Karyology of three evolutionarily hexaploid southern African species of yellowfish, *Labeobarbus* Rüppel, 1836 (Cyprinidae)

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The karyotypes of three species of yellowfish, namely *Labeobarbus marequensis* (A. Smith, 1841), *L. capensis* (A. Smith, 1841) and *L. polylepis* (Boulenger, 1907), were examined by Giemsa staining using an approach improved for the description of high chromosome numbers. In each case, 2n = 150; no heteromorphic chromosomes were detected; chromosomes in all morphological categories ranged smoothly from large to small, with no distinctly large submetacentric pairs; and metacentric chromosomes showed little variation in size. *Labeobarbus marequensis* had 26 metacentric (m), 44 submetacentric (sm), 42 subtelocentric (st) and 38 acrocentric (a) chromosomes and a fundamental number (FN) of 262; *L. capensis* had 16 m, 58 sm, 42 st and 34 a chromosomes and FN = 266; and *L. polylepis* had 18 m, 60 sm, 42 st and 30 a chromosomes and FN = 270. These results, combined with published literature, imply that *Labeobarbus* Rüppel, 1836 is an evolutionarily hexaploid African lineage and support its removal from synonymy with the evolutionarily tetraploid Asian genus *Tor* Gray, 1834. A review of fundamental numbers for conspecific *Labeobarbus* species examined in different studies implicated karyological technique as a confounding factor in assessing details of karyotypes, leading to recommendations for future karyological studies of barbine fishes. Potential synapomorphies are pointed out in karyological characters of species within *Labeobarbus*.

Key words: **Pisces, Barbinae, cytogenetics, hexaploidy, taxonomy, phylogenetics.**

INTRODUCTION

Species of the closely related pan-African barbine genera *Labeobarbus* Rüppel, 1836 and *Varicorhinus* Rüppel, 1836 generally grow distinctively large, often greatly exceeding 150 mm in length, and have distinctive scales with parallel striae on the exposed part. However, *V. beso* Rüppel, 1835, the type species of *Varicorhinus,* has radially striate scales, so the definition of the genus and its relationship to *Labeobarbus* need attention. These fish also show strong ontogenetic heterochrony, resulting in large changes in morphology as they grow, such that there is no strict link between their morphology and their phylogenetic relationships (Mina *et al.* 2001). This taxonomic challenge is reflected in the synonymy of *V. nyasensis* Worthington, 1933 with *L. johnstonii* Boulenger, 1907 (Banister & Clarke 1980) and the synonymy of *V. nasutus* Gilchrist & Thompson, 1911 with *L. marequensis* (A. Smith, 1841) (Tweddle & Skelton 1998). While the Asian genus *Capoeta* Valenciennes 1842 was resurrected from synonymy with *Varicorhinus* in 1969, it was only recently suggested that *Labeobarbus* should be restored from synonymy with the Asian genus *Tor* Gray, 1834 to full generic status (Golubtsov & Krysanov 1993; Berrebi *et al.* 1996; Nagelkerke & Sibbing 2000; Skelton 2001, 2002).

Karyological investigations of barbine fish have provided additional characters for resolving relationships of barbine cyprinids (Oellermann & Skelton 1990; Berrebi *et al.* 1996; Naran *et al.* 2006). *Labeobarbus* has seven southern African representatives, informally grouped into small-scaled and large-scaled species (Jubb 1967; Gaigher 1975; Skelton 2001). The first karyological study of *Labeobarbus* focussed on five small-scaled species from South Africa and reported their hexaploid evolutionary origin (Oellermann & Skelton 1990). *Labeobarbus marequensis* is a large-scaled, southern African species with variable mouth forms that range from normal to horny-lipped (Tweddle & Skelton 1998). Its karyotype was examined using a preparation technique (Naran *et al.* 2006) that allows detailed imaging of the chromosomes, and compared to karyotypes of the small-scaled species

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L. capensis and *L. polylepis* prepared in the same way.

Our results also provide an opportunity to review the known karyotypes of *Labeobarbus, Varicorhinus, Capoeta* and *Tor* species, including hexaploid species of '*Barbus*' (*sensu* Berrebi *et al.* 1996) from Africa that have been suggested to belong in *Labeobarbus* (Golubtsov & Krysanov 1993; Nagelkerke & Sibbing 2000).

MATERIALS & METHODS

Four males and one female of *L. marequensis* were collected from the Marico River (25°552'S, 25°53'E). In addition, a male of *L. capensis* from the Rondegat River (25°47'S 26°22'E) and a female of *L. polylepis* from the Elands River (25°33'S, 26°36'E) were sampled for comparison. Voucher samples (SAIAB 52700, SAIAB 52705 and SAIAB 53163, respectively) are housed in the collection of the South African Institute for Aquatic Biodiversity (SAIAB, formerly the J.L.B. Smith Institute of Ichthyology), Grahamstown, South Africa.

Mitosis was stimulated by intraperitoneal injection of a baking yeast solution. After 48 hours colchicine was injected (0.01% wt/vol at 0.1 ml/g body weight), and two hours later the fish were killed by overdose with L-phenoxyethanol. Chromosome preparation followed the method described by Naran *et al.* (2006): kidney tissue was removed, macerated and placed in a hypotonic solution of 0.4% NaCl for 30 minutes and fixed in Carnoy's solution; the cell suspension was dropped onto microscope slides, air-dried, and stained with 4–6% Giemsa stain for five minutes. Meiotic figures of *L. marequensis* and *L. capensis* were prepared similarly from testis cells.

Bromides were made of each spread and the arm lengths of each chromosome in a spread were measured from these with Vernier callipers. Using the ratio of these arm lengths, chromosomes were classified into metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a) following Levan *et al.* (1964). The FN value was calculated using the formula FN = 2(m + sm + st) + a. A karyotype of *L. polylepis* was prepared using MicroImage (Olympus) to clean the image and Ikaros (MetaSystems) to arrange the chromosome images into a figure.

RESULTS

A typical karyotype (Fig. 1) showed chromosomes morphologies that included all categories and ranged smoothly in size from largest to smallest. No obviously heteromorphic elements, suggesting the presence of well-differentiated sex-chromosomes, were present in *L. marequensis.* Chromosome counts (Table 1) showed a modal chromosome number of $2n = 150$ for each species. Although other counts (Table 1) were also observed, they were evidently preparation artefacts (Collares-Pereira 1985; Ráb & Roth 1989). All three species had about twice as many acrocentric chromosomes as metacentrics, and these categories together formed only about a third of the total chromosome number (Table 2). This resulted in similar fundamental numbers of 262–270 arms (Table 3).

Meiotic figures of *L. marequensis* (Fig. 2) and *L. capensis* showed only bivalent synaptic pairing; meiotic cells of *L. polylepis* were not examined.

DISCUSSION

As is characteristic of cyprinids, these three species had very small chromosomes, making it difficult to find physical markers to identify particular chromosomes. The three karyotypes showed a gradual change in chromosomes size and centromere position from median to terminal (Fig. 1), as is typical of cyprinids. There was no pair of large submetacentric chromosomes like those found in *Capoeta capoeta sevangi* De Philippi, 1865 or the European tetraploid *Barbus sensu stricto* species (Kryzanov 1999). *Labeobarbus marequensis* had fewer submetacentric chromosomes and slightly more metacentric chromosomes than *L. capensis* and *L. polylepis.* The division of the southern African *Labeobarbus* into large- and small-scaled groups might be supported by such cytogenetic differences, but our data are too few relative to the diversity of species in the genus to do more than point out the possibility.

The karyotypes were dominated by biarmed chromosomes (Fig. 1), in contrast to the previous study of these species (Oellermann & Skelton 1990), which found a distinct predominance of telocentric chromosomes and lower fundamental numbers in *L. capensis* and *L. polylepis* (Table 3). Unfortunately, discordant fundamental numbers have been reported whenever a species has been examined more than once (Table 3). Within studies there can be ambiguity about the classification of 6% or more of the acrocentric chromosomes in a spread (e.g. Table 2; see also Naran *et al.* 2006), primarily due to their small size, but this alters fundamental numbers by less than 8%, while variation between studies can be 10–35% (Table 3).

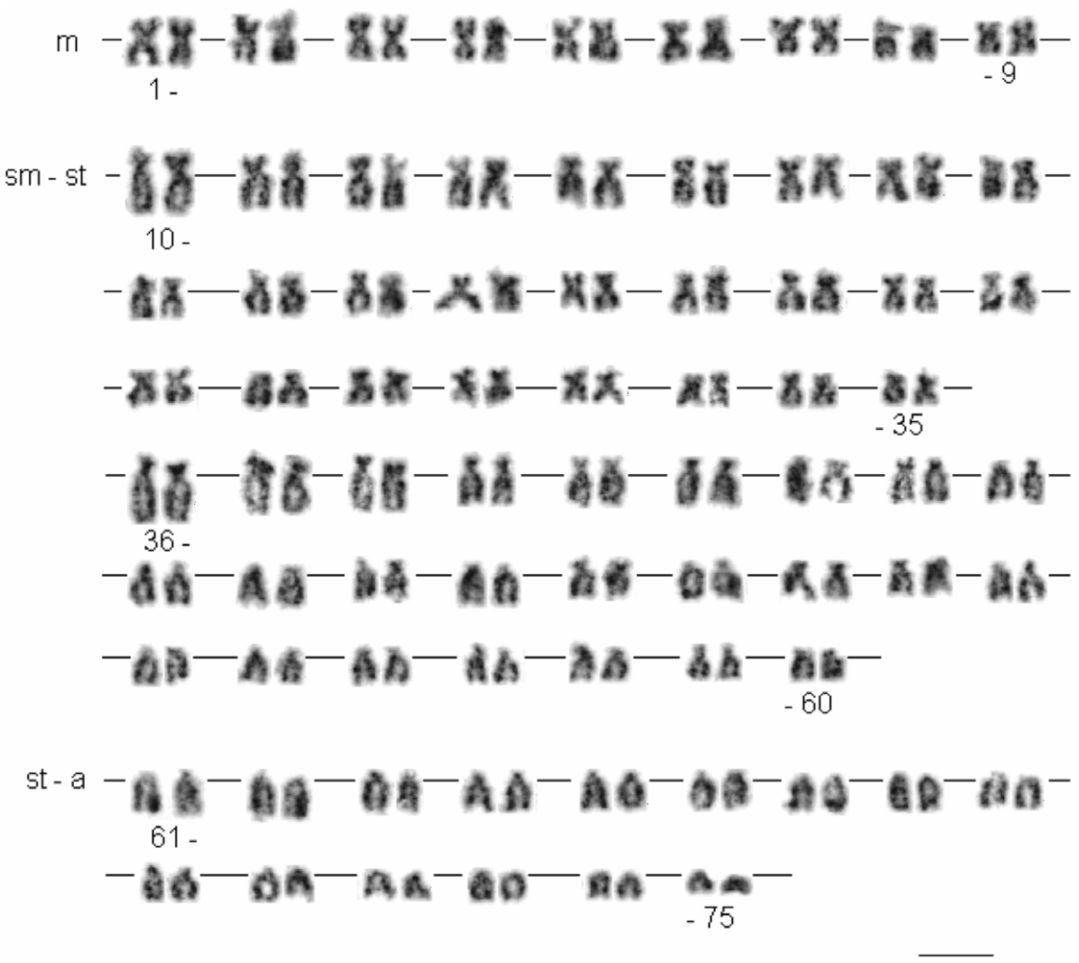

Fig. 1. Photokaryotype of a mitotic cell of a female of *Labeobarbus polylepis* (Elands River; SAIAB 53163). m = metacentric, sm + st = submetacentric and subtelocentric, a = acrocentric. Scale bar = 5 µm.

These differences may reflect variability between conspecific populations in some cases, but could also be attributed to disparities in the preparation techniques of different studies (Collares-Pereira 1985; Ráb & Roth 1989; Ráb & Collares-Pereira 1995) and perhaps to taxonomic problems (Kryzanov & Golubtsov 1996), which would preclude meaningful comparisons across the literature until these problems are mitigated. We therefore avoid interspecific comparisions of chromosome morphology. The preparation method used in this study (Foresti *et al.* 1992; Naran *et al.* 2006) provided a clear image of the chromosomes and their centromere positions, and is recommended for future studies.

To date, all representatives of *Labeobarbus* that have been karyotyped have a diploid complement of 148 or 150 chromosomes (Table 3), an apparent synapomorphy that is interpreted as an evolutionarily hexaploid karyotype (Ohno 1970; Oellermann & Skelton 1990; Guégan *et al.* 1995; Machordom & Doadrio 2001; Tsigenopoulos *et al.* 2002; Leggat & Iwama 2003). Molecular data suggest that *Labeobarbus* and the African evolutionarily hexaploid barbs referred to '*Barbus*' *sensu* Berrebi *et al.* (1996) form a monophyletic lineage (Machordom & Doadrio 2001; Tsigenopoulos *et al.* 2002). The clade is widespread through Africa, indicating that the event that gave rise to hexaploidy is ancient, predating the origin of *Labeobarbus* (Table 3). The long time available for the hexaploid genome to become functionally diploid through mutation would account for why no tetravalent or hexavalent synapsis was found in

Table 1. Frequencies of cells with particular chromosome numbers in samples of kidney cells from three species of *Labeobarbus*.

Species	Cells sampled	Chromosome number						
		<146	146	147	148	149	150	>150
L. marequensis	35	6	1	–	–	–	27	1
L. capensis	17	4	–	1	1	2	9	–
L. polylepis	15	3	–	–	1	–	11	–

Table 2. Modal (and minimum and maximum) number of chromosomes in each morphological category of three *Labeobarbus* species. m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric.

Species	Cells sampled	Morphological categories		
		m	sm + st	a
L. marequensis	9	26 (16–28)	44 + 42 (84–94)	38 (36–40)
L. capensis	6	16 (14–18)	58 + 42 (100–104)	34 (34–36)
L. polylepis	9	18 (14–20)	60 + 42 (88–104)	30 (30–48)

meiotic cell spreads (e.g. Fig. 2). A chromosome number of 148 may represent a synapomorphy for *L. aeneus* and *L. kimberleyensis* (Table 3) and for two West African species (Table 3), but given the geographical separation of these two groups, its occurrence in species from the two regions may be a homoplasy.

Species of the Asian genus *Tor* are evolutionarily tetraploid (Table 3), which, along with the biogeographical distinctness of the two genera, supports the taxonomic restoration of *Labeobarbus* to full generic status (Skelton 2001, 2002). However, molecular phylogenetic studies indicate that the characteristically hexaploid genus *Capoeta* (Table 3) forms a clade within the tetraploid taxon *Luciobarbus* Heckel, 1843 (Tsigenopoulos *et al.* 2003); similarly, *Labeobarbus* may have arisen from within *Tor*. A molecular phylogenetic study of *Tor* and *Labeobarbus* is needed to resolve their taxonomic status.

Since *Capoeta* is nested within *Luciobarbus* (Tsigenopoulos *et al.* 2003) and the latter is phylogenetically distinct from *Labeobarbus* (Machordom & Doadrio 2001; Tsigenopoulos *et al.* 2002, 2003),

Fig. 2. Photograph of a meiotic spread of a male of *Labeobarbus marequensis* (Marico River; SAIAB 52700). Scale bar = 5 µm.

Table 3. Published fundamental number (FN), diploid number and number of chromosomes in each morphological category for various African species of *Labeobarbus, Varicorhinus, Tor* and *Capoeta*. The name '*Barbus*' is used *sensu* Berrebi *et al.* (1996) to indicate species that do not belong in the genus *Barbus sensu stricto,* but which are awaiting taxonomic revision. m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric.

Species	Origin	2n	m	sm	st	a	FN	Source
L. aeneus (Burchell, 1822)	South Africa	148	48			100	196	Oellermann & Skelton 1990
L. capensis (A. Smith, 1841)	South Africa	150	58			92	196	Oellermann & Skelton 1990
	South Africa	150	16	58	42	34	266	This study
L. kimberleyensis (Gilchrist & Thompson, 1913)	South Africa	148	56			92	204	Oellermann & Skelton 1990
L. marequensis (A. Smith, 1841)	South Africa	150	26	44	42	38	262	This study
L. natalensis (Castelnau, 1861)	South Africa	150	50			100	200	Oellermann & Skelton 1990
L. polylepis (Boulenger, 1907)	South Africa	150	56			94	206	Oellermann & Skelton 1990
	South Africa	150	18	60	42	30	270	This study
'*Barbus*' *bynni bynni* (Forskål, 1775)	Ethiopia	150		70		80	220	Golubtsov & Kryzanov 1993 Kryzanov & Golubtsov 1996
'*Barbus*' *bynni occidentalis* Boulenger, 1911	West Africa	148						Guégan *et al.* 1995
	West Africa	150						Guégan & Morand 1996
'Barbus' bynni waldroni Norman, 1935	West Africa	150						Guégan & Morand 1996
'*Barbus*' *canis* (Valenciennes, 1842)	Israel	150	76		24	50	226	Gorshkova *et al.* 2002
'*Barbus*' *ethiopicus* Zolezzi, 1939	Ethiopia	150	40			110	190	Golubtsov & Kryzanov 1993
'*Barbus*' *intermedius* Rüppel, 1835	Ethiopia	150	90			60	240	Golubtsov & Kryzanov 1993
	Ethiopia	150	66			84	216	Golubtsov & Kryzanov 1993 Kryzanov & Golubtsov 1996
'*Barbus*' *parawaldroni* Lévêque, Thys van den Audenaerde & Traoré, 1987	West Africa	150						Guégan & Morand 1996
'*Barbus*' *petitjeani* Daget, 1962	West Africa	150	36		90	24	186	Guégan *et al.* 1995
'*Barbus*' *sacratus* Daget, 1963	West Africa	150						Guégan & Morand 1996
'*Barbus*' *wurtzi* Pellegrin, 1908	West Africa	148						Guégan *et al.* 1995
V. beso Rüppel, 1835	Ethiopia	150	66			84	216	Golubtsov & Kryzanov 1993 Kryzanov & Golubtsov 1996
V. nelspruitensis Gilchrist & Thompson, 1911	South Africa	150						Oellermann & Skelton 1990
C. capoeta sevangi De Filippi 1865	Russia	150	10		30	110	190	Krysanov 1999
C. capoeta umbla (Heckel, 1843)	Turkey	150	86			64	236	Kiliç Demírok & Ünlü 2001
C. damascina (Valenciennes, 1842)	Israel	150	76			74	228	Gorshkova *et al.* 2002
C. trutta (Heckel, 1843)	Turkey	150	70			80	220	Kiliç Demírok & Ünlü 2001
T. douronensis (Valenciennes, 1842)	China	100						Zan *et al.* 1986
T. putitora (Hamilton, 1822)	India	100						Rishi & Haobam 1984
T. sinensis Wu, 1977	China	100						Zan *et al.* 1986

hexaploidy must have arisen independently in Eurasian *Capoeta* and African *Labeobarbus*. The pair of large submetacentric chromosomes found in *C. c. sevangi* and the European tetraploid *Barbus sensu stricto* species (Kryzanov 1999) is an apparent karyological synapomorphy for this clade. The absence of such chromosomes and the occurrence of 150 chromosomes (Table 3) in species of *Varicorhinus,* the occurrence of this genus in Africa, and the placement of *V. maroccanus* (Günther, 1902) in the same clade as the African hexaploids (Machordom & Doadrio 2001) support its taxonomic distinction from *Capoeta,* and suggest that it is related to *Labeobarbus*. Given the taxonomic interchanges between *Varicorhinus* and *Labeobarbus* (Banister & Clarke 1980; Tweddle & Skelton 1998), these two genera may even be found to represent a single clade, a possibility already suggested by the limited molecular phylogenetic data available (Machordom & Doadrio 2001). Comprehensive molecular phylogenetic studies of *Varicorhinus* are a priority for resolving these questions and

indicating which karyological character states are apomorphic.

Since hexaploidy may arise in a variety of ways (Oellermann & Skelton 1990; Leggat & Iwama 2003), a phylogenetic approach is needed to discover the mechanism underlying its evolution. A molecular approach to this problem has been used successfully in polyploid frogs (Evans *et al.* 2004) and a selection of barbine fish (Machordom & Doadrio 2001; Tsigenopoulos *et al.* 2002, 2003). A further step in this direction for barbine karyology would be to identify particular chromosome arms using banding patterns, chromosome painting or fluorescent *in situ* hybridization (FISH), and to use the information they yield about shared duplications, fusions, translocations and inversions in phylogenetic analyses.

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