotypes of two subspecies of the cutthroat trout *Salmo clarki* and concluded that each (*Salmo c. bouvieri* and *Salmo c. lewisi*) represent distinct evolutionary lines in the Rocky mountain regions of the U.S.A. Dorafeygva and Rukhkyan (1982) used karyological data in addition to morphological and ecological information to reconstruct the sequence of divergence of the four subspecies *Salmo ischchan aestivalis*, *Salmo i. darilewskii*, *Salmo i. gegarkuni* and *Salmo i. ischchan* in the Sevan Lake region. On the basis of geological history and karyological data, Rukhkyan (1984) showed that the Alabalakh trout (found exclusively in the Sevan Lake) was a relict of *Salmo trutta* rather than of *Salmo ischchan*, as was previously thought.

Ishii and Yabu (1985) discovered that *Eleginus gracilis* has a diploid number of 26, which is considerably less than the rest of the species of this gadid genus, whose diploid numbers fall between 38 to 48. This data could have important taxonomic implications.

Passakas and Tesch (1980) and Bieniarz *et al*. (1981) have used karyology to sex the yellow eel (*Anguilla anguilla*), which experiences sex reversal. Fish karyology has not yet produced the same calibre of work found in other phyla, but is becoming an important addition to ichthyology. Although much of fish karyology is aimed at recording karyotypes, the above examples show that there is a growing number of workers using

karyology as a tool for studying genetics, hybridization, polyploidy, sex and sex reversal and taxonomy. Karyology and other cytotaxonomic studies are a useful supplement to taxonomy based on morphometric and meristic studies.

Although the yellowfish appear to be very similar physically, the results of the multivariate analyses hint at patterns of stronger affinity occurring amoungst the species. In the following section karyology is used to clarify these patterns, and to provide further characters for comparison of the yellowfish species.

No karyological work has been published on southern African cyprinids. The family is represented by a number of different species besides the yellowfish. Jubb (1967), following Boulenger (1911), divided Barbus into a group of large species with longitudinally striated scales (e.g. yellowfish, B. marequensis) or radiately striated scales (B. serra); and small species with radiately striated scales. The small species where further grouped into those with dorsal spine not serrated (e.g. B. trimaculatus), those with dorsal spine serrated (e.g. B. argenteus, B. trevelyani) and species with an unserrated flexible dorsal spine (e.g. B. anoplus). Skelton (1988) removed the redfin barbs from this latter group and placed them in the genus Pseudobarbus (e.g. P. afer, P. burgi). The southern African Cyprinidae also include species of the genera Varicorhinus (e.g. V. nelspruitensis) and Labeo (e.g. L. capensis and L. umbratus).

Chromosome counts were taken from specimens of representative species for each of the above cyprinid groups. These chromosome numbers are necessary to form a base against which the yellowfish can be compared.

MATERIALS AND METHODS

Specimens

The yellowfish specimens used for karyology were mostly sexually immature juveniles, ranging from about six months to two years old. The collection sites, methods and sources of the specimens used are tabulated below. All specimens karyotyped were lodged in the JLB Smith Institute of Ichthyology (RUSI).

TABLE 10. RUSI catalogue number, localities and sources of the yellowfish specimens used for karyological studies.

SPECIES	CAT. No.	LOCALITY	SOURCE
Barbus aeneus	28407/8	Kibusie R., Great Kei RS.	self collected, seine net
B. capensis	28403/4	Olifants R., S.W. Cape	CDNEC*, Clanwilliam hatchery
B. kimberley-ensis	28400/1	P.K.LeRoux Dam, Orange R.	self collected, gill net CDNEC, Amalinda hatchery
B. natalensis	28402	Mgeni R.	NPB**, Pietermaritzburg
B. polylepis	28406	Dorps R., Olifants R.S., Transvaal	self collected, electro-fisher

*Cape Department of Nature and Environmental Conservation
**Natal Parks Board

The number of specimens karyotyped for each yellowfish species is listed in table 11.

TABLE 11. Number of yellowfish specimens karyotyped.

SPECIES	SPECIMENS
B. aeneus	15
B. capensis	15
B. kimberleyensis	8
B. natalensis	11
B. polylepis	4

Figure 26 shows the localities from which the yellowfish specimens were collected.

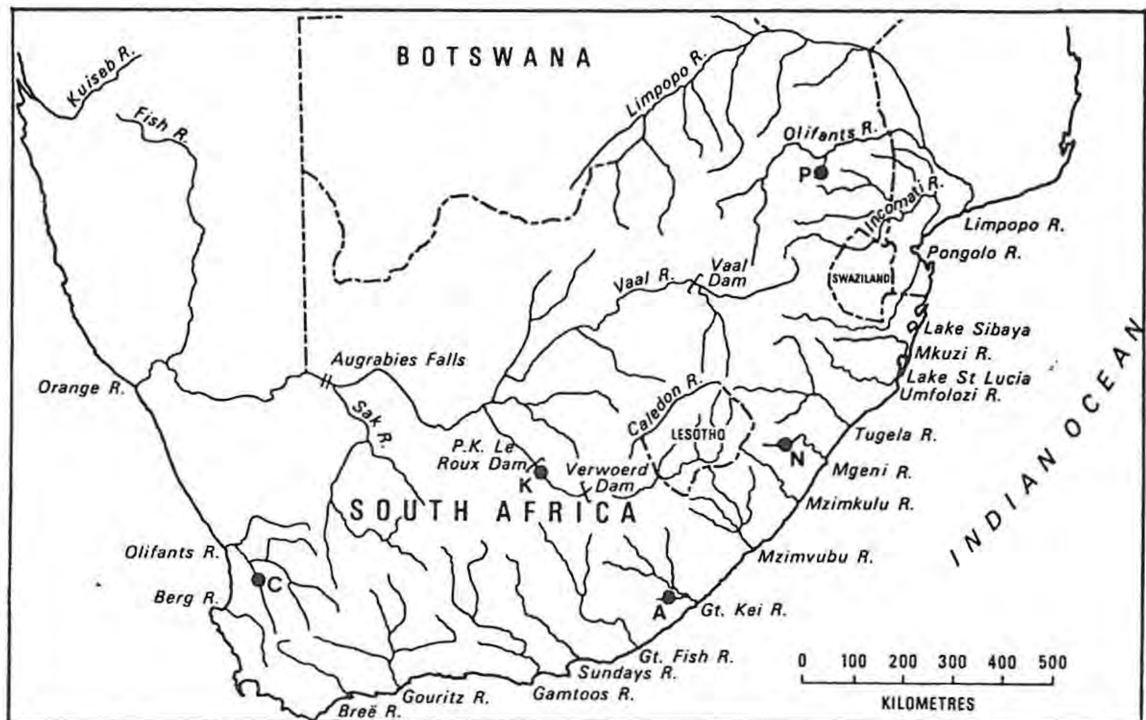


Figure 26. Sample sites of yellowfish populations used for karyology. A= B.aeneus, Kibusie River; C= B.capensis, Olifants River; K= B.kimberleyensis, P.K. Le Roux Dam, Orange River; N= B.natalensis, Mgeni River and P= B.polylepis, Dorps River.

While yellowfish were the targeted species during collection trips, other cyprinids were also caught and the chromosome numbers of these specimens were obtained for outgroup comparisons. Table 12 lists the collecting localities, sources and RUSI catalogue numbers of the outgroup species.

TABLE 12. RUSI catalogue numbers, localities and sources of the cyprinid species used for comparative purposes.

SPECIES	CAT. No.	LOCALITY	SOURCE
Barbus anoplus	28416	Dorps R., Olifants R.S. N.E. Transvaal	self collected, electrofisher
B. argenteus	28417	Incomati R.S., E.Transvaal	TNCD*, Lydenburg hatchery
B. marequensis	28411	Blyde R., Merry Pebbles holiday resort	self collected, electrofisher
B. serra	28405	Olifants R., S.W. Cape	CDNEC, Clanwilliam hatchery
B. trevelyani	28419	Keiskamma R.S.	M. Maychiso, Transkei U.
B. trimaculatus	28418	JLB Smith Institute	D. Weeks, Rhodes U.
Labeo capensis	28409	P.K.LeRoux Dam, Orange RS.	Self collected, Gill nets
L. umbratus	28410	P.K.LeRoux Dam, Orange RS.	Self collected, gill nets
Pseudobarbus afer	28415	Blinderkloof R., Swartkops River System	Self collected, electrofisher
P. burgi	28413	Redlinghuis, Verlorevlei River	CDNEC, Jonkershoek hatchery
Varicorhinus nelspruitensis	28412	Blyde R., Pilgrims Rest	self collected, electrofisher

*Transvaal Nature Conservation Department

The number of specimens karyotyped for each outgroup species is listed in table 13.

TABLE 13: Number of specimens karyotyped for the outgroup cyprinid species.

SPECIES	No.*	SPECIES	No.
B. anoplus	5	L. capensis	3
B. argenteus	1	L. umbratus	1
B. marequensis	5	P. afer	4
B. serra	5	P. burgi	8
B. trevelyani	1	V. nelspruitensis	3
B. trimaculatus	1		

*No.= Number of specimens karyotyped.

Chromosome isolation

Chromosomes were isolated according to the method described by Kligerman and Bloom (1977), as modified by Oellermann (1985). The method is as follows:

1. Colchicine solution was injected into the abdominal cavity of the specimen, at 0.01 ml solution per gram of wet body weight. The colchicine solution consisted of 0.1 gram of colchicine powder dissolved into 100 ml distilled water. There was no limit to this solution's shelf life.

2. The injected specimen was placed into a small, highly aerated tank for four to six hours. The water temperature was kept 2 to 5°C above that of the holding tanks to stimulate cell activity. Rivilin et al. (1986) recommended feeding the specimen during this period as a further stimulus to cellular activity.

3. The fish was killed by pithing. The whole gill arches were immediately removed from the gill cavity and teased apart with forceps. The specimen was then preserved in 10 % formalin for voucher purposes. The gill tissue was placed into a test-tube containing a 0.4% potassium chloride (KCL) hypotonic solution for about 40 minutes to swell the cells. The volume of the solution was about ten times that of the tissue. The hypotonic solution was made up of 0.4 grams of

KCL crystals dissolved into 100 ml of boiling distilled water. The solution was allowed to cool before use.

5. The hypotonic solution was removed and the gill tissue fixed by gently pouring Carnoy solution into the test-tube. Carnoy solution consisted of 50 ml glacial acetic acid in 150 ml absolute methanol. The solution was freshly prepared and well shaken before use. After 15 minutes this was replaced with fresh solution. The tissue could be stored for a limited period (one week) in Carnoy solution before the chromosome material began to deteriate. Kligerman and Bloom (1977) recommended that the tissue be refrigerated if a longer storage time was needed. Best results were obtained if the tissue was used within 24 hours of fixing.

Chromosomes were obtained as follows:

1. The tissue was divided into small pieces, placed in 10 to 15 drops of 50% acetic acid (50 ml glacial acetic acid diluted with 50 ml distilled water) in a watchglass and macerated using a scalpel and forceps.

2. The cell suspension was taken up into a bulb-dropper and three or four drops were released onto a heated (40°C) glass microscope slide from a height of about five centimeters. The suspension was allowed to rest on the slide for 30 seconds, and then was withdrawn back into the dropper, leaving a circular deposit on the slide. Two circular deposits were made per slide.

3. Once dry, the slides were placed into a stain bath containing fresh 5% Giemsa solution for 10 to 15 minutes. They were then removed and washed in running tap water before being dried on a slide warmer for at least 24 hours. Giemsa solution was provided by the South African Medical Research Institute (SAIMR) at Settlers hospital,

Grahamstown. The solution had a shelf life of about 3 months. The 5% Giemsa solution was freshly made up before staining and consisted of five milliliters Giemsa solution in 100 milliliters boiling tap water. This solution was filtered while hot, and allowed to cool before staining.

4. A coverslip was fixed over the slide with DPX mountant. Preparations were viewed under a binocular Nikon Optiphot compound light microscope. Chromosome photomicrographs were taken through an oil immersion 100x objective lens, in combination with a 10x ocular lens. Ilford Pan F monochrome film (ASA 50) was used at a shutter speed of 0.25 to 0.50 seconds. The film was developed in Ilford ID 11.

5. Chromosome counts were made directly from the slide and from photographic prints. All slides used in this work are lodged and catalogued in the JLB Smith Institute of Ichthyology (RUSI) collection.

The high number and small size of the yellowfish chromosomes made them difficult to count. Each photographed chromosome spread was recounted at least three times. A transparent sheet was placed over the photograph, and each chromosome was traced onto the sheet using water insoluble marker pens. The chromosomes were then counted using different colour marker pens to mark the bi- and uniarmed chromosomes. Schwartz and Maddock (1986) divided elasmobranch chromosomes into atelocentric and telocentric groups, as they considered any other division of the chromosomes as fairly arbitrary. Other authors have come to similar conclusions, and have divided the chromosomes into biarmed and uniarmed groups.

The ten most well defined chromosome spreads from each species were used to estimate the Fundamental Number (FN) of the karyotype as follows:

$$FN = 2(n1) + n2 ,$$

where n1 is the number of biarmed and n2 is the number of uniarmed chromosomes.

The final karyotype for each species was taken from the best defined chromosome spread. The chromosomes were cut from the photograph and paired according to their type and size into a photokaryotype. These karyotypes were traced on a light table and drawn for presentation in this work.

Silver-staining of nucleolar organizer region sites

Nucleolar organizer regions (NORs) are sites on a chromosome which represent the DNA sequences for the transcription of the 18S and 28S ribosomal RNA genes (Goodpasture and Bloom, 1975). The sites appear as secondary constrictions on metaphase chromosomes. Silver-staining allows these sites to be seen because the non-histone nuclear proteins associated with the NOR sites reduce the silver ions (Gold and Ellison, 1982). These proteins are present only if the NOR site was active during the previous interphase, thus only recently active NOR sites are stained (Howell *et al.*, 1975).

Five slides for each yellowfish species were stained with silver nitrate to find the number of NOR sites on the chromosomes. The method used was based on Howell and Black (1980); four drops of a 50% aqueous silver nitrate solution were pipetted onto the prepared slide followed by two drops of a colloidal developer, for each circular deposit of cells. The silver nitrate solution consisted of four grams of silver nitrate crystals dissolved in 8 ml distilled water. Two grams of powdered gelatin was dissolved in 100 ml hot distilled water, and one milliliter pure formic acid was added to make up the colloidal developer. The solutions were mixed on the slide and coverslips were placed over the cell deposits.

The slide was then placed onto a slide warmer set at 70°C. Once the solution on the slide had turned a rich golden-brown the slide was removed and washed under a running tap until the coverslips and excess stain were removed. The slide was dried on a slide warmer and then counterstained for two to five minutes using a 5% Giemsa solution.

This method was later modified following Gold and Ellison (1982), by introducing a 5% (w/v) sodium thiosulfate fixing solution. The slide was fixed in this solution for four to five minutes prior to counterstaining.

Silver-staining of nucleoli

One hundred silver-stained (following the above procedure) interphase cells were counted for two specimens of each yellowfish species, and the number of nucleoli occurring in each cell was recorded. Phillips *et al*. (1986) used the number of nucleoli per interphase cell to predict whether the cells were haploid, diploid or triploid. Their experiment was emulated to test the ploidy level of the yellowfish. The same procedure was followed for two *Labeo capensis* specimens (2N= 48) and two *B. serra* specimens (2N= 102) to provide control data.

RESULTS

Chromosome numbers and karyotypes

The yellowfish species can be divided into two groups on the basis of their diploid (2N) chromosome number. Barbus capensis, B. natalensis and B. polylepis each have 150 chromosomes, while B. aeneus and B. kimberleyensis have 148 chromosomes. Figure 27 (a-e) shows the percentage variation about the modal chromosome number for each species.

Chromosome number varied greatly, mostly due to chromosomal loss from the spreads. The large number of chromosomes caused great difficulty in spreading the chromosomes sufficiently for counting without losing chromosomes from the spread. The chromosome isolation technique improved greatly in this respect over the two years the yellowfish were collected and karyotyped. Barbus aeneus specimens were the first to be karyotyped, and initially appeared to have a bi-modal chromosome number of 90 and 136. However this was an artifact of chromosome loss through the yet to be perfected isolation technique. Specimens from the same populations were karyotyped toward the end of the project and were found to have 148 chromosomes. This observation emphasizes the importance of rechecking data and the danger of subjective analysis of karyotypes.

A prime example of the subjective nature of chromosome analysis was shown by the different karyotypes of Aristichthys nobilis presented by Marian and Krasznai (1979) and Beck et al. (1980). In each case the chromosome number reported was the same, but the assigned arm ratios were different. Differences in chromosome characterization are often found among studies of the same species by different authors (Garcia et al., 1987).

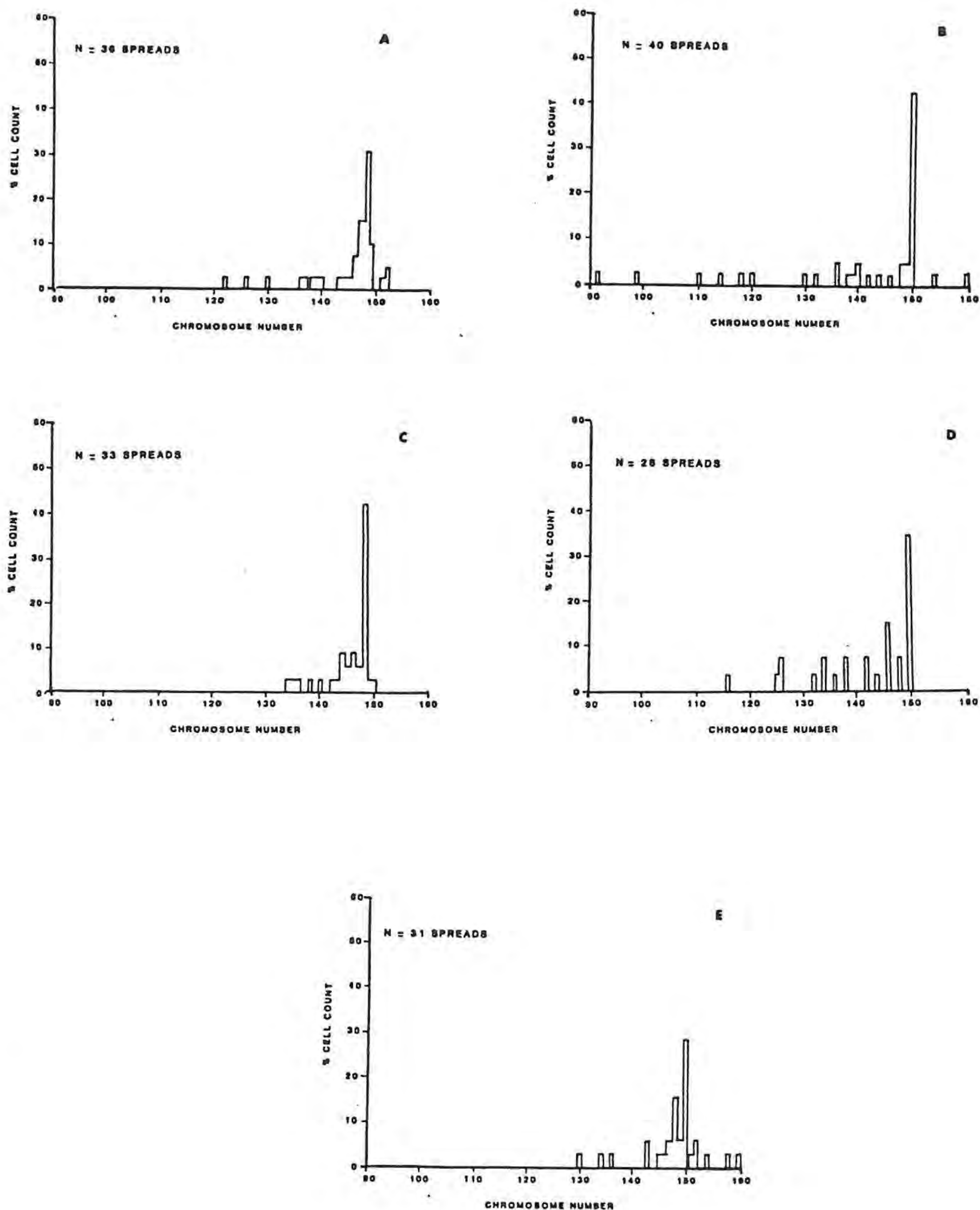


Figure 27: Percentage spreads with various chromosome numbers for (a) B. aeneus, (b) B. capensis, (c) B. kimberleyensis, (d) B. natalensis and (e) B. polylepis

Difficulties experienced in obtaining accurate and consistent counts were overcome by applying the counting method described in the materials and methods section. Counts made from the slides under the microscope were usually at least 10 chromosomes less than when counted from the photographs. Mistaken chromosome identification (e.g. a biarmed chromosome counted as two uniarmed chromosomes) and chromosome imports from other spreads probably resulted in chromosome counts higher than the modal number.

No sexual dimorphism was observed within the yellowfish karyotypes. Any major dimorphism in chromosome pairs has probably been masked by the multiple chromosomes introduced by polyploidy.

Any classification of the yellowfish chromosomes above the level of biarmed or uniarmed would be highly subjective. The small size of the chromosomes made them difficult to measure objectively. Variable uptake of colchicine by the cells could also lead to differences in chromosome density and therefore arm size (Fisher and Rachlin, 1972). The chromosomes themselves could be unstable (polymorphic) as Giles *et al.* (1985) found in the species *Gobius paganellus*. For these reasons chromosome analysis beyond chromosome number was based on Fundamental Number (FN) (see page 62).

Table 15 shows the estimated number of biarmed chromosomes for 10 spreads from each yellowfish species, and table 16 summarizes their modal arm ratios. The modal FN value for each species was relatively similar, and decreased from *B. polylepis* through *B.capensis*, *B. kimberleyensis*, *B. natalensis* to *B. aeneus*.

TABLE 15: Estimated number of biarmed chromosomes in 10 spreads for the five yellowfish species.

SPREAD	AEN (148)	CAP (150)	KIM (148)	NAT (150)	POL (150)
1	40	44	46	41	48
2	41	46	52	42	54
3	46	48	52	42	54
4	46	50	54	48	56
5	48	54	55	50	56
6	48	58	56	50	56
7	49	58	56	52	56
8	52	58	56	52	56
9	58	68	57	53	58
10	60	70	58	54	58
	--	--	--	--	--
MODE	48	58	56	50	56

where: AEN= B. aeneus CAP= B. capensis KIM= B. kimberleyensis NAT= B. natalensis POL= B. polylepis

TABLE 16: Estimated number of biarmed chromosomes, uniarmed chromosomes and Fundamental Numbers for the 5 yellowfish species.

SPECIES	2N	BI-ARMED	UNI-ARMED	FN
B. aeneus	148	48	100	196
B. capensis	150	58	92	208
B. kimberleyensis	148	56	92	204
B. natalensis	150	50	100	200
B. polylepis	150	56	94	206

The yellowfish karyotype appears to have evolved along two pathways. The Orange-Vaal River species are separated from the rest by having two chromosomes less than the other species, most likely due to a Robertsonian translocation (centric fusion). Further differentiation between the species appears to be an increase or decrease in the number of bi-armed chromosomes. Taxonomic and evolutionary implications of this are explored in the following discussion section.

The representative spread and drawn karyotype for each yellowfish species is shown in figures 28 to 32.

A

0.1 µm

B

2N=48+100=148

Figure 28: The chromosome photomicrograph (a) and drawn karyotype (b) of Barbus aeneus.

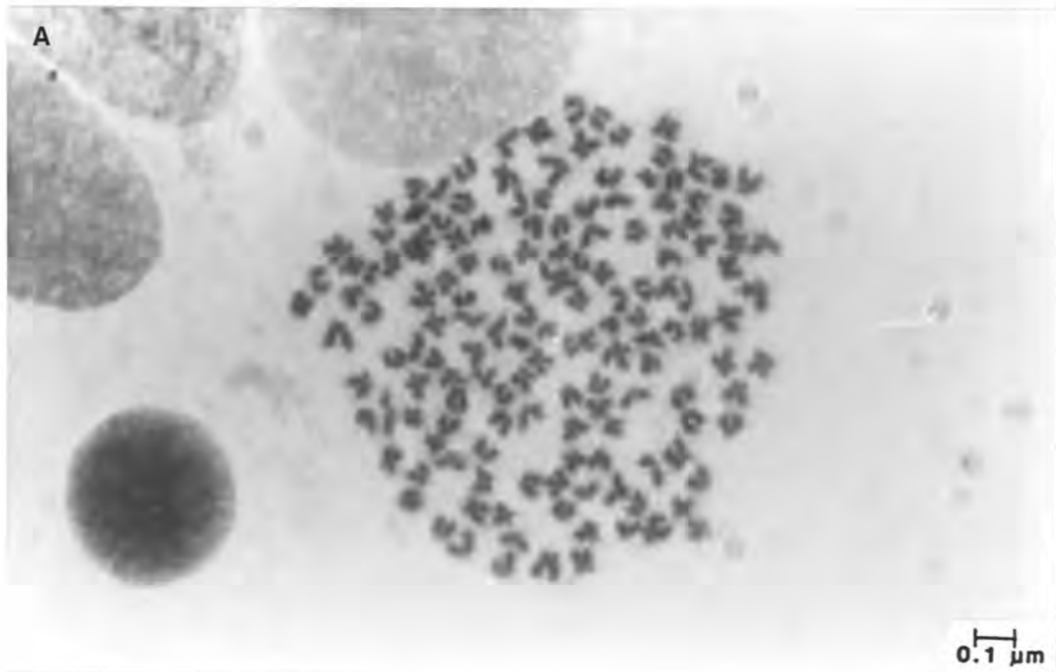
A

0.1 µm

2N=58+92=150

B

Figure 29: The chromosome photomicrograph (a) and drawn karyotype (b) of Barbus capensis.



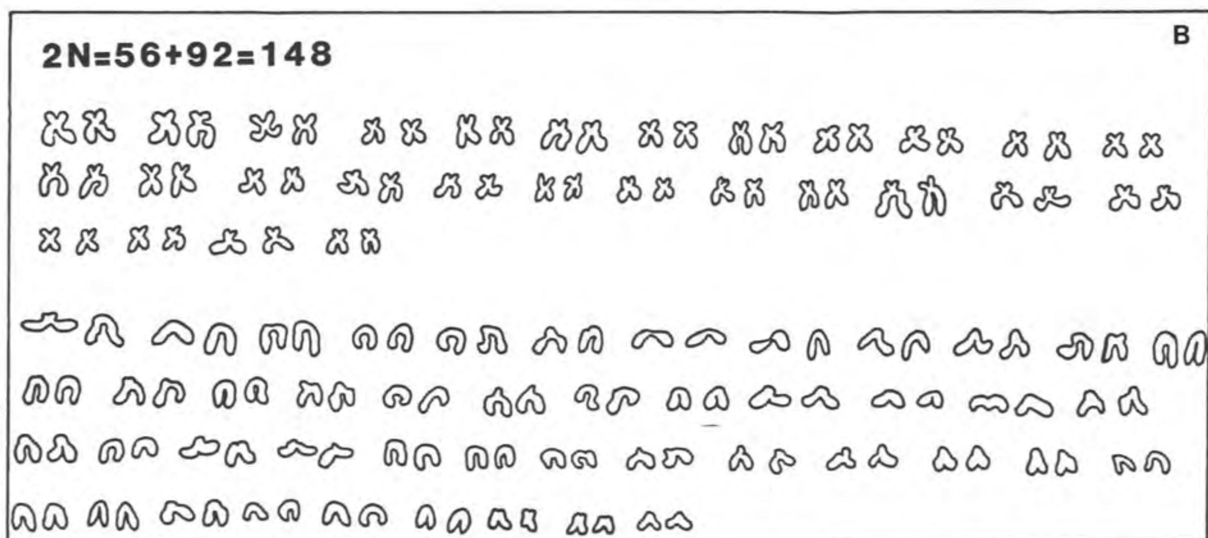


Figure 30: The chromosome photomicrograph (a) and drawn karyotype (b) of Barbus kimberleyensis.

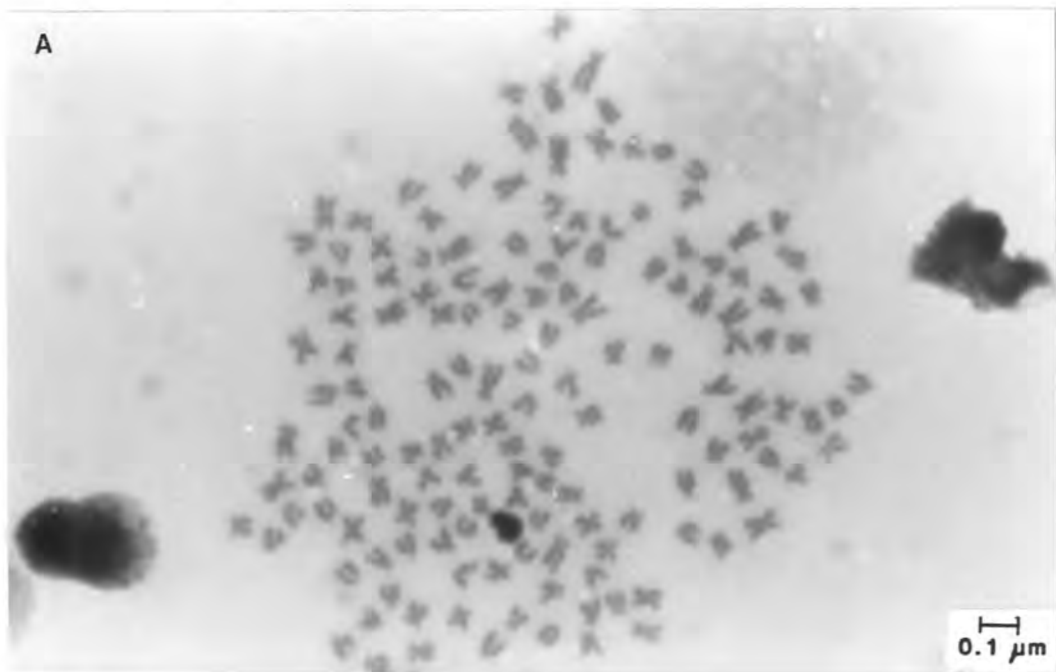
A

0.1 μm

B

2N=50+100=150

Figure 31: The chromosome photomicrograph (a) and drawn karyotype (b) of Barbus natalensis.



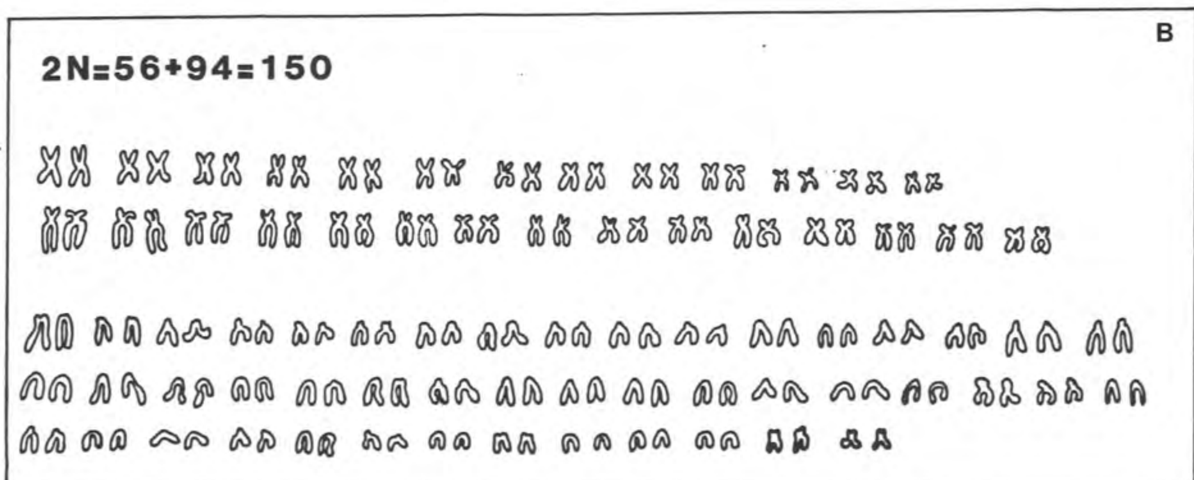


Figure 32: The chromosome photomicrograph (a) and drawn karyotype (b) of Barbus polylepis.

The yellowfish chromosome numbers are high when compared to the average cyprinid chromosome number (2N= ± 50). Table 16 shows the distribution of chromosome number in the counted chromosome spreads of the outgroup species. There are three sets of species with greatly differing chromosome numbers.

TABLE 16. Chromosome number counts for the outgroup species.

SPECIES (No. specimens)	No. SPREADS	2N CHROMOSOME COUNTS												
		40	41	42	43	44	45	46	47	48	49	50	51	52
B. anoplus (5)	12						1	1		10				
B. argenteus (1)	2													2
B. trimaculatus (1)	3			1				1		1				
L. capensis (3)	13					3		2	1	6				
L. umbratus (1)	16			1	2	1	1		2	9				

SPECIES (No. specimens)	No. SPREADS	2N CHROMOSOME COUNTS																
		<	90	1	2	3	4	5	6	7	8	9	100	01	02	03	104	<
B. serra (5)	18	1											3	3	8		2	1
B. trevelyani (1)	10		1	1		1		2	3	1	1							
P. afer (4)	16	2	1	1		1	3		8									
P. burgi (8)	20	7	1				1		7			1		1				2

SPECIES (No. specimens)	No. SPREADS	2N CHROMOSOME COUNTS																				
		130	1	2	3	4	5	6	7	8	9	140	1	2	3	4	5	6	7	8	9	150
B. marequensis (5)	20	2		2		6	1	2				1	1	1		1						3
V. nelspruitensis (3)	14	1			1	1	1			4		2			1	1			1	1		

Each group represents a different level of polyploidy; that is an increase in the number chromosome sets making up a chromosome complement. The polyploidic nature of the yellowfish and southern African cyprinids in general is

discussed in detail in the following discussion section.

Silver staining of NORs

Difficulty was experienced in silver-staining nucleolar organizer regions in the yellowfish. The slide preparations generally turned out poorly, with excess silver deposition screening the chromosome NORs. The preparations for Barbus natalensis were clear, but the chromosomes themselves appeared inundated with silver-stain. Fourteen silver-stained spreads were obtained from two B. capensis specimens. Although the spreads were not stained strongly enough to show which types of chromosomes had the NOR sites, the sites appeared well defined. Table 17 shows that 50% of the spreads contained two pairs of NOR sites in the chromosome complement (indicating the presence of four NOR-bearing chromosomes), but one spread was found with as many as four pairs (which could be an artifact of staining). Thus it appears that B. capensis has a genome with multiple NOR sites.

TABLE 17: Number of silver-stained Nucleolar Organizer Region pairs per spread in Barbus capensis.

No. NOR PAIRS	No. CHROMOSOME SPREADS
1	4
2	6
3	3
4	1
TOTAL No. SPREADS:	14

Silver-staining of nucleoli

Silver-staining showed that a range of from one to six nucleoli were present in the interphase cells of the yellowfish. Barbus natalensis also had four spreads with seven nucleoli (table 18).

The four cells with seven nucleoli may be an artifact of slide

preparation, as there was much silver-stained "interference" on the slide. This may also explain why the nucleoli counts of the second B. natalensis specimen were skewed to a higher modal number than the rest of the yellowfish specimens.

The second B. capensis specimen only had one to four nucleoli per cell. Phillips et al. (1986) found that in rapidly dividing tissue each NOR of a NOR pair produces a nucleolus; but each pair tends to only produce one nucleolus in slow developing tissue. Perhaps the lower nucleoli counts in this specimen were due to lower cellular activity, and visa-versa for the second B. natalensis specimen. Less than 10% of the cells in any of the specimens (besides the second B. natalensis specimen) had six nucleoli. Gold (pers. comm.) has noted that it is rare to find metaphases with all of the chromosomal NORs silver-stained in specimens with mutiple NORs.

TABLE 18: The number of silver-stained nucleoli per cell for 2 specimens of each yellowfish species, Barbus serra and Labeo capensis.

NUCLEOLI	AEN		CAP		KIM		NAT		POL		SER		L.CAP	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	14	14	16	31	17	22	10	0	18	18	41	43	64	58
2	29	32	28	42	27	20	32	17	30	30	38	41	34	41
3	18	23	20	24	22	24	23	21	25	24	18	14	2	2
4	22	17	18	3	14	19	19	15	18	16	2	2	0	0
5	9	8	11	0	8	8	8	22	5	6	0	0	0	0
6	7	6	7	0	2	7	8	21	4	6	0	0	0	0
7	0	0	0	0	0	0	0	4	0	0	0	0	0	0
TOTAL SPREADS:	100	100	100	100	100	100	100	100	100	100	100	100	100	100

where: AEN= B. aeneus CAP= B. capensis KIM= B. kimberleyensis SER= B. serra NAT= B. natalensis POL= B. polylepis L.CAP= Labeo capensis

The nucleoli counts followed a similar trend in the rest of the yellowfish specimens examined. The modal number was two nucleoli per cell and occurred in an overall average of 29% of

the cells of all the specimens. The overall average number of cells with three and four nucleoli was 22% and 18% respectively, while only 7% of the cells had five or six nuleoli.

Labeo capensis produced results one would expect from a diploid species; 61% of the cells had a single nucleolus and 37% had two nucleoli. Two percent of the cells showed three silver-stained nucleoli. The extra nucleolus could be from an overlapping cell (it was sometimes difficult to differentiate between two silver-stained cells) or an artifact of silver-stained debris on the slide. The B. serra specimens had 42% of the cells with one nucleolus, 40% with two nucleoli, 16% with three nucleoli and 2% of the cells with four nucleoli.

The number of nucleoli occurring in the cells of a species may provide information on the level of polyploidy of a species, and on the extent of diploidization (Ohno, 1970) to have occurred. However, Gold (pers. comm.) has warned that (i) the number of silver-stained interphase nucleoli is always a minimum estimate of the actual number of chromosomal NORs, and (ii) it is rarely possible to enumerate all chromosomal NORs via counting stained interphase nucleoli.

The relationship between number of nucleoli per cell, chromosome number and polyploidy are discussed further in the next section.

DISCUSSION

Polyploidy

A surprisingly large number of teleost fish belonging to diverse orders have karyotypes consisting of 48 acrocentric chromosomes (chromosomes with terminally located centromeres) (Ohno, 1970). This chromosome complement has been generally accepted as the primitive fish karyotype (Nogusa, 1960; Post, 1965; Chen, 1967; Roberts, 1967). Khuda-Bukhsh et al. (1986) however have proposed that 24 chromosomes may be the primitive fish karyotype. This takes into consideration the low chromosome numbers found in some families, e.g. Belontidae (2N= 16), Galaxiidae (2N= 22) and Cyprinodontidae (2N= 16-26) (Khuda-Bukhsh et al., 1986). However, of these examples only the Galaxiidae are considered "lower teleosts" (Skelton, pers comm.).

Fish chromosome numbers can vary from a low of six haploid chromosomes in Gonostoma bathyphilum (Post, 1974) to 206 chromosomes in the tetraploid (evolutionary octoploid) Carassius auratus langsdorfii (Murayama et al., 1986).

Cyprinid genera are well represented in karyological studies. Most of the nearly 450 species of cyprinids karyotyped thus far have a chromosome number of 50 (Gold pers. comm.). A second, very much smaller cluster of cyprinid species have around 100 chromosomes per cell (fig. 33).

Ohno et al. (1967) noted the dichotomy in cyprinid chromosome number and they proposed that the family consisted of species with diploid and tetraploid origins. Klose et al. (1969) showed that the isoenzyme 6-PGD was duplicated in the cyprinid species which they used as examples of the tetraploid group. Thus at some stage during their evolution these species experienced a polyploidic event (Mayr et al., 1986) which doubled their chromosome numbers to produce the tetraploid

condition. The term "polyploidic event" is used to include all possible ways in which polyploidy may have been induced i.e. environmental changes and hybridization.

Cyprinid species included in the second group occur in the genera Aeroscheilus, Aulopyge, Barbus, Carassius, Cyprinus, Tor and Schizotharaeichthys (Mayr et al., 1986). Barbus serra, B. trevelyani and the red fin species Pseudobarbus afer and P. burgi karyotyped in this study are thus tetraploids. Fifteen of the 24 species of tetraploid cyprinids listed by Suzuki and Taki (1986) are in the subfamily Barbinae.

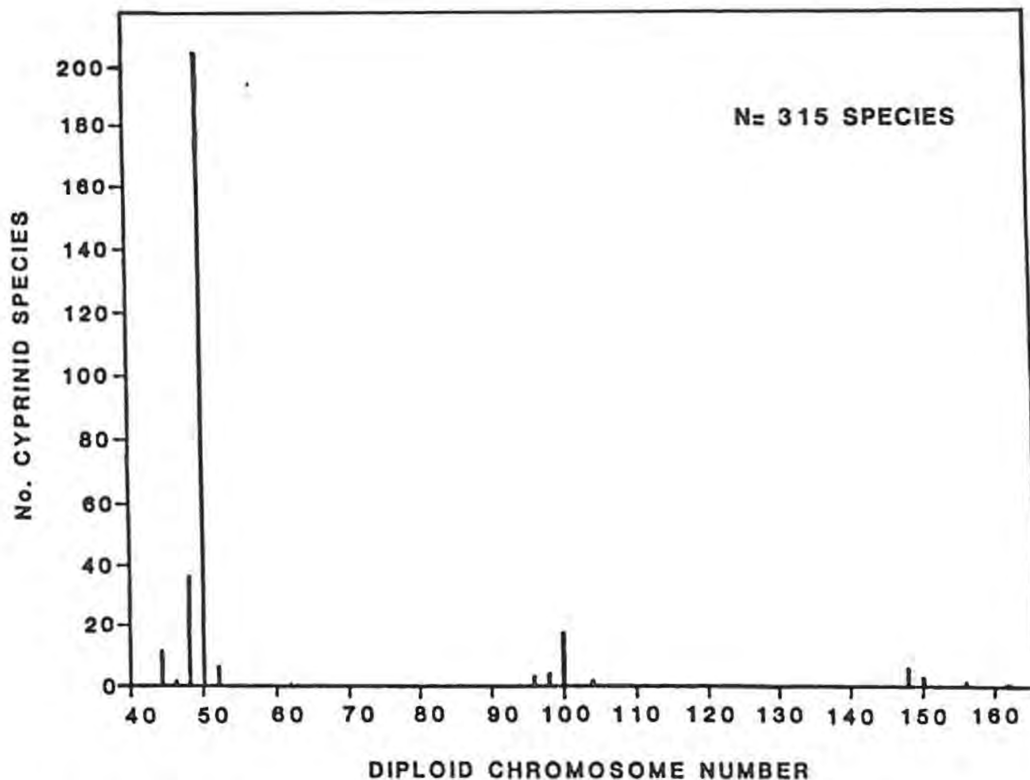


Figure 33. The diploid chromosome number of cyprinid species, determined from the literature as given in Appendix two.

Tetraploidy can arise from incomplete meiosis II during the development of male and female gametes (autotetraploidy). Both sperm and egg would then each contain a diploid complement of chromosomes, and in combination produce a tetraploid zygote. Don and Avtalion (1988) have found that by giving tilapia eggs a temperature shock (a sharp decrease in temperature),

tetraploidy is induced in the embryos, some of which survive to adulthood.

Allotetraploidy arises when two species hybridize. The resulting genome may consist of a number of chromosomes which cannot be equally partioned during the telophase (eg. triploid or pentaploid). Spontaneous doubling of the chromosome number may then occur, producing a viable euploid genome, albeit tetraploid (Gibby, 1981).

Viable polyploid species can only occur in the lower vertebrates, primarily amphibians and fish. This is because the heterologous sex chromosomes of higher vertebrates (particularly mammals) prevents further polyploidic evolution (Wolf et al., 1969). Only 72 species of the fishes karyotyped to date showed heterologous sex chromosomes (Ojima and Kojima, 1985). The low frequency of polyploid gametes and the lower probability of finding a mate of the same polyploidic level probably limit the production of viable polyploid progeny in the fishes.

Polyploidy has played an important role in the evolution of the fishes, as it provides redundant gene loci (Becak et al., 1966). Natural selection favours the conservation of important gene loci. For instance, although small mutations have occurred to the kinetic properties of the enzyme lactate dehydrogenase, its basic structure has not been altered through 300 million years of vertebrate evolution (Ohno, 1970). Thus the gene loci coding for lactate dehydrogenase must have been strongly conserved throughout vertebrate evolution. Polyploidy should cause a number of redundant gene loci to be present in the genome, due to the increase in chromosome number and genetic material in the nucleus of a cell. Redundant gene loci are more likely to escape the natural selective pressure to conserve active loci and can then accumulate mutations (Wolf et al. 1969).

The autotetraploid cell contains four homologous chromosomes. Such cells should therefore contain quadrivalents during meiosis. A gradual reversion from a quadrivalent to a bivalent state in an autotetraploid genome may occur, and has been called the process of diploidization (Ohno et al., 1967). The gene redundancy of a tetraploid (which started out as a diploid) appears to establish a gradual diploid state in the genome, either by divergence of DNA sequences or by chromosomal rearrangements. Structural heterogeneity must be created among the four original homologues (Shaver, 1963), as the preferential separation of one quadrivalent into two bivalents is the prerequisite for functional diversification and diploidization (Ohno, 1970). If the species experienced allotetraploidy this step would be unnecessary, as only bivalents would be formed.

Diploidization is relatively far advanced in evolutionary polyploid cyprinids (Klose et al., 1969). Woods and Buth (1984) found that the tetraploid Cyprinus carpio retained 52% of the duplicate enzyme loci tested, while the closely related tetraploid Carassius auratus only retained 19%. The lower number of duplicate enzyme loci (i.e. higher degree of diploidization) is considered the apomorphic state.

Phillips et al. (1986) found that about 75% of the cells in three species of triploid fishes (rainbow trout, coho salmon and chinook salmon) had three nucleoli. By similar extrapolation a tetraploid fish should have many cells with four nucleoli. However B. serra only had four nucleoli in 2% of the cells counted. Most of the cells contained one or two nucleoli, as was expected for diploid fishes such as Labeo capensis (table 14). One is tempted to use these results as evidence of the process of diploidization in the species; but could also merely be evidence of cellular inactivity in the specimens examined. I have used the term "evolutionary tetraploid" to distinguish species such as B. serra, which have tetraploid chromosome numbers but

probably no longer have tetrapolid genome characteristics.

Naturally occurring triploid populations are rare in fishes (Beck et al., 1980) and under normal conditions the meiotic efficiency of fish gonadal tissue limits the occurrence of spontaneous triploid specimens (Gold, 1986). Natural populations of triploids have been reported in the cyprinodont genera Poecilia and Poeciliopsis, and in the cyprinids Carassius (Schultz, 1980) and Rutilus (Collares-Pereira, 1985a). A single triploid individual has been found in a natural population of Hesperoleucus symmetricus (Gold and Avise, 1977) and in a natural population of Pimephales promelas (Gold, 1986). Cherfas (1972) found that six percent of the eggs in the bisexual silver crucian carp studied were spontaneous diploids. Fertilization of these eggs by the haploid male gametes would produce triploid fishes.

In artificial and hatchery conditions fishes are easily induced to produce triploid zygotes. Hot and cold temperature shocks (Lincoln and Scott, 1983; Krasznai and Marian, 1986; Wolters et al., 1982), hydrostatic pressure (Lou and Purdom, 1984) and chemicals such as colchicine (Denton, 1973) have been used to induce triploidy. Hybridization of two closely related species can also lead to triploidy (Beck et al., 1980).

By using a marker chromosome in the newt Pleurodeles waltlii Ferrier and Joylet (1978) were able to show that triploidy results from the retention of the second polar body during oogenesis. The egg remains diploid, and the addition of the haploid sperm to the egg produces a triploid zygote. Aneuploid gametes (gametes which do not have the same chromosome numbers) are expected from the triploid offspring, which should therefore have low viability or be sterile (Gjedrem et al., 1977; Beck et al., 1980).

Some triploid populations have overcome the problem of

aneuploidy by unisexual reproduction. Unisexual vertebrates reproduce either by parthenogenesis (females reproduce without males) or gynogenesis (sperm needed to stimulate egg development, but does not fuse with egg). Parthenogenesis has not yet been reported as a means of unisexual reproduction in amphibians and fishes, although it has been found in unisexual snakes and lizards (Dawley et al., 1987).

Murayama et al. (1986) described the process of gynogenesis in a triploid population of Carassius auratus langsdorfii. The formation of the first polar body was skipped, and eggs were produced with a full chromosome complement by a single homoeotype meiosis. Injected male sperm remained compacted and did not fuse with the female pronucleus.

Figure 33 shows a further group of cyprinid species centered around 150 chromosomes. This group is made up of B. marequensis, V. nelspruitensis, a group of cyprinids from China belonging to the genus Schizothorax, and the southern African yellowfishes. The six Schizothorax species have chromosome numbers of 148 (Zan et al., 1985; Zan et al., 1986). These species have three times the chromosome content of a diploid cyprinid, thus are of hexaploidic origin.

In figure 34 I have attempted to depict the possible ways in which hexaploidy may develop. Four scenarios are possible; the first three involve autopolyploidy, and the last is based on allopolyploidy.

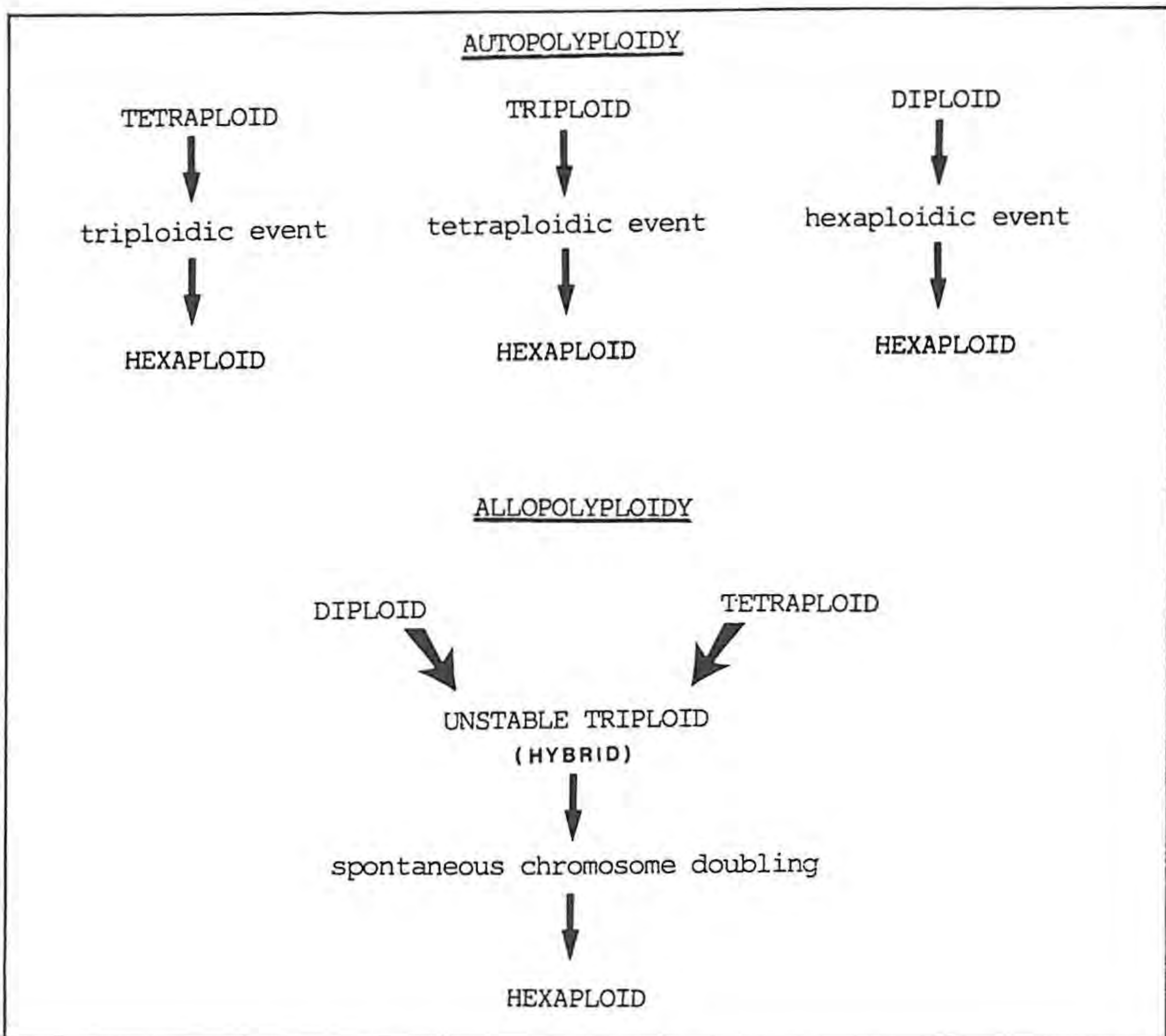


Figure 34. Possible scenarios for the development of hexaploidy in the yellowfish.

The first scenario depicts a tetraploid species which has experienced a triploidic event, giving rise to a hexaploid genome. This is unlikely, as it would lead to either a sterile population, or a species that propagated through gynogenesis. No unisexual reproductive behaviour has been reported in the yellowfish. Gynogenesis in a naturally occurring population should eventually lead to the extinction of male triploid individuals. The diploid male line could only survive in the presence of diploid bisexual females. Ecological studies such as that undertaken on the Orange-Vaal River yellowfish by Mulder (1973) and breeding programmes involving the yellowfish at Amalinda, Clanwilliam and Lydenburg hatcheries have shown that the five species reproduce bisexually.

Zan et al. (1986) have found a population of hexaploid Cyprinus carpio bibelio reproducing gynogenetically which is evidence that this pathway has been followed at least once. There is however a high ratio of hexaploid males present in the population. The sperm has a 50% reduction in DNA content to that of the male somatic cells and therefore must be undergoing full meiosis. If the female gonads redeveloped meiosis then perhaps the population would evolve to a bisexually reproducing hexaploid species.

The second possible pathway depicted in figure 34 shows a triploid species which has experienced chromosome doubling (due to a tetraploidic event) as a means of overcoming sterility due to aneuploidy. The chromosome doubling gives rise to a hexaploid complement of chromosomes. This pathway could be an alternative to gynogenesis for a triploid species.

The third scenario describes a single-step increase in ploidy from diploid to hexaploid. Such a large jump in ploidy level is unlikely. Artificial induction of hexaploidy through environmental manipulation has not been reported in cyprinids.

The most likely way in which the yellowfish developed hexaploidy involves a combination of both autopolyploidy and allopolyploidy (scenario four). If a diploid and a tetraploid species hybridized, the product would be an unstable triploid hybrid. Should the hybrid experience spontaneous chromosome doubling, the result would be a hexaploid. Spontaneous chromosome doubling after hybridization appears to be fairly common in plants (Gibby, 1981) but has not as yet been reported in fishes. I consider this scenario the most likely because closely related Barbus seem to hybridize readily, and spontaneous chromosome doubling ensures that sexual reproduction is not limited by aneuploidy.

Whatever the means, the resultant complex hexaploid genome would probably undergo the process of diploidization, allowing a large amount of redundant genetic material the opportunity for mutation. Tetraploid and hexaploid species have four and six chromosome sets respectively. The possible combinations of dominant and recessive genes are thus doubled and tripled. In this way the heterozygosity of a polyploid species is greatly increased. This large gene reservoir may explain the plastic nature of the intraspecific yellowfish phenotype, and could also account for their resilience to major phenotypic changes in the face of diverse habitats.

There is a strong correlation between DNA content and cell size, thus the more the chromosomes the larger the cells of an organism (Van't Hof, 1974). This may lead to a size increase and change in growth patterns of the organism or its organs, or to a decrease in the number of cells in the organism. The latter would mean physiological and biochemical changes to the organism. The southern African hexaploid group are all relatively large species. Perhaps size increase due to hexaploidy provided the impetus for these species to evolve to larger sizes than most of the other species of genus Barbus.

The advantages of polyploid cyprinids has been summed up by Uyeno and Smith (1972) as larger size, a longer life, faster growth and greater ecological adaptability than the majority of cyprinids.

Silver-stained NORs and Nucleoli

A single pair of NOR chromosomes have been found for most species of fish from which NOR sites are known (Gold, 1984; Feldberg and Bertello, 1985; Rivilin et al., 1985; Thode et al., 1985a; Thode et al., 1985b; Amemiya et al., 1986; Gold and Amemiya, 1986; Uwa and Magtoon, 1986; Feldberg et al., 1987; Thode, 1987). Feldberg and Bertello (1985) reported a

single NOR chromosome in the species Chaetobranchopsis australe. The NOR site on the other chromosome of the homologous pair may have been genetically inactive, or lost through gene deletion.

Gold (pers. comm.) has reported that about 40% of the fishes examined by his team have multiple NORs, including such examples as Campostoma anomalum (Gold, 1984), Hybopsis aestivalis and Notropis ardens (Gold and Amemiya, 1986). Takai and Ojima (1986) reported as many as eight NOR sites in Zacco temmincki. Species with multiple NOR chromosomes are considered as apomorphic to those with a single pair of NOR chromosomes (Gold, 1984). Amemiya et al. (1984) have tentatively described the most pleisomorphic state for North American cyprinids as a single pair of acrocentric chromosomes with their NOR sites located terminally on the short arms. Only one species of the 35 Asian cyprinids reported by Takai and Ojima (1986) did not have the NORs located terminally on the short arm of the NOR-bearing chromosome.

If the yellowfish are hexaploids, one would expect the genome to contain multiple NOR sites. The expected number of NOR chromosome pairs is around three, depending on the extent of diploidization or gene duplication to have occurred in the hexaploid genome. Unfortunately, this expectation is based on the assumption that the diploid cyprinids closely related to the yellowfish have only one pair of NORS in the genome. Only eight of the 35 cyprinid species listed by Takai and Ojima (1986) had multiple NOR sites, none of which were evolutionary polyploid. Takai and Ojima (1986) did however observe that the evolutionary tetraploid Carassius auratus and Cyprinus carpio had far larger NOR sites than the diploid species they karyotyped. They proposed that after the tetraploidization event the species had two pairs of NOR chromosomes. During the formation of quadrivalents unequal crossing over led to large portions of the NORS of one pair being translocated to the other pair.

The 14 B. capensis silver-stained cells showed one to four pairs of chromosomes with NOR sites, and had a mode of two pairs (Table 17). The expected result if the species was an evolutionary hexaploid (i.e. a hexaploid karyotype in the advanced stages of diploidization) would be one to three NOR pairs, with the mode occurring at one or two pairs. It is possible that the fourth pair of NORs recorded in one of the B. capensis chromosome spreads was an artifact of excess silver-staining. The results may be evidence of polyploidy, but information is needed on the number of NORs occurring in closely related diploid species.

By extrapolation of Phillips et al.'s (1986) silver-stained nucleoli data one would expect the yellowfish and other evolutionary hexaploid species to show one to six nucleoli per cell. The results obtained in the present work (table 18) indicate that this prediction is correct. The low percentage of yellowfish cells with five or six nucleoli may be an indication of the level of diploidization which has occurred within the yellowfish genomes, but could also reflect the cellular activity of the specimens examined. The evolutionary tetraploid B. serra appeared to have a genome which had almost completely reverted to diploidy, with very few cells showing three or four nucleoli.

The results of the silver-staining experiments were not enough to provide evidence that the large chromosome number found in the yellowfishes is due to polyploidy, or to demonstrate that diploidization has or is still occurring in the polyploid species examined. However the yellowfishes do have a genome structure expected for hexaploid fishes and structural changes and functional diversification of the chromosome homologues would eventually give rise to a pair of chromosomes per linkage group (Ohno, 1970).

Amemiya and Gold (1986) have recently developed a method of viewing NOR sites in fishes using Chromomycin A_3 (CMA) which is used in amphibian NOR research. CMA stains the guanine-cytosine rich DNA of the NOR sites, thereby staining inactive NOR sites as well. Although not tried here this method may prove more successful in finding and mapping the multiple NOR sites expected in the genomes of yellowfish and other polyploid species.

Evolutionary and taxonomic significance of the southern African Barbus karyotypes.

The species grouped under the genus Barbus are probably of polyphyletic origin. Skelton (1988) has recently removed the redfins from Barbus and placed them in genus Pseudobarbus. Chromosome counts of Pseudobarbus afer and P. burgi presented in this study showed a chromosome number of 96, placing these redfins with the group of cyprinids showing tetraploidic origins. Barbus serra and B. trevelyani also manifested tetraploidic origins, whereas B. anoplus, B. argenteus and B. trimaculatus were clearly diploid. Table 19 provides a summary of the levels of ploidy expressed by the karyotyped Barbus and Pseudobarbus species.

Table 19. Groups of karyotyped Barbus and Pseudobarbus species sharing common levels of ploidy.

DIPLOID	TETRAPLOID	HEXAPLOID
B. anoplus	B. serra	B. aeneus
B. argenteus	B. trevelyani	B. capensis
B. trimaculatus	P. afer	B. kimberleyensis
	P. burgi	B. natalensis
		B. polylepis
		B. marequensis

In the introduction to the karyology section I described

Jubb's (1967) subdivision of the Barbus genus into five groups on the basis of size, scale striation and type of dorsal spine. Table 20 shows that by dividing the example species into these groups a different relationship is apparent to that of similar ploidy levels.

Table 20: Groups of Barbus and Pseudobarbus species following Jubb (1967).

GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V
B. aeneus	B. serra	B. trimac.	B. argenteus	B. anoplus
B. capensis			B. trevelyani	P. afer
B. kimberley.				P. burgi
B. natalensis				
B. polylepis				
B. marequensis				

These tables show clearly that Jubb's (1967) group of large, longitudinally striated scale bearing species of Barbus (Group I) in fact defines the hexaploid group of barbs. Varicorhinus nelspruitensis, which is large and has both longitudinally striated scales and a chromosome number of hexaploidic origin, should also be included in this group. Groenewald (1958) expressed doubt as to the validity of distinguishing between the genus Varicorhinus and Barbus. The species showing hexaploidic origins in southern Africa are a well defined group, and could be placed in their own genus. On a purely karyotypic basis the group differs from the type species of genus Barbus i.e. Barbus barbus, which has a chromosome number of 100.

The rest of the example species used were not correspondingly grouped when Jubb's (1967) criteria and levels of ploidy were compared. The levels of ploidy may be a more accurate means of grouping these species, as Jubb's (1967) distinguishing features did not necessarily represent phylogeny or evolutionary relationships, but were used as characters in a dichotomous key. There is the possibility that tetraploidy

occurred in more than one *Barbus* evolutionary line, and not all the species within the group are closely related. On the other hand, less than 10% of the almost 300 cyprinid species listed in Appendix two were of tetraploid origins. Thus there is a strong probability that tetraploid species of the same genus occurring in the same region are closely related.

Chromosome numbers and karyotypes of all the species of southern African *Barbus* are necessary before one can comment further on the importance of levels of ploidy in the group.

Only major changes in chromosome number through polyploidy have been discussed so far. Most interspecific karyological information comes from smaller changes in chromosome number due to Robertsonian translocations, and changes in chromosome shape by pericentric inversions.

Robertsonian translocations occur when centromeres of two non-homologous chromosomes fuse together, producing a metacentric chromosome; or when a metacentric chromosome's centromere disassociates to produce two acrocentric chromosomes. The combination of Robertsonian translocations and pericentric inversions (the inversion of a length of chromosome, including the centromere, after two breaks occur in the chromosome) can lead to numerous rearrangements in the positions of the centromeres on the chromosomes and in chromosome numbers (Denton, 1973). These chromosome aberrations probably led to the diverse karyotypes of the modern fishes.

Vitturi *et al*. (1986) observed a population of *Seriola dumerili* in the process of karyotypic change through Robertsonian translocation. They found two karyotypes within the population; a 48 acrocentric chromosome subpopulation and a 46 acrocentric plus one metacentric chromosome subpopulation. A Robertsonian translocation could account for this variation in chromosome number.

Assuming the diploid chromosome number for cyprinids to be 50, a centric fusion would account for the 48 chromosomes of Barbus anoplus, Labeo capensis, and L. umbratus while a centric disassociation could produce the 52 chromosomes of B. argenteus. Karyotypic pathways at higher levels of ploidy are more difficult to follow as one cannot be sure of the chromosome number of the original diploid ancestors. The redfin species' (Pseudobarbus afer and P. burgi) chromosome number of 96 may have been derived from a diploid ancestral species with 48 chromosomes, or one with 50 chromosomes. The latter would produce a tetraploid population with 100 chromosomes, from which four Robertsonian fusions could give rise to the 96 chromosomes of the redfins.

I postulate that the ancestral hexaploid karyotype for the yellowfish was 150 chromosomes, as this number is the most common in the yellowfish and it can be directly derived from the diploid number of 50 chromosomes found in most cyprinid species. A centric fusion probably led to the separation of the Orange-Vaal river species (B. aeneus and B. kimberleyensis) with 148 chromosomes.

It is generally thought that the most evolved species in a group are those with the least chromosomes (Thode et al., 1985b), and highest proportion of biarmed chromosomes (Denton, 1973). Dorofeyeva and Ruhkhyan (1982) found that the most generalized populations of Sevan Lake trout had a greater number of chromosomes and biarmed elements than the specialized populations. A decrease in gene material and arm ratio occurred as these trout evolved. Decreasing chromosome number and increasing fundamental number (arm ratio) were the major trends in the elasmobranch evolution proposed by Schwartz and Maddock (1986).

Garcia et al. (1987) anticipated two basic lines of evolution in the Blennidae genus. The first evolutionary line followed an increase in arm ratio for a constant chromosome number,

an increase in arm ratio for a constant chromosome number, while the second followed a decrease in chromosome number for a more or less constant arm ratio.

Cano et al. (1982) found that there was a contradiction in their results as to the degree of chromosomal and morphological evolution on the one hand and the expected nuclear and DNA content on the other in the genus Blennius. The morphologically and karyotypically derived species did not necessarily have the least amount of nuclear material. They argued that both increases and decreases in genome size had occurred during the evolution of the group. Species with derived karyotypes are not necessarily advanced in other ways. The relatively primitive stickleback Apeltes quadracus had mostly biarmed chromosomes while its specialized relative Culaea inconstans had mostly uniarmed chromosomes (Chen and Ebeling, 1974).

The yellowfish can be separated into two primary groups on the basis of chromosome number, that of Barbus aeneus and B. kimberleyensis (the Orange-Vaal River species), and B. capensis, B. natalensis and B. polylepis.

I have attempted to interpret yellowfish relationships in figure 35 using chromosome number and median fundamental number, following the guidelines used in the previous examples. These are:

a) A decrease in chromosome number is apomorphic
b) An increase in bi-armed chromosomes is apomorphic

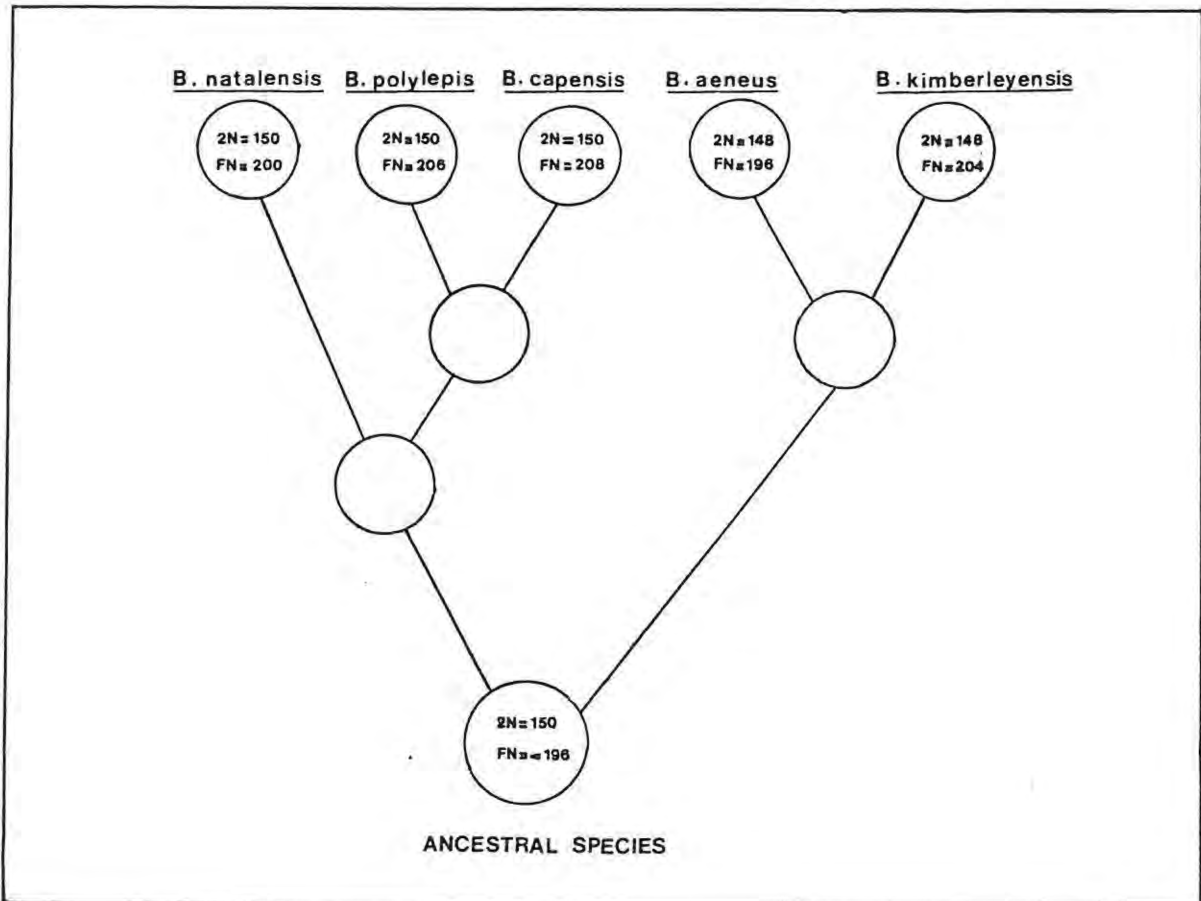


Figure 35: A cladogram showing yellowfish relationships based on karyotypic data.

This cladogram shows very different relationships between the yellowfish to those expected for the group. It has been generally assumed that B. aeneus was the species most like the ancestral yellowfish, and the Cape, Transvaal and Natal species were more derived. On the basis of median fundamental number it seems that B. natalensis is quite distinct from B. polylepis, and the latter species is very close to B. capensis. Zan et al. (1986) found it was impossible to determine karyotypic differences between the hexaploid Schizothorax species due to the large intraspecific variations in chromosome shape. The differences in fundamental numbers presented in this work may merely be artifacts of the chromosome isolation technique used. However, the strong similarities between B. capensis and B. polylepis shown in the ANOVA, principle component and discriminant function analyses of traditional morphometrics strengthens the feasiblity of

such a relationship. Furthermore, electrophoretic studies on the yellowfish species also show that B. capensis and B. polylepis are the most genetically similar of the group (Mulder, pers comm.).

Although Barbus natalensis and B. polylepis were not as similar karyotypically as they were phenotypically only small populations at the opposite ends of the ranges of the two species were sampled. An extensive karyological comparison of B. natalensis and B. polylepis may show that the reported differences between the two species is merely clinal variation, or that they are diverging subspecies. However, the suggestion made on the basis of morphometrics and meristics for the synonymizing of B. natalensis and B. polylepis must be retracted for the present.

E. CONCLUSION

The present study has contributed positively to southern African yellowfish systematics and taxonomy. Application of different morphometrical methods and statistical tests confirms the close similarity of the species. However the analyses also expose clearly the two subgroups (B. aeneus, B. kimberleyensis and B. natalensis, B. polylepis, B. capensis) within the yellowfish.

The karyology of the yellowfish has been surprizingly interesting and taxonomically useful. In the first place karyology corroborates the findings of the morphometrical studies in that (a) the yellowfish have similar karyotypes, and (b) the main difference within the group is between B. aeneus and B. kimberleyensis with 148 chromosomes and B. natalensis, B. polylepis and B. capensis with 150 chromosomes.

The high chromosome numbers found in the yellowfish arose through hexaploidy. Hexaploidy may be important phylogenetic evidence for the monophyletic status of the yellowfish. However, further comparison with the karyotypes of the other African and Asian Barbus with parallel-striated scales is necessary before the extent and significance of hexaploidy within cyprinids is known. Hexaploidy may also be strong evidence to include B. marequensis and Varicorhinus nelspruitensis within the southern African yellowfish lineage.

Preliminary studies on Barbus in southern Africa show that the genus consists of at least three levels of ploidy; diploid, tetraploid and hexaploid. These ploidy levels may play an important role in further taxonomic studies of this genus, thus it is vital to broarden the karyological database of all the cyprinids of this region, for affective outgroup comparisons.

Concerning techniques, the chromosome isolation technique applied in the present study works well under both laboratory and field conditions. Unfortunately the polyploidic nature of the southern African cyprinids and associated problems in isolating numerous small chromosomes warrants further research into cell culture techniques. An important innovation would be the equal uptake of mitotic inhibitor by all the cells. The variation in chromosome size and shape due to unequal dosage tends to mask any physical differences between the very small chromosomes of a hexaploid species. Chromosome banding is effected by changes in chromosome size as well, and probably will be of little use in yellowfish karyology. NOR studies using silver and Chromomycin A_3 stains are important for further karyological research in southern Africa, however tutelage by an expert in this field is necessary, as NOR staining has proved a difficult technique to master.

These and other developments indicate strongly the need for extending karyology as a field of taxonomic and systematic research on African freshwater fishes.

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APPENDIX 1

Table 21. The means and standard deviations of the ratio-transformed traditional morphometric data.

CHARACTER	AENEUS	CAPENSIS	KIMBERLEY.	NATALEN.	POLYLEPIS
HL	27.2±1.5	29.4±1.9	27.2±1.8	28.2±1.7	29.0±2.6
SnL	8.1±0.8	9.3±0.6	5.0±1.9	9.4±0.8	9.1±0.9
OD	7.0±0.7	6.7±0.8	4.2±1.1	6.6±1.3	6.6±1.5
PO	12.5±0.6	14.1±0.7	9.2±4.0	13.4±0.6	14.1±1.5
POPO	4.4±0.5	6.4±0.5	3.7±1.8	5.9±0.5	6.2±0.8
IO	7.1±0.6	8.3±0.5	4.5±1.9	9.0±0.6	9.1±0.6
PreD	53.0±1.2	55.6±1.5	52.1±2.6	54.5±1.8	52.7±2.3
DFL	29.5±1.8	22.6±1.5	25.2±1.9	22.9±1.9	23.5±3.1
BD	26.1±1.6	24.1±1.1	23.8±2.6	25.8±2.0	24.9±1.9
BW	14.7±1.1	12.2±1.0	14.0±0.9	12.8±1.4	12.3±1.9
CPL	18.9±1.9	18.1±0.9	18.2±0.8	18.7±1.2	18.9±1.6
CPD	9.9±0.7	9.6±0.3	8.9±0.4	11.9±0.9	11.2±0.9
ABarb	5.3±0.8	3.3±1.3	4.8±0.6	4.5±0.9	3.7±2.0
PBarb	6.3±1.0	5.0±1.2	5.9±0.9	5.7±0.7	5.9±0.7
PFPF	27.0±2.0	27.3±1.4	28.0±2.5	27.1±1.2	25.6±3.0
PFAF	20.2±1.7	16.7±1.0	18.8±1.2	18.7±1.3	18.1±2.9

Table 22. The means and standard deviations of the ratio-transformed Truss morphometric data.

DISTANCE	AENEUS	CAPENSIS	KIMBERLEY.	NATALEN.	POLYLEPIS
ST-SO	22.9±2.0	23.0±1.7	21.4±1.2	22.9±1.8	22.6±1.8
SO-DFO	35.7±4.2	32.0±2.2	34.7±2.5	32.6±1.2	31.7±1.5
DFO-DFB	14.7±1.3	14.2±0.8	14.6±0.9	13.1±0.8	13.4±0.9
DFB-UCP	28.2±2.9	33.0±1.4	30.2±0.8	35.9±1.8	35.2±1.8
UCP-LCP	10.2±1.0	10.3±0.7	10.8±1.1	12.4±0.7	11.4±0.6
LCP-AFO	22.7±2.0	23.5±1.1	24.1±1.0	24.0±1.1	23.4±1.1
AFO-PFO	22.5±2.1	22.5±1.4	23.0±1.5	23.2±1.0	23.6±1.7
PFO-BJ	42.9±1.7	44.5±1.3	43.6±1.9	41.9±1.5	43.0±1.7
BJ-ST	11.6±2.0	11.3±1.2	10.4±1.3	11.1±0.9	13.5±1.8
SO-BJ	18.2±1.8	19.0±1.5	17.4±0.7	19.2±1.5	19.2±1.2
DFO-PFO	25.2±3.4	23.5±1.7	24.0±2.2	25.4±1.8	23.8±1.5
DFB-AFO	19.5±3.3	21.0±2.3	19.1±1.5	23.0±1.5	23.3±1.3
BJ-DFO	48.2±2.5	46.2±2.0	48.5±1.4	47.0±1.3	46.1±1.8
PFO-DFB	25.7±2.4	22.7±1.8	24.6±1.6	24.4±1.7	23.7±1.4
AFO-UCP	26.0±1.8	26.1±1.8	27.7±1.0	28.8±1.4	27.2±1.3
SO-PFO	38.6±1.5	38.6±1.5	38.1±1.2	38.8±1.0	38.7±1.4
DFO-AFO	30.9±3.3	32.1±1.3	31.0±2.0	34.4±1.9	33.1±2.9
DFB-LCP	32.9±3.2	36.6±1.5	33.6±1.0	39.3±1.9	38.6±1.5

APPENDIX 2

The following table consists of the species and chromosome numbers used to produce figure 37. The karyotypes of the species were included where possible.

Table 23. The chromosome numbers and karyotypes of various species belonging to the cyprinid family.

SPECIES	2N	M	SM	A	SOURCE
Abbottina rivularis	50	24	24	2	LI et al. (1984)
Abramis ballerus	52				ARAI (1982)
Abramis brama	50				WOLF et al. (1969)
Acanthobrama simoni	48	9	13	2	LI et al. (1983)
Acanthorhodeus chankaensis	44	14	14	18	HONG et al. (1983)
Acanthorhodeus longipinnis	44				ARAI (1982)
Acanthorhodeus macropterus	44	14	18	12	HONG et al. (1983)
Acanthorhodeus tonkinensis	44				HONG et al. (1983)
Acheilognathus cyanostigma	44				ARAI (1982)
Acheilognathus lanceolata	48				ARAI (1982)
Acheilognathus limbata	48				ARAI (1982)
Acheilognathus moriokae	44				ARAI (1982)
Acheilognathus tabira	44				ARAI (1982)
Acrossocheilus labiatus	50				ARAI (1982)
Acrossocheilus yunnanensis	50	18	16	16	ZAN et al. (1986)
Alburnoides bipunctatus	50				ARAI (1982)
Alburnus albidus alborella	52				ARAI (1982)
Alburnus alburnus	50				ARAI (1982)
Amblypharyngodon mola	50				ARAI (1982)
Anabarilius alburnops	48				ARAI (1982)
Anabarilius andersoni	48				ARAI (1982)
Anabarilius grahami	48				ARAI (1982)
Anabarilius macrolepis	48				ARAI (1982)
Anaecypris hispanica	50	10	36	4	COLLARES-PEREIRA (1985b)
Aristichthys nobilis	48				BECK et al. (1980)
Aspius aspius	52				ARAI (1982)
Barbus aeneus	148				THIS PAPER
Barbus anoplus	48				THIS PAPER
Barbus argenteus	52				THIS PAPER
Barbus barbus	100				WOLF et al. (1969)

Table 23. The chromosome numbers and karyotypes of various species belonging to the cyprinid family (contd.).

SPECIES	2N	M	SM	A	SOURCE
Barbus capensis	150				THIS PAPER
Barbus kimberleyensis	148				THIS PAPER
Barbus marequensis	134-150				THIS PAPER
Barbus meridionalis	100				ARAI (1982)
Barbus natalensis	150				THIS PAPER
Barbus polylepis	150				THIS PAPER
Barbus tetrazona	50				WOLF et al. (1969)
Barilius barila	50				KHUDA-BUKHSH et al. (1986)
Barilius bendelisis	50				KHUDA-BUKHSH et al. (1986)
Bola bola	50				ARAI (1982)
Campostoma anomalum	50				GOLD et al. (1980)
Carassioides cantonensis	100				ZAN et al. (1986)
Carassius auratus	162	33	53	76	ZAN et al. (1986)
Carassius auratus	156				ZAN et al. (1986)
Carassius auratus	104				WOLF et al. (1969)
Carassius carassius	100				ARAI (1982)
Catla catla	50				ARAI (1982)
Chondrostoma kneri	50				COLLARES-PEREIRA (1983)
Chondrostoma lemmingi	50	12	32	6	COLLARES-PEREIRA (1985b)
Chondrostoma lusitanicum	50				COLLARES-PEREIRA (1983)
Chondrostoma phoxinus	50				COLLARES-PEREIRA (1983)
Chondrostoma soetta	50				ARAI (1982)
Chondrostoma toxostoma	50				COLLARES-PEREIRA (1983)
Cirrhinus reba	48				ARAI (1982)
Clinostomus elongatus	50				UYENO AND SMITH (1972)
Coreius guichenoti	50	16	22	12	LI et al. (1984)
Coreius heterodon	50	16	22	12	LI et al. (1984)
Ctenopharyngodon idella	48				BECK et al. (1980)
Culter erythropterus	48	16	26	6	LI et al. (1983)
Cyprinus carpio	100	12	40	48	ZAN et al. (1986)
Cyprinus carpio chilia	100	22	30	48	ZAN et al. (1986)
Cyprinus carpio gibelio	156	42	74	40	ZAN et al. (1986)
Cyprinus longipectralis	100	22	30	48	ZAN et al. (1986)
Cyprinus megalophthalmus	100	22	30	48	ZAN et al. (1986)
Cyprinus micristius fuxianensis	100	22	30	48	ZAN et al. (1986)
Cyprinus pellegrini	100	22	30	48	ZAN et al. (1986)

Table 23. The chromosome numbers and karyotypes of various species belonging to the cyprinid family (contd.).

SPECIES	2N	M	SM	A	SOURCE
Cyprinus yunnanensis daliensis	100	22	30	48	ZAN et al. (1986)
Danio aequipinnatus	50				KHUDA-BUKHSH et al. (1986)
Danio albolineatus	50				ARAI (1982)
Danio devario	50				ARAI (1982)
Danio devario	50				KHUDA-BUKHSH et al. (1986)
Danio malabaricus	50				ARAI (1982)
Danio rerio	50				ARAI (1982)
Dionda episcopa	50				GOLD et al. (1981)
Ericymba buccata	50				GOLD et al. (1980)
Erythroculter dabryi	48	16	28	4	LI et al. (1983)
Erythroculter ilishaeformis	48	16	26	6	LI et al. (1983)
Erythroculter mongolicus	48	14	28	6	LI et al. (1983)
Erythroculter oxycephaloides	48	20	24	4	LI et al. (1983)
Esomus danrica	50				ARAI (1982)
Exoglossum maxillingua	48				GOLD et al. (1980)
Garra gotyla	50				KHUDA-BUKHSH et al. (1986)
Garra lamta	50				KHUDA-BUKHSH et al. (1986)
Gila bicolor	50				GOLD AND AVISE (1977)
Gila nigrescens	50				JENKIN AND GOLD (Unpubl.)
Gila orcutti	50				GOLD et al. (1980)
Gnathopogon argentatus	50	22	26	2	LI et al. (1984)
Gnathopogon elongatus elongatus	50				ARAI (1982)
Gnathopogon sihuensis	50	22	24	4	LI et al. (1984)
Gobio albipinnatus vladykovi	50				ARAI (1982)
Gobio gobio	50				ARAI (1982)
Gobio kessleri banaticus	50				ARAI (1982)
Gobio uranoscopus	52				ARAI (1982)
Hemibarbus barbus	50				ARAI (1982)
Hemibarbus labeo	50	16	16	20	LI et al. (1984)
Hemibarbus longirostris	50	18	18	14	LI et al. (1984)
Hemibarbus maculatus	50	16	14	20	LI et al. (1984)
Hemiculter bleekeri bleekeri	48	16	26	6	LI et al. (1983)
Hemiculter leucisculus	48	16	26	6	LI et al. (1983)

Table 23. The chromosome numbers and karyotypes of various species belonging to the cyprinid family (contd.).

SPECIES	2N	M	SM	A	SOURCE
Hemitremia flammea	50				AMEMIYA AND GOLD (1987)
Hesperoleucus symmetricus	50	42			GOLD AND AVISE (1977)
Hiugobio chensienensis	50	24	24	2	HONG et al. (1984)
Hybognathus hayi	50	10	36	4	LI et al. (1983)
Hybognathus nuchalis	50				GOLD et al. (1981)
Hybognathus placitus	50				AMEMIYA AND GOLD (1987)
Hybopsis aesivalis	50	12	32	6	LI et al. (1983)
Hybopsis amblops	50	16	30	4	GOLD et al. (1981)
Hybopsis storeriana	50				AMEMIYA (1987)
Hypophthalmichthys molitrix	48				MARIAN AND KRASZNAI (1979)
Ischikauia steenackeri	48				ARAI (1982)
Labeo bata	50				ARAI (1982)
Labeo calbasu	50				ARAI (1982)
Labeo capensis	48				THIS PAPER
Labeo diplostomus	50				KHUDA-BUKHSH et al. (1986)
Labeo umbratus	48				THIS PAPER
Lavinia exilicauda	50	42		8	GOLD AND AVISE (1977)
Lepidomeda albivallis	50				GOLD et al. (1980)
Lepidomeda mollispinis	50				GOLD et al. (1980)
Lepidomeda vittata	50				GOLD et al. (1980)
Leuciscus cephalus	50				AL-SABTI (1987)
Leuciscus cephalus cabeda	50				ARAI (1982)
Leuciscus idus	50				GOLD et al. (1980)
Leuciscus leuciscus	50				ARAI (1982)
Leuciscus souffia muticellus	50				ARAI (1982)
Leuciscus svallize	50				ARAI (1982)
Leuciscus turskyi	50				ARAI (1982)
Meda fulgida	50				GOLD et al. (1980)
Megalobrama amblycephala	48	18	26	4	LI et al. (1983)
Megalobrama terminalis	48	14	26	8	LI et al. (1983)
Microphysogobio tafangensis	50				ARAI (1982)
Microphysogobio yaluensis	50				ARAI (1982)
Moroco jouyi	52				ARAI (1982)
Moroco lagowskii	50				ARAI (1982)
Moroco oxycephalus	50				ARAI (1982)
Moroco steindachneri	50				ARAI (1982)
Morulius chrysophekadion	50				ARAI (1982)

Table 23. The chromosome numbers and karyotypes of various species belonging to the cyprinid family (contd.).

SPECIES	2N	M	SM	A	SOURCE
Mylopharodon conocephalus	50	44		6	GOLD AND AVISE (1977)
Neolissocheilus sumatranus	98				ARAI (1982)
Nocomis asper	50				AMEMIYA (1987)
Nocomis leptocephalus	50	14	28	8	GOLD *et al*. (1981)
Notemigonus cryosoleucas	50	44		6	GOLD AND AVISE (1977)
Notropis albeolus	50				ZOCH AND GOLD (Unpubl.)
Notropis amabilis	50	14	34	2	GOLD *et al*. (1981)
Notropis analostanus	50				ZOCH AND GOLD (Unpubl.)
Notropis ardens	50				GOLD *et al*. (1980)
Notropis atherinoides	50	16	32	2	GOLD *et al*. (1980)
Notropis atrocaúdalis	50	10	36	4	GOLD *et al*. (1980)
Notropis baileyi	50				GOLD *et al*. (1988)
Notropis bellus	50				AMEMIYA AND GOLD (1987)
Notropis boops	50				GOLD *et al*. (Unpubl.)
Notropis braytoni	50				AMEMIYA AND GOLD (1987)
Notropis buchanani	50				AMEMIYA AND GOLD (1986)
Notropis callistius	50				GOLD *et al*. (1980)
Notropis camurus	50	18	24	4	GOLD *et al*. (1981)
Notropis cardinalis	50				ZOCH AND GOLD (Unpubl.)
Notropis cerasinus	50				ZOCH AND GOLD (Unpubl.)
Notropis chalybaeus	50				AMEMIYA (1987)
Notropis chrosomus	50				AMEMIYA AND GOLD (1987)
Notropis coccogenis	50				ZOCH AND GOLD (Unpubl.)
Notropis dorsalis	50				Gold *et al*. (Unpubl.)
Notropis cornutus	50				GOLD *et al*. (1980)
Notropis crysocephalus	50				GOLD *et al*. (1980)
Notropis formosus	50				GOLD *et al*. (Unpubl.)
Notropis fumeus	50				GOLD *et al*. (1980)
Notropis galacturus	50				GOLD *et al*. (1988)
Notropis gibbsi	50				GOLD *et al*. (Unpubl.)
Notropis girardi	50				AMEMIYA (1987)
Notropis hubbsi	50				AMEMIYA AND GOLD (1989)

Table 23. The chromosome numbers and karyotypes of various species belonging to the cyprinid family (contd.).

SPECIES	2N	M	SM	A	SOURCE
Notropis jemezanus	50				AMEMIYA AND GOLD (1987)
Notropis lepidus	50				AMEMIYA AND GOLD (1987)
Notropis longirostris	50				GOLD *et al*. (1980)
Notropis lutrensis	50				GOLD *et al*. (1980)
Notropis maculatus	50				AMEMIYA AND GOLD (1989)
Notropis nubilus	50				GOLD *et al*. (1988)
Notropis oxyrhynchus	50				GOLD *et al*. (1980)
Notropis petersoni	50				GOLD *et al*. (Unpubl.)
Notropis pilsbryi	50				GOLD *et al*. (1988)
Notropis potteri	50	16	32	2	GOLD *et al*. (1981)
Notropis proserpinus	50				AMEMIYA AND GOLD (1987)
Notropis sabinae	50				GOLD *et al*. (1980)
Notropis roseipinnus	50				GOLD *et al*. (1980)
Notropis shumardi	50				GOLD *et al*. (1980)
Notropis signipinnis	50				GOLD *et al*. (1980)
Notropis spilopterus	50				GOLD *et al*. (1988)
Notropis stilbius	50				GOLD *et al*. (1980)
Notropis stramineus	50	16	34	0	GOLD *et al*. (1981)
Notropis texanus	50				GOLD *et al*. (1980)
Notropis trichroistius	50				AMEMIYA (1987)
Notropis umbratilis	50				GOLD *et al*. (1980)
Notropis venustus	50				GOLD *et al*. (1980)
Notropis volucellus	50				GOLD *et al*. (1980)
Notropis welaka	50				AMEMIYA AND GOLD (1989)
Notropis whipplei	50				GOLD *et al*. (1988)
Notropis zonatus	50				ZOCH AND GOLD (Unpubl.)
Notropis zonistius	50				ZOCH AND GOLD (Unpubl.)
Opsariichthys uncirostris	78				ARAI (1982)
Opsopoeodus emiliae	50				GOLD (1984)
Orthodon microlepidotus	50	44		6	GOLD AND AVISE (1977)
Pachychilon pictum	50				ARAI (1982)
Parabramis pekinensis	48	14	26	8	LI *et al*. (1983)
Paracanthobrama guichenoti	50	18	20	12	LI *et al*. (1984)

Table 23. The chromosome numbers and karyotypes of various species belonging to the cyprinid family (contd.).

SPECIES	2N	M	SM	A	SOURCE
Paracheilognathus imberbis	44	14	18	12	HONG *et al.* (1983)
Paracheilognathus rhombea	44				ARAI (1982)
Paraphoxinus adspersus	50				ARAI (1982)
Paraphoxinus alepidotus	50				ARAI (1982)
Paraphoxinus croaticus	50				ARAI (1982)
Paraphoxinus metohiensis	50				ARAI (1982)
Paraphoxinus pstrossi	50				ARAI (1982)
Percocypris pingi	98	42	30	26	ZAN *et al.* (1986)
Phenacobius mirabilis	50	18	28	4	LI *et al.* (1983)
Phonixus cumberlandensis	50				JOSWIAK *et al.* (1980)
Phoxinus eos	50				GOLD *et al.* (1980)
Phoxinus erythrogaster	50				GOLD *et al.* (1980)
Phoxinus neogaeus	50				JOSWIAK *et al.* (1980)
Phonixus oreas	50				JOSWIAK *et al.* (1980)
Phoxinus phoxinus	50				ARAI (1982)
Pimephales notatus	52				GOLD *et al.* (1980)
Pimephales promelas	50	14	34	2	GOLD *et al.* (1981)
Pimephales vigilax	50	24	24	2	LI *et al.* (1983)
Plagiognathops microlepis	48	9	13	2	LI *et al.* (1983)
Plagopterus argentissimus	50				GOLD *et al.* (1980)
Pogonichthys macrolepidotus	50	44		6	GOLD AND AVISE (1977)
Poropuntius lucustris	50	10	18	22	ZAN *et al.* (1986)
Pseudobarbus afer	96				THIS PAPER
Pseudobarbus burgi	96				THIS PAPER
Pseudogobio esocinus	50				ARAI (1982)
Pseudogobio vaillanti	50				HONG *et al.* (1984)
Pseudoperilampus typus	44				ARAI (1982)
Pseudorasbora parva	50	18	22	10	LI *et al.* (1984)
Pseudorasbora pumila pumila	50				ARAI (1982)
Ptychocheilus grandis	50	42		8	GOLD AND AVISE (1977)
Ptychocheilus lucius	50				GOLD *et al.* (1980)
Puntius arulius	50				ARAI (1982)
Puntius binotatus	50				ARAI (1982)
Puntius chola	50				ARAI (1982)
Puntius conchonius	50				ARAI (1982)
Puntius cumingi	50				ARAI (1982)
Puntius everetti	50				ARAI (1982)
Puntius fasciatus	50				ARAI (1982)
Puntius filmentosus	50				ARAI (1982)
Puntius japonicus	50				ARAI (1982)
Puntius lateristriga	50				ARAI (1982)

Table 23. The chromosome numbers and karyotypes of various species belonging to the cyprinid family (contd.).

SPECIES	2N	M	SM	A	SOURCE
Puntius migrofasciatus	50				ARAI (1982)
Puntius oligolepis	50				ARAI (1982)
Puntius orphoides	50				ARAI (1982)
Puntius partipentazona	50				ARAI (1982)
Puntius pentazona	50				ARAI (1982)
Puntius schwanenfeldi	50				ARAI (1982)
Puntius tetrazona	50				ARAI (1982)
Puntius ticto	50				ARAI (1982)
Puntius titteya	50				ARAI (1982)
Puntius conchonius	50				KHUDA-BUKHSH *et al*. (1986)
Puntius melanampyx	50				KHUDA-BUKHSH *et al*. (1986)
Puntius sophore	50				ARAI (1982)
Pungtungia herzi	50				ARAI (1982)
Rasbora buchanani	50				KHUDA-BUKHSH *et al*. (1986)
Relictus solitarius	50				HUBBS *et al*. (1974)
Rhinichthys atratulus	50				GOLD *et al*. (1980)
Rhinichthys cataractae	50				GOLD *et al*. (1980)
Rhinichthys evermanni	50				GOLD *et al*. (1980)
Rhinichthys osculus	50				JENKIN AND GOLD (Unpubl.)
Rhinogobio cylindricus	50	14	22	14	HONG *et al*. (1984)
Rhinogobio typus	50	14	22	14	HONG *et al*. (1984)
Rhodeus atremius	46				ARAI (1982)
Rhodeus ocellatus	48	10	24	14	HONG *et al*. (1983)
Rhodeus sericeus	48				HONG *et al*. (1983)
Rhodeus sericeus amarus	48				ARAI (1982)
Rhodeus sinensis	48				HONG *et al*. (1983)
Rhodeus suigensis	46				ARAI (1982)
Rohtee cotio	48				ARAI (1982)
Rutilus arcasi	50	16	30	4	COLLARES-PEREIRA (1985b)
Rutilus macrolepidotus	50	14	32	4	COLLARES-PEREIRA (1985b)
Rutilus rubilio	50				ARAI (1982)
Rutilus rutilus	50				WOLF *et al*. (1969)
Salmostoma bacaila	50				ARAI (1982)
Sarcocheilichthys kianensis	50	18	22	10	HONG *et al*. (1984)

Table 23. The chromosome numbers and karyotypes of various species belonging to the cyprinid family (contd.).

SPECIES	2N	M	SM	A	SOURCE
Sarcocheilichthys nigripinnus	50	18	22	10	HONG et al. (1984)
Sarcocheilichthys parvus	50	18	22	10	HONG et al. (1984)
Sarcocheilichthys sinensis	50	18	22	10	HONG et al. (1984)
Sarcocheilichthys variegatus	50				ARAI (1982)
Saurogobio dabryi	50	18	24	8	HONG et al. (1984)
Saurogobio dumerili	50	18	24	8	HONG et al. (1984)
Saurogobio gymnocheilus	50	18	24	8	HONG et al. (1984)
Scardinius erythrophthalmus	50				GOLD et al. (1980)
Schizothorax grahami	148	52	30	66	ZAN et al. (1986)
Schizothorax lissolabiatus	148	38	32	78	ZAN et al. (1986)
Schizothorax niger	98				KHUDA-BUKHSH et al. (1986)
Schizothorax schizothorax	148				ZAN et al. (1986)
Schizothorax taliensis	148	48	30	70	ZAN et al. (1986)
Schizothorax yunnanensis	148	48	28	72	ZAN et al. (1986)
Semotilus atromaculatus	50	22	24	4	GOLD et al. (1981)
Semotilus corporalis	52				GOLD et al. (1980)
Semotilus margarita	50				GOLD et al. (1980)
Sinocyclocheilus grahami	96	22	36	38	ZAN et al. (1986)
Sinocyclocheilus maculatus	96	18	32	46	ZAN et al. (1986)
Spinibarbus caldwelli	100				ZAN et al. (1986)
Spinibarbus denticulatus	100				ZAN et al. (1986)
Spinibarbus sinensis	100	14	44	42	ZAN et al. (1986)
Tanakia tanago	50				ARAI (1982)
Tanichthys albonubes	50				ARAI AND TOKORO (1986)
Tiaroga cobitis	50				JENKIN AND GOLD (Unpubl.)
Tinca tinca	48				WOLF et al. (1969)
Tor brevifilis	50	14	16	20	ZAN et al. (1986)
Tor douronensis	100	22	30	48	ZAN et al. (1986)
Tor khudree	100				KHUDA-BUKHSH et al. (1986)
Tor mosal	100				KHUDA-BUKHSH et al. (1986)
Tor putitora	100				KHUDA-BUKHSH et al. (1986)
Tor sinensis	100	18	30	52	ZAN et al. (1986)
Tor tor	100				KHUDA-BUKHSH et al. (1986)
Tribolodon ezoe	50				ARAI (1982)
Tribolodon hakonensis	50				ARAI (1982)

Table 23. The chromosome numbers and karyotypes of various species belonging to the cyprinid family (contd.).

SPECIES	2N	M	SM	A	SOURCE
Varicorhinus nelspruitensis	138-148				THIS PAPER
Vimba vimba carinata	50				ARAI (1982)
Vimba vimba vimba	50				ARAI (1982)
Xenocypris argentea	48	10	13	1	LI *et al*. (1983)
Xenocypris davidi	48	9	13	2	LI *et al*. (1983)
Zacco platypus	48				ARAI (1982)
Zacco temmincki	48				ARAI (1982)