

Karyology of the redbin minnows, genus *Pseudobarbus* Smith, 1841 (Teleostei: Cyprinidae): one of the evolutionarily tetraploid lineages of South African barbines

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Received 11 April 2003. Accepted 1 December 2005

The karyotypes of six species of *Pseudobarbus* Smith, 1841, namely *P. afer* (Peters, 1864), *P. asper* (Boulenger, 1911), *P. burchelli* Smith, 1841, *P. burgi* (Boulenger, 1911), *P. phlegethon* (Barnard, 1938) and *P. tenuis* (Barnard, 1938), were examined by conventional Giemsa staining and described. All six karyotypes have $2n = 100$ chromosomes, dominated by bi-armed chromosomes, as does the only other member of the genus, *P. quathlambae* (Barnard, 1938). Sex-related intraspecific karyotype variation was not found. The shared chromosome numbers and general similarity of the karyotypes (FN = 186–192) provide a new synapomorphy to support their monophyly, which is already indicated by anatomical and mtDNA markers. Karyotype evolution within the genus has been accompanied by chromosomal inversions and centromeric shifts. Comparison of the diploid number found in *Pseudobarbus* with other African barbine cyprinines, which have in the region of $2n = 50$ and lower FNs, suggests a tetraploid evolutionary origin of the genus, possibly by allotetraploidy.

Key words: *Pseudobarbus*, Barbinae, tetraploidy, cytogenetics, karyotype

INTRODUCTION

Cyprinids are a major component of the freshwater ichthyofauna of Africa (Skelton *et al.* 1991; Skelton 2001). Biogeographically, the southern African representatives have been grouped into temperate (Cape and/or Karoid) and tropical (Zambezi) elements (Skelton 2001). Cyprinids comprise about 80% of the temperate region's fish fauna, and the majority of these are barbine cyprinines (*sensu* Howes 1991), placed in the genera *Barbus* Cuvier & Cloquet, 1816, *Pseudobarbus* Smith, 1841, *Labeobarbus* Rüppel, 1836, and *Labeo* Cuvier, 1817 (Skelton *et al.* 1991; Skelton 1994, 2001). The available karyological studies showed that these African barbine lineages include three ancestral ploidy levels: diploid (e.g. Wolf *et al.* 1969; Ráb *et al.* 1995; Golubtsov & Krysanov 1993; Naran 1997); tetraploid (Naran 1997); and hexaploid (Oellermann & Skelton 1990; Golubtsov & Krysanov 1993; Guégan *et al.* 1995; Krysanov & Golubtsov 1996; Naran 1997; Berrebi & Ráb 1998).

The first data on the phylogenetic relationships, based on mtDNA markers (Machordom &

Doadrio 2001; Tsigonopoulos *et al.* 2002) suggest several conclusions. First, the genus of 'true' *Barbus s. str.* is tetraploid and is confined to the European region (Berrebi *et al.* 1996; Tsigonopoulos & Berrebi 2000); African species still assigned to the genus must be transferred to other genera. Second, the genus *Labeobarbus*, comprising a number of African and Middle Eastern evolutionarily hexaploid species, is monophyletic (Machordom & Doadrio 2001; Durand *et al.* 2002; Gorskova *et al.* 2002; Tsigonopoulos *et al.* 2002). Third, the endemic South African genus *Pseudobarbus* is monophyletic (Machordom & Doadrio 2001) and may be of evolutionarily tetraploid origin (Oellermann & Skelton 1990). Finally, several other southern African barbine species ('*B. andrewi*', '*B. serra*', '*B. erubescens*', '*B. calidus*' and '*B. trevelyani*') are also tetraploid, but do not form a monophyletic group, and several new genera should be erected for them (Tsigonopoulos *et al.* 2002).

The genus *Pseudobarbus* is a distinct lineage of seven species, endemic to temperate South Africa (Skelton 1988). The monophyly of the genus is strongly supported by morphological characters

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Table 1. Specimens of redfin minnows (*Pseudobarbus* spp.) used in this study. All material is now housed in the collection of the South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa.

Species	Male	Female	?	Voucher number	Locality	
<i>P. afer</i>	2	3	1	SAIAB 52709	Bezuidenhoutskraal	33°41'S 23°35'E
	2	1	0	SAIAB 52710	Blindekloof	33°41'S 25°18'E
<i>P. asper</i>	7	2	0	SAIAB 52712	Groot River	33°28'S 24°42'E
<i>P. burchelli</i>	3	1	1	SAIAB 52713	Bainskloof	33°33'S 19°09'E
	2	1	1	SAIAB 52714	Wolvekloof	33°34'S 19°08'E
<i>P. burgi</i>	3	1	0	SAIAB 52715	Wemmers River	33°50'S 19°07'E
<i>P. phlegethon</i>	2	2	1	SAIAB 52716	Thee River	33°47'S 19°07'E
<i>P. tenuis</i>	3	1	1	SAIAB 52717	Grobbelaars River	33°22'S 22°01'E

(their lack of predorsal supraneural bones, the presence of distinct nuptial tubercles on breeding males, distinctive pharyngeal teeth and gut morphology) and genetic traits (cytochrome *b* sequences). Six of the species are threatened (Skelton 1987) and many populations have been extirpated by bass or trout and by habitat destruction. Commonly referred to as 'redfin minnows' because the base of the fins in adults are bright scarlet, they are small (generally less than 150 mm SL), with a flexible, soft primary dorsal ray and radially striated scales (Skelton 1988).

The chromosomes of the various species of *Pseudobarbus* have not been investigated beyond a preliminary study of *P. quathlambae* by Oellermann (1988) and Oellermann & Skelton (1990) that indicated that the redfin karyotype might be ancestrally tetraploid. We report the karyotypes of the remaining six species.

MATERIALS & METHODS

Details of the species, locality, number and sex, and voucher numbers of examined specimens are listed in Table 1. Specimens were kept alive in aquaria until required. Voucher samples are housed in the collection of the South African Institute for Aquatic Biodiversity (SAIAB), formerly the J.L.B. Smith Institute for Ichthyology, Grahamstown, South Africa.

Chromosome preparation followed Collares-Pereira's protocol (Foresti *et al.* 1992). Briefly, the fish were stimulated into mitosis by a baking yeast injection 48 hours before examination, and held at 23–24°C. After two hours of colchicine treatment (0.01% wt/vol at 0.1 ml/g body weight, injected), the fish were overdosed with L-phenoxyethanol and kidney (and occasionally gill) 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 dripped onto microscope slides, air-dried, and stained with 4–6% Giemsa stain for five minutes. Meiotic figures were prepared in the same way from gonad cells.

Chromosomes were classified following Levan *et al.* (1964) and the fundamental number (FN) was calculated using the formula $FN = 2(m + sm + st) + a$, where *m* = metacentric, *sm* = submetacentric, *st* = subtelocentric, and *a* = acrocentric.

RESULTS

A typical karyogram is illustrated in Fig. 1. Chromosome counts from all six species (Table 2) showed a modal diploid chromosome number of $2n = 100$. Hypomodal and hypermodal counts (Table 2) were also observed, evidently caused by preparation artefacts (Collares-Pereira 1985; Gui *et al.* 1986; Ráb & Roth 1989). The karyotype compositions and FN values are summarized in Table 3. No heteromorphic elements indicating sex chromosomes were seen in either sex in all six species. Meiotic figures of males of all six species showed 50 bivalents only.

DISCUSSION

Based on an examination of the karyotype of *P. quathlambae*, Oellermann (1988) and Oellermann & Skelton (1990) suggested that all species of the genus *Pseudobarbus* have $2n = 100$, a speculation that is now validated. In cyprinids, species and/or groups with about 50 pairs of chromosomes are of evolutionarily tetraploid level. To date, taxa of this or higher ploidy level occur only among the barbina and schizothoracine cyprinids (Collares-Pereira 1994).

The six species examined have remarkably similar karyotypes, e.g. very similar proportions of chromosome morphologies (Table 3). Intraspecific

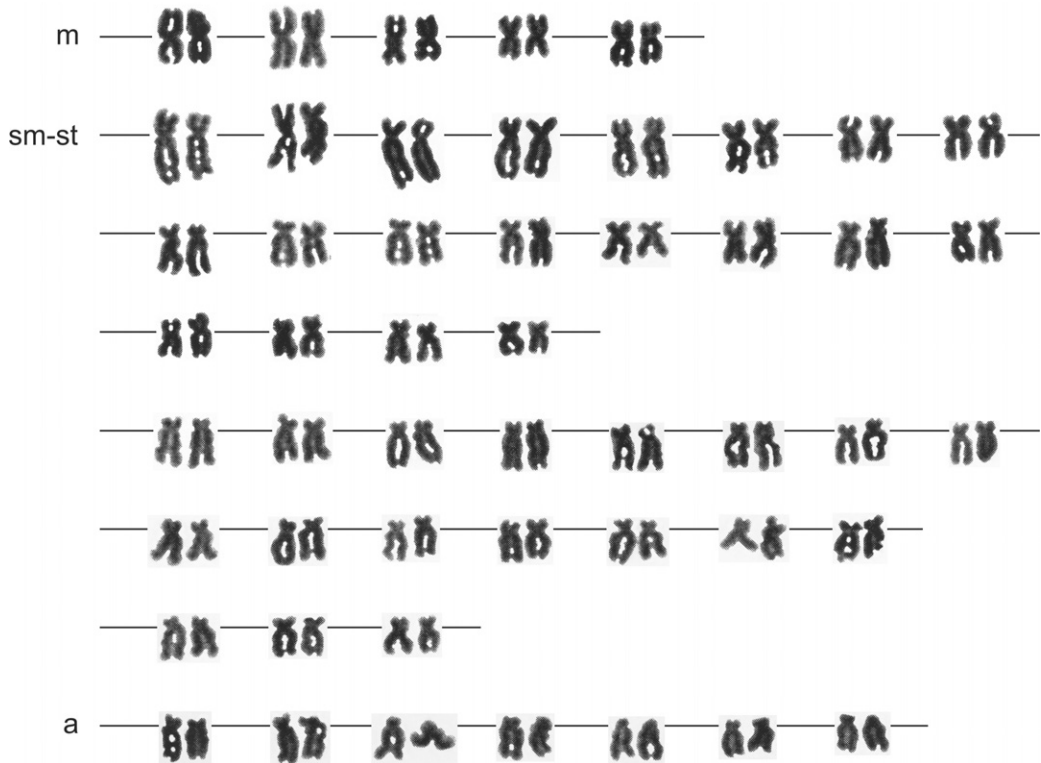


Fig. 1. Photokaryotype of a female *Pseudobarbus burchelli* (Wolvekloof). m = metacentric, sm+st = submetacentric/subtelocentric, a = acrocentric.

variation in karyotype morphology can be ascribed to ambiguities in classification due to borderline centromere positions caused by cell-to-cell variation in the extent of chromosome contraction, which is a general problem in the description of the relatively small chromosomes of cyprinids (Collares-Pereira 1985; Ráb & Roth 1989; Collares-Pereira & Ráb 1995). Undoubtedly, the types of rearrangement differentiating the karyotypes are

small inversions and centromeric shifts, as in other polyploid cyprinids. Oellermann used a different technique to prepare chromosome spreads of *P. quathlambae* that resulted in more chromosome condensation. The results cannot, therefore, be compared rigorously with those reported here, but it is probable that if the chromosomes of *P. quathlambae* had been less condensed, fewer of them would be scored as acrocentrics, and the

Table 2. Frequencies of chromosome counts from kidney cells of seven species of redfin barb (*Pseudobarbus* spp.). Bez = Bezuidenhoutskraal; BI = Blindekloof; B = Bainskloof; S = Wolvekloof.

Species	Cells sampled	Diploid chromosome number						
		90	92	94	96	98	100	102
<i>P. afer</i> (Bez)	40	–	–	1	2	6	30	1
(BI)	49	–	–	1	3	4	41	–
<i>P. asper</i>	110	–	5	5	8	16	72	4
<i>P. burchelli</i> (B)	75	–	–	1	10	18	46	–
(S)	36	4	1	1	4	5	21	–
<i>P. burgi</i>	38	1	1	1	6	8	20	1
<i>P. phlegethon</i>	50	3	3	2	6	8	28	–
<i>P. tenuis</i>	45	2	1	2	5	7	28	–

Table 3. Fundamental number (FN) and modal (and minimum and maximum) number of chromosomes in each morphological category for the seven redfin barbids (*Pseudobarbus* spp.). m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric.

Species	n (cells)	FN	Morphological categories		
			m	sm + st	a
<i>P. afer</i> (Bez)	20	190	12 (10–14)	40 + 38 (40–42; 38–38)	10 (8–10)
(Bl)	40	190	12 (10–14)	42 + 36 (36–44; 34–40)	10 (8–14)
<i>P. asper</i>	24	192	14 (10–14)	46 + 32 (32–48; 32–34)	8 (8–12)
<i>P. burchelli</i> (B)	28	186	10 (8–12)	42 + 34 (38–42; 32–36)	14 (10–18)
(S)	19	186	10 (8–14)	40 + 36 (36–46; 32–38)	14 (10–16)
<i>P. burgi</i>	12	188	14 (8–14)	36 + 38 (32–36; 38–48)	12 (10–14)
<i>P. phlegethon</i>	19	192	14 (10–14)	40 + 38 (38–48; 30–42)	8 (8–10)
<i>P. tenuis</i>	17	186	14 (12–14)	40 + 32 (34–42; 32–36)	14 (12–16)

karyotype would then differ little from those reported here.

Except for *Pseudobarbus afer*, the species of this genus are confined to a single drainage system or neighbouring drainages (Skelton 2001). To investigate intraspecific geographical variation, two populations each of *P. afer* and *P. burchelli* were examined. Only small differences were found (Table 3), probably due to the misclassification of a few chromosomes caused by minor differences in their state of contraction in different cells (Collares-Pereira & Ráb 1995). There is thus no compelling macro-karyological evidence of geographical differentiation in these two *Pseudobarbus* species, a pattern also found in almost all other tetraploid cyprinines investigated so far (Collares-Pereira 1994).

There were no heteromorphic chromosomes in the karyotypes of either sex. However, the presence of sex-determining systems among cyprinids is not well understood (Devlin & Nagahama 2002), forestalling further speculation.

Pseudobarbus is a monophyletic clade (Machordom & Doadrio 2001; Tsigenopoulos *et al.* 2002) characterized by tetraploidy, but whether this condition arose by auto- or allopolyploidy is not clear. No tetravalents (or even a proportion of them), which would indicate autotetraploidy, were found in meiotic figures. It has been suggested that, with sufficient time for mutational divergence, tetraploid genomes undergo functional diploidization (Ohno 1970; Allendorf & Thorngard 1985). If autotetraploidy occurred in the ancestor of the *Pseudobarbus* lineage, then both copies of the duplicated genome could undergo independent chromosomal rearrangements and mutation so that tetravalent figures no longer form. The

absence of tetravalents therefore suggests that the polyploidy event was either historically remote or involved hybridization and allopolyploidy. Chenuil *et al.* (1999) suggested that allopolyploidy had occurred in the tetraploid members of *Barbus s. str.* None of the karyotypes presented here could be arranged into morphologically homomorphic quadruplets, and other approaches such as fluorescent *in situ* hybridization (FISH) will be needed to explore this problem further.

ACKNOWLEDGEMENTS

We thank Anton Bok and Wayne Haselau (both formerly of East Cape Nature Conservation) and Stuart Thorne (Western Cape Nature Conservation) for providing fish and permits to collect fish; Rob Cross and Neill Canon (both formerly of the Rhodes University Electron Microscopy Unit) for help in making the photokaryotypes; John Keulder (formerly of Rhodes University) for preparing bromides; Roger Bills (SAIAB) and two anonymous reviewers for constructive comments on the manuscript; and the National Research Foundation for funding.

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