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A new redfin species, *Pseudobarbus skeltoni* (Cyprinidae, Teleostei), from the Cape Floristic Region, South Africa

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Abstract

A new cyprinid species, *Pseudobarbus skeltoni* sp. nov, is described from material recently collected in the upper Riviersonderend River (a major tributary of the Breede River system) and the Krom River (a tributary of the Molenaars River in the upper Breede River) in the Cape Floristic Region of South Africa. The new species is readily distinguished from congeners, except *P. burgi* and *P. burchelli*, by having two pairs of prominent oral barbels. *Pseudobarbus skeltoni* can be distinguished from *P. burgi* and *P. burchelli* by the following combination of characters: distinctive terminal (*vs.* subterminal) mouth in adults; mouth inferior in sub-adults and young adults of *P. skeltoni* but lower lips are unretracted (*vs.* retracted) and lack a distinct cartilaginous plate; snout prominent; more slender head (head depth 64.2% of HL, *vs.* 70.1% of HL in *P. burchelli* and 74.1% of HL in *P. burgi*); and a longer head relative to standard length (30.5 % *vs.* 26.8% in *P. burchelli* and 25.8% in *P. burgi*). The new species attains the largest size of any *Pseudobarbus*. The restricted distribution and the small remaining population sizes of *P. skeltoni* indicate that this species is highly threatened and requires immediate conservation attention.

Key words: Breede River system, endemic, cytochrome *b*, morphology

Introduction

There are a number of cyprinid minnows belonging to the genera *Barbus* and *Pseudobarbus* in southern Africa that have bright red fins. Fishes of the genus *Pseudobarbus*, currently represented by seven species (reviewed by Skelton, 1988), all have redfins and a soft or flexible primary dorsal spine. They occur in rivers associated with the Cape Floristic Region (CFR) of South Africa, with one species endemic to the highlands of Lesotho.Two of the species have two pairs of barbels, whilst the other five species have a single pair of barbels, apart from occasional rudimentary secondary barbs in *P. tenuis* (Skelton 1988).

The double barbed redfins occur in the Verlorenvlei, Berg, Heuningnes, Breede, Duiwenhoks and Goukou river systems in the western and south-western CFR. The first species of *Pseudobarbus* was described by Smith (1841) as *Barbus burchelli*, but the origin of the specimens used for this description is unknown, and there is no known type material (Barnard 1943). The illustration that accompanied the species description, however, clearly shows that the species had red fins and two pairs of barbels. Castelnau described *Gnathendalia vulnerata* in 1861 and Steindachner (1870) described *Barbus multimaculatus* from the Breede River system. Both descriptions were of species with two pairs of barbels. Valenciennes in Cuvier and Valenciennes (1842) described *Barbus gobionides* that was placed in synonymy with *Gnathendalia vulnerata* by Günther in 1868. However, Barnard (1943) declared that *Barbus gobionides* was a *nomen dubium*. Boulenger (1911) placed *Barbus multimaculatus* in synonymy with *Gnathendalia vulnerata* in 1905, a decision that was accepted by Barnard (1943), Jubb (1965) and Skelton (1988).

Boulenger (1911) also described a redfin species with two pairs of barbels from the Berg River system and called it *Barbus burgi*. Barnard (1943), working with a better understanding of the distribution of the species, had to decide whether the Breede or Berg species should be placed in synonymy with Smith's *Barbus* (*Pseudobarbus*)

burchelli. He decided to place *Barbus burgi* in synonymy with *Barbus burchelli* and therefore recognised *Barbus vulneratus* for the Breede River system. Jubb (1965), however, reversed this decision and placed *Gnathendalia vulnerata* in synonymy with Smith's *Barbus* (*Pseudobarbus*) *burchelli*. According to Jubb (1965), this was done after P. H. Greenwood examined the skins of Castelnau's types of *Gnathendalia vulnerata*, which he compared to Smith's description of *Barbus* (*Pseudobarbus*) *burchelli* and concluded that they should be placed in synonymy. However, Jubb (1965) gave no justification why *Barbus burgi* does not also agree with Smith's description and why Barnard's (1943) arrangement was not kept. When Skelton (1988) defined a monophyletic redfin genus, he raised Smith's (1841) subgenus name to a full generic name and accepted Jubb's (1965) nomenclatural changes to maintain taxonomic stability. A specimen from the Tradou catchment was assigned as neotype material for *P. burchelli*.

Thus, *P. burchelli* and *P. burgi* are the two currently recognised species of the double barbed redfins with a flexible primary dorsal spine in the CFR. The current distribution of *P. burchelli* is considered to encompass four isolated river systems (the Breede, Heuningnes, Duiwenhoks and Goukou) on the south coast of South Africa, while *P. burgi* occurs in two river systems (the Berg and Verlorenvlei) on the west coast (Skelton 1988). Broad intraspecific morphological variation between populations of these species has been documented. Skelton (1988) reported that Verlorenvlei specimens of *P. burgi* had a much longer gut than specimens from the Berg River system. Skelton (1988) also reported variation in gut length and a range of exceptional lip development in *P. burchelli*.

Molecular studies have uncovered hidden diversity in both *P. burgi* and *P. burchelli*. Bloomer and Impson (2000) found deep genetic divergence in *P. burgi* between the Berg and Verlorenvlei River systems. Three historically isolated genetic lineages of *P. burchelli* have been identified in a previous study (Swartz *et al.* 2009; Swartz *et al.* submitted): a widespread 'Breede' lineage from the Breede, Duiwenhoks and Goukou river systems, a restricted 'Tradou' lineage that is only found in the Tradou catchment of the Breede River system and a restricted 'Heuningnes' lineage that is only found in the Heuningnes River (Swartz *et al.* 2009; Swartz *et al.* submitted). Genetic analyses revealed that specimens collected during a recent survey in 2009 from the Riviersonderend River (a major tributary of the Breede River) and the Krom River (a tributary of the Molenaars River) do not belong to any of these lineages although they are geographically located within the distribution of the widespread 'Breede' lineage of *P. burchelli*. Morphological comparison of the newly collected material presents several consistent differences with descriptions of *P. burchelli* and *P. burgi*, indicating existence of a previously unrecognised *Pseudobarbus* species in the CFR. The formal description of this species (nicknamed the Giant redfin) is presented under the designation *Pseudobarbus skeltoni* **sp. nov.**

Material and methods

Sample collection. Specimens were collected during comprehensive surveys in the rivers of the south-western CFR during low flow conditions between November 2008 and December 2009. A combination of sampling methods was used: electric fishing, seine nets, fyke nets, gill nets or snorkelling with a hand net; depending on habitat, depth and water clarity and conductivity. Fishes were anaesthetised using clove oil (0.2 ‰). Small pieces of muscle tissue were placed in 95% ethanol in the field, and then transferred to -20°C in the laboratory. Source specimens were preserved in 10% formalin in the field and were transferred to 70% ethanol in the laboratory for storage. The research was conducted under permit from CapeNature (permit number: AAA-004-000205-0035). The permit is issued only after the approval of methods by a review panel.

Molecular analyses. Detailed methods for DNA extraction, amplification, sequencing and analyses of a fragment of the mitochondrial cytochrome *b* gene are presented in Swartz *et al.* (2009). A total of 165 sequences of *P. burchelli* (41 from Swartz *et al.* (submitted) and a further 124 generated by Chakona *et al.* (in preparation)) collected from across the species' range were compared to 12 sequences of *Pseudobarbus skeltoni* **sp. nov.** Phylogenetic distance analyses (not shown) were done to identify unique lineages as in Swartz *et al.* (submitted). Representatives of the *P. burchelli* lineages were then compared to all other *Pseudobarbus* lineages using a Bayesian analyses performed in MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001) similar to the analysis performed in (Swartz *et al.* 2009), but based only on cytochrome *b* data (GenBank number EU341714 - EU341754). *Barbus calidus* ((Genbank number AF180846.1) and *B. erubescens* (Genbank number AF180845.1), both serrated tetraploid barbs with red fins and known to be part a sister lineage to *Pseudobarbus* (Tsigenopoulos *et*

al. 2002), were used as outgroups. Bayesian posterior probabilities and branch lengths were estimated using the generalised time reversible model of DNA substitution with gamma-distributed rate variation across sites. One cold and three heated Monte Carlo Markov chains were run for one million generations. The log-likelihood scores were plotted to establish when the runs became stable, which occurred before 5,000 generations. The first 10,000 generations were therefore discarded as burn-in to ensure that only optimal trees were being sampled. Optimal trees were sampled every 100 generations, yielding 10,000 trees on which posterior probabilities and branch lengths were based.

Morphological analyses. Morphometric and meristic characters follow Hubbs and Lagler (1958) and Skelton (1988) unless otherwise specified. Morphological measurements were taken using digital calipers and recorded to the nearest 0.1 mm. Standard length was measured from the premaxillary symphysis (anterior tip of the snout) to the point of flexure of the caudal fin. Predorsal length was measured from the tip of the snout to the base of the primary dorsal fin ray. Prepectoral length is distance from the anterior tip of the snout to the base of the first pectoral fin ray. Prepelvic length is the distance from the tip of the snout to the anterior base of the first pelvic fin ray. Preanal length extends from the anterior tip of the snout to the base of the first anal fin ray. Fin height was measured from the anterior base of the fin to the distal tip of the longest ray.

External counts (scales and fin rays) were made under a dissecting microscope. The predorsal scale count is the number of scale rows between the posterior margin of the head at the nape and the anterior base of the first dorsal spine. The lateral-line-to-pelvic and lateral-line-to-anal scale count records the number of scale rows between the lateral line scale row and the base of the respective fin. Single scales interposed between regular rows were excluded. Postcranial meristics (i.e., predorsal vertebrae, abdominal/precaudal vertebrae, preanal vertebrae and caudal vertebrae) were taken from radiographs following criteria described by Skelton (1988). The total vertebrae include the four Weberian vertebrae and a single ural centrum. Predorsal and preanal vertebrae include the four Weberian vertebrae and all vertebrae in advance of the respective leading pterygiophore. Precaudal vertebrae include the four Weberian vertebrae and all vertebrae in advance of the first caudal vertebrae (i.e. the vertebrae opposite the leading anal pterygiophore). The caudal vertebrae include a single ural centrum and all vertebrae before the last precaudal vertebrae. The entire branchial basket including pharyngeal bones was dissected out to from two specimens (male and female) to count gill rakers and pharyngeal teeth.

Counts and measurements from the present study were compared with data for *Pseudobarbus burchelli* (n = 143) and *P. burgi* (n = 108) using raw data from Skelton (1980, 1988). The allometric effects of body size on morphological measurements were removed using the normalisation procedure described by Lleonart *et al*. (2000). The normalised values (Z_{ij}) for each individual were computed using the following equation:

$$Z_{ij} = Y_{ij} \left[\frac{X_0}{X_j} \right]^{b_i}$$

where: Y_{ij} is the i variable of individual j, X_0 represents a reference value of size of the independent variable (e.g. standard length) to which all individuals are increased or reduced, X_j is the value of the independent variable (e.g. standard length) for individual j and b_i is the parameter of the allometric ratio between the independent variable and the dependent variable i (e.g. predorsal length) (see Lleonart *et al*. 2000). The parameter b_i was estimated using the equation: $\ln Y = \ln a + b \ln X + \varepsilon$ (Lleonart *et al*. 2000). This is a linear equation with an additive error. The value of b_i was thus estimated using standard linear regression of the logarithmically transformed X and Y.

This normalisation approach completely removes all effects of size due to allometric growth and adjusts the shape of the individuals to the new size (Lleonart *et al*. 2000). Measurements on the head were normalised with head length as the independent variable. The X_0 used was 17 mm, which was the average head length of all individuals ($n = 245$). Measurements on the body were normalised with standard length as the independent variable. The X_0 used was 62 mm, which was the average standard length for all individuals ($n = 245$). Body size

was not considered for the counts because meristic characters were not significantly correlated with standard length (results not shown).

Principal component analysis (PCA) was performed using the correlation matrix to explore the separation of the specimens based on the normalised morphometric data (Lleonart *et al*., 2000) and raw meristic characters (excluding those that are invariant such as the number of anal and pelvic fin rays). Because the normalisation approach completely removes all information related to size (Lleonart *et al*. 2000), the scores on all axes (including PC1) were considered. Statistical analyses were done using PAST.

Institutional abbreviations follow Eschmeyer (2010). Other abbreviations are AMGP—Albany Museum, Grahamstown (fish collection); SAIAB—South African Institute for Aquatic Biodiversity.
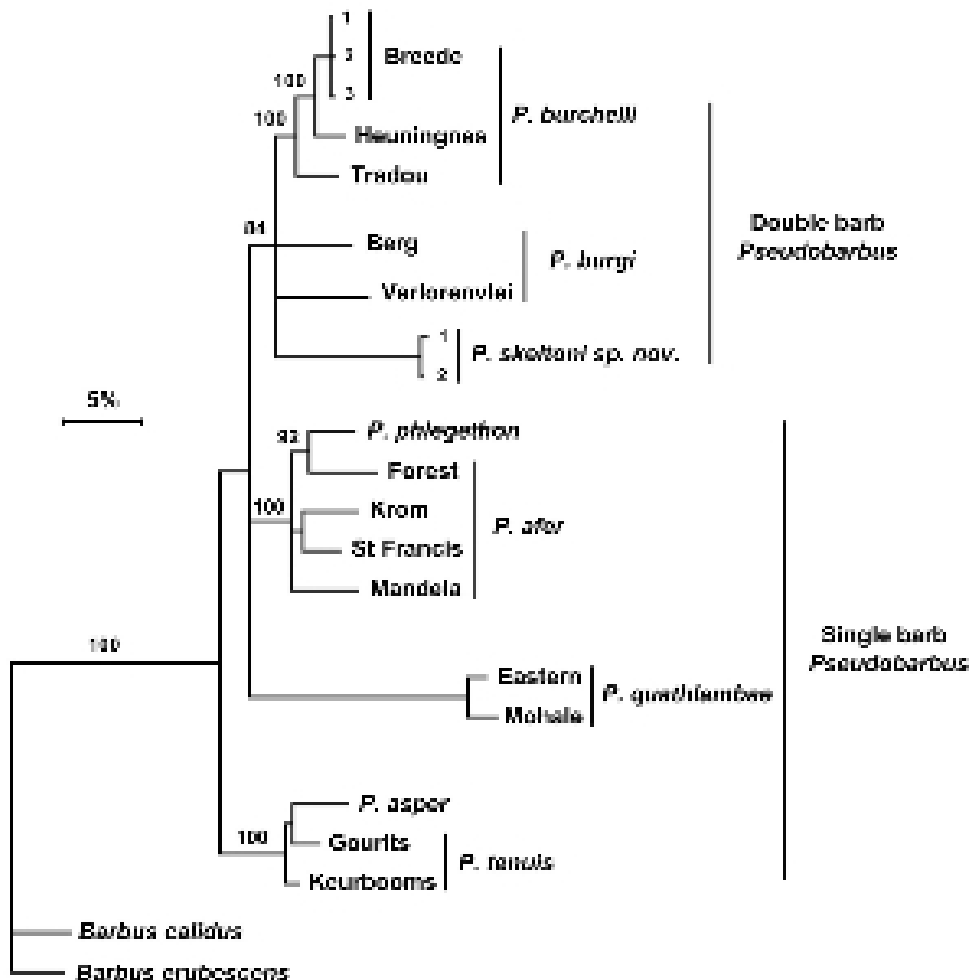

FIGURE 1. Bayesian phylogram with Bayesian posterior probabilities, showing the genetic distance between *Pseudobarbus skeltoni* **sp. nov.** compared to all other *Pseudobarbus* lineages. Although phylogenetic relationships among *Pseudobarbus* lineages should be based on Swartz *et al*. (2009) due to more data that were analysed, it is clear from the present study that *P. skeltoni* is closely related to *Pseudobarbus* lineages that have two distinct pairs of barbels. More genes will have to be analysed to resolve the phylogenetic relationships within this group.

Results

Molecular data. Bayesian and distance phylogenetic analyses confirmed the existence of six divergent *Pseudobarbus* lineages that have two pairs of barbels (Fig.1). These lineages correspond to the newly discovered *P. skeltoni* **sp. nov.** that is distinct from two previously recognised lineages of *P. burgi* (Bloomer & Impson 2000; Swartz *et al*. 2009) and three previously recognised lineages of *P. burchelli* (Swartz *et al*. 2009). The monophyly of these double-barbed redfins is strongly supported (Bayesian posterior probability = 1.00). Deep phylogenetic divergences (2.58–9.99%) were found among lineages collected from the Breede, Duiwenhoks, Goukou and Heuningnes River systems, while minor genetic divergences were found within lineages (0.30–0.71%).

Morphological data. Principal components analysis (PCA) of normalized morphometric and raw meristic characters shows *P. skeltoni* **sp. nov.** well separated from *P. burchelli* and *P. burgi* (Fig. 2). The first three linear contrasts of morphometric and meristic characters explained 54.7% of the observed variation, with the first PCA axis (6.77 eigenvalue, 32.3% of total variation) primarily contrasting differences in head length, head depth, predorsal length, number of lateral line scales, number of scale rows between lateral line and dorsal fin, and scale size as reflected by the number of scale rows around the caudal peduncle and the number of predorsal scale rows (Table 1). The second PCA axis (2.63 eigenvalue), primarily contrasting differences in the length of the anterior barbel, explained 12.5% of total variation. Caudal peduncle depth was found to load heavily on the third PCA axis (2.08 eigenvalue, 9.9% of total variation) (Table 1). Specimens of *P. skeltoni* were associated negatively with PCI, describing individuals characterised by relatively longer heads, relatively longer predorsal distance, a higher number of scales along the lateral line, generally a higher number of scale rows between lateral line and dorsal fin and a higher number of circumpeduncular scale rows. Specimens of *P. burgi* were associated positively with PCI, describing individuals with deeper heads relative to head length.
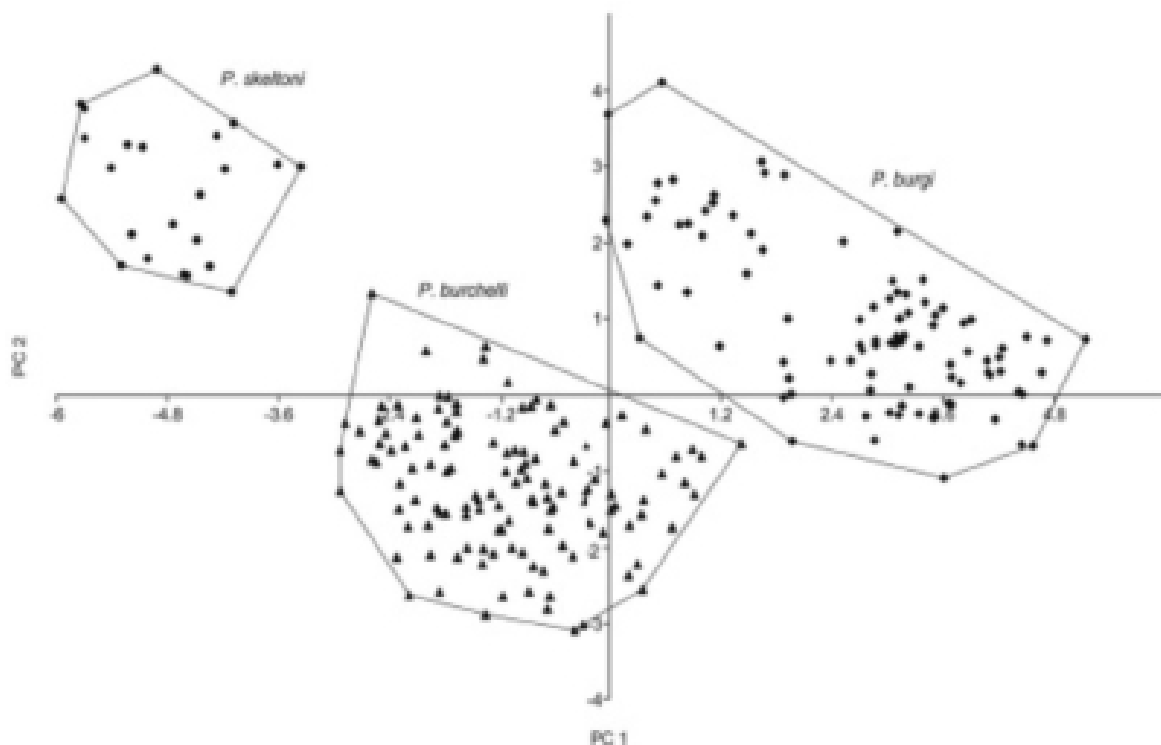

FIGURE 2. Scatter plot of PCI against PCII for a PCA carried out on 14 normalised morphometric and seven raw meristic characters for all examined specimens (*n* = 245). The cluster of *P. skeltoni* sp. nov include 12 recently collected specimens from the Riviersonderend and Krom Rivers as well as 11 specimens from two samples collected in the Wit River (see text for details).

TABLE 1. Factor loadings for the first three principal component (PC) axes of a PCA carried out on 14 morphometric and seven meristics of all examined specimens ($n = 245$).

	PCI	PCII	PCIII
Head length	-0.7308	0.4315	-0.1570
Head depth	0.7002	0.0915	-0.3191
Inter obit	0.4183	-0.0724	-0.4357
Post orbit	0.2937	0.1324	0.3014
Predorsal length	-0.7267	0.1059	-0.1941
Dorsal fin base	0.4887	-0.2048	-0.2863
Dorsal fin height	0.4456	0.2522	-0.2619
Body depth	0.3466	0.5083	-0.6212
Body width	-0.4436	-0.1907	-0.5953
Caudal peduncle length	0.4275	-0.4921	-0.1013
Caudal peduncle depth	-0.2985	-0.2478	-0.7639
Anterior barbel	-0.5477	-0.7401	-0.0645
Posterior barbel	-0.3008	-0.6053	-0.2070
Pelvic to anal fin length	0.4181	0.5015	0.0506
Dorsal unbranched rays	-0.4561	-0.1813	0.2064
Lateral line scales	-0.7469	0.0117	0.0780
Scale rows between lateral line and dorsal fin	-0.8432	0.1967	0.0219
Scale rows between lateral line and pelvic fin	-0.5608	0.5248	-0.1723
Scale rows between lateral line and anal fin	-0.6451	0.2319	-0.0537
Scale rows around caudal peduncle	-0.7291	0.3115	-0.0994
Predorsal scale rows	-0.7942	-0.1875	0.0845

Pseudobarbus skeltoni **sp. nov.**
(Figure 3; Table 2)

Type material. Holotype: South Africa: Western Cape Province: SAIAB 187243, mature male, 158.2 mm standard length (SL), collected on 5 December 2009 by A. Chakona and J. Merron using fyke nets at the pool-riffle interphase at the foot bridge just below gauging weir in the Riviersonderend River upstream of Theewaterskloof Dam, Breede River system, at altitude 327 m (34°03' 44.3" S, 19°04' 13.8" E).

Paratypes: South Africa: Western Cape Province: SAIAB 187244, BMNH 2012.9.17.1, USNM408375, same data as for holotype; SAIAB 187241, collected on 3 December 2009 by A. Chakona, J. Merron and M. Jordaan using seine and hand net in a deep pool (33°43' 22" S, 19°06' 46.6" E), Krom River, tributary of the Molenaars River, Breede River system; SAIAB187242, collected 200 m upstream of locality for SAIAB 187241, same date and collectors; SAIAB 122627, collected from the Wit River (Bainskloof), Breede River system by P.H. Skelton and P.H. Greenwood on 14 October 1975; SAIAB 187238 collected from the Wit River (Bainskloof), Breede River system by Farquharson. Hologenetype (GenBank number KF222578) and paragenetype sequences (GenBank numbers KF222577, KF222579–KF222588) have been deposited in GenBank following the definitions of Chakrabarty (2010).

Diagnosis. *Pseudobarbus skeltoni* **sp. nov.** reaches the largest size of any *Pseudobarbus* species and is distinguished from its congeners, except *P. burchelli* and *P. burgi*, by possessing two pairs of prominent oral barbels. The new species is distinguished from *P. burchelli* and *P. burgi* by the combination of the following features: distinctive terminal mouth in adults (versus subterminal in all size classes of *P. burchelli* and *P. burgi*) (Fig. 4); juveniles, sub-adults and young adults of *P. skeltoni* have subterminal mouths (as in *P. burchelli* and *P. burgi*), but their lower lips are unretracted and they lack the cartilaginous plate found in both *P. burchelli* and *P.*

burgi (Fig. 5); a typically longer head relative to standard length (30.5 % *vs.* 26.8% in *burchelli* and 25.8% in *burgi*); shallow head depth (64.2% of HL compared to 70.1% of HL in *P. burchelli* and 74.1% of HL in *P. burgi*); mature sexually active males develop two clusters of large conical tubercles on the snout, with 22 - 30 tubercles in each cluster (versus 5–10 tubercles per cluster in *P. burchelli* and 10–15 tubercles per cluster in *P. burgi*; multiple rows of large conspicuous tubercles above each nare and antero-dorsal edge of each orbit (*vs.* single row in congeners) (Fig. 4). Comparisons among the species are given in Table 2.

Description. Proportional measurements and meristics are summarised in Table 2. Body fusiform, dorsal surface slightly convex and ventral surface flattened from anterior base of pelvic fins to tip of lower lip; head relatively large and slightly depressed; head length greater than body depth. Adults have distinct terminal mouths; juveniles, sub-adults and young adults have inferior mouths, but lower lip unretracted and lack cartilaginous plate (Fig.5C). Two pairs of simple and well-developed barbels, both pairs are longer than the orbit diameter in adults. Eyes relatively small, located dorsolaterally, closer to tip of snout than to the caudal margin of gill cover, interorbital profile flat, and snout prominent.
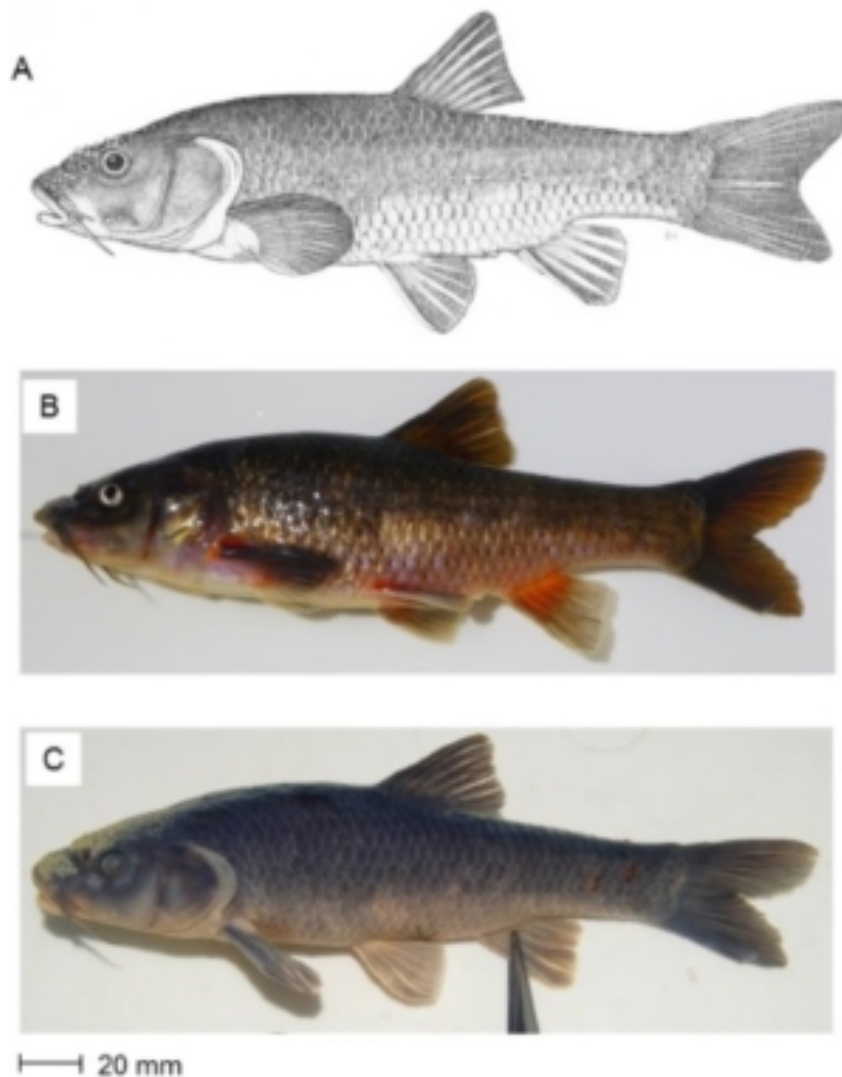

FIGURE 3. *Pseudobarbus skeltoni* **sp. nov.** (holotype, SAIAB 187243, 158.2 mm SL): A. illustration by Elain Heemstra of SAIAB; B. lateral view showing live colouration; and C. lateral view showing colouration of alcohol preserved specimen.

TABLE 2. Comparisons of the morphometric measurements and meristic counts of three species of *Pseudobarbus* with two pairs of barbels. For morphometric characters, the range is given first, with the mean and standard deviation in parentheses. For the meristic characters, the range is given first, with the mode in parentheses. For pharyngeal teeth, the teeth pattern is given and the number in parentheses refers to the number of specimens examined.

	P. skeltoni	*P. burchelli*	*P. burgi*
No. of specimens	23	129	93
Standard length (SL) (mm)	28.5–163.4	30.0–151.7	34.0–109.0
Head length (HL) (mm)	8.6–51.7	8.1–45.8	10.0–25.0
Percentage of SL (%)			
Head length	28.5–35.6 (30.5; 1.5)	23.9–30.2 (26.8; 1.0)	22.9–29.4 (25.8; 1.5)
Predorsal length	50.4–56.6 (53.3; 1.7)	45.6–55.3 (51.2; 1.4)	44.6–53.4 (48.8; 2.1)
Dorsal fin base	10.9–14.0 (12.0; 0.8)	11.8–15.2 (13.4; 0.7)	11.7–15.4 (13.7–0.8)
Dorsal fin height	17.9–23.9 (21.2; 1.8)	19.2–25.9 (22.9; 1.4)	16.1–28.4 (24.6; 2.5)
Body depth	22.8–28.8 (25.6; 1.3)	21.1–31.4 (25.6; 1.6)	20.9–32.0 (27.6; 1.9)
Body width	14.8–21.9 (17.9; 1.5)	13.7–21.6 (17.1; 1.6)	9.8–18.6 (15.3; 1.8)
Caudal peduncle length	20.8–24.5 (22.4; 0.8)	22.2–28 (25.0; 1.2)	22.5–27.7 (25.1; 1.3)
Percentage of HL (%)			
Head depth	55.9–69.7 (64.2; 3.1)	61.7–76.8 (70.1; 2.6)	67.7–79.3 (74.1; 2.4)
Inter–obit	23.9–32.3 (28.1; 2.1)	25.4–36.5 (31.3; 2.1)	27.8–40.0 (33.5;2.3)
Snout length	32.7–41.7 (38.0; 2.2)	30.9–41.5 (36.5; 1.9)	30.0–42.1 (35.5;3.1)
Post orbit	39.4–47.4 (45.4; 1.8)	40.6–50.7 (45.2; 1.9)	38.7–52.2 (46.8; 2.1)
Anterior barbel length	1.2–33.3 (20.3; 9.5)	5.1–27.2 (16.5; 4.3)	0.9–9.1 (4.5; 1.9)
Posterior barbel length	1.2–43.8 (27.5; 11.9)	14.3–48.0 (28.4; 5.6)	7.0–29.2 (19.9; 3.7)
Orbit diameter	16.4–31.4 (21.5; 4.4)	19.2–34.9 (27.7; 2.8)	22.4–35.4 (29.3; 3.1)
Percentage of caudal peduncle length (%)			
Caudal peduncle depth	46.7–61.3 (53.4; 3.6)	42.6–61.0 (49.4; 3.5)	36.2–57.1 (46.9; 4.0)
Dorsal fin rays	iii–iv (iv), 7–8 (7)	iii–iv (iv), 6–8 (7)	iii–iv (iii), 6–8 (7)
Anal fin rays	iii, 5	iii–iv (iii), 4–6 (5)	ii–iv (iii), 5–6 (5)
Pectoral fin rays	13–14	12–16 (14)	13–18 (14–15)
Pelvic fin rays	8	7–8 (8)	7–9 (8)
Lateral line scales	36–39 (38)	29–39 (36)	28–37 (30–34)
Scale rows between lateral line and dorsal fin	6–7 (7)	5–7 (6)	4–6 (5)
Scale rows between lateral line and pelvic fin	5–7 (5 – 6)	4–6 (4)	3–5 (4)
Scale rows between lateral line and anal fin	4–6 (5)	4–6 (4)	3–5 (4)
Caudal peduncle scale rows	15–18 (16–17)	12–18 (12)	12–16 (12)
Predorsal scale rows	17–21 (18–19)	14–22 (17–19)	12–18 (15)
Total vertebrae	36–38 (37)*	34–38 (36–37)	35–39 (36–37)
Precaudal vertebrae	19–21 (20)*	17–20 (18–19)	17–20 (19)
Caudal vertebrae	16–18 (17)*	15–20 (17–18)	16–20 (18)
Predorsal vertebrae	12*	11–13 (12)	10–12 (11–12)
Preanal vertebrae	20–22 (21)*	18–22 (19–20)	19–21 (20)
Pharyngeal teeth	2,3,5-5,3,2 (2)	2,3,5-5,3,2 (61)	2,3,5-5,3,2 (30)

*Twelve individuals of *P. skeltoni* were examined for vertebral counts.

FIGURE 4. Lateral view of the heads of (a) *P. skeltoni* **sp. nov.** (holotype, SAIAB 187243, 158.2 mm SL), (b) *P. burchelli* (neotype, SAIAB 126387, 95.9 mm SL) and (c) *P. burgi* (PG2076, 93.5 mm SL). Note the position of mouth, and a large eye relative to head length in *P. burchelli* and *P. burgi*. Also note the clear differences in tubercle expression of mature males of the three species.
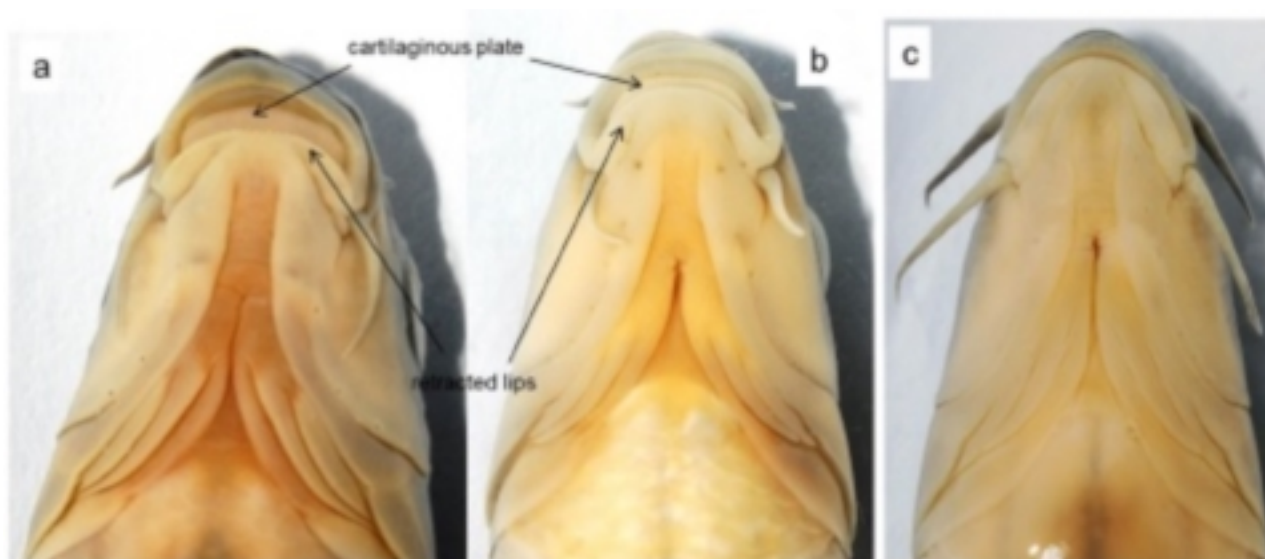

FIGURE 5. Ventral view showing the difference in the mouth forms of (A) *P. burchelli* (specimen from RUSI 058390), (B) *P. burgi* (specimen from SAIAB 186124) and (C) *P. skeltoni* **sp. nov.** subadult (specimen from SAIAB 187241). Both *P. burgi* and *P. burchelli* have retracted lips exposing a cartilaginous plate, but lips are unretracted in *P. skeltoni* and cartilaginous plate is absent (based on seven specimens examined).

Mature breeding males develop large prominent conical tubercles on the snout and top of the head. Two clusters of tubercles develop on the snout, with 22 to 30 tubercles in each cluster in large individuals. The tubercles measure up to 2.2 mm basal diameter. Multiple rows of tubercles develop above each nare, with 6 to 16 tubercles per cluster. Multiple rows of tubercles also develop above the antero-dorsal edge of each orbit, with up to 12 tubercles per cluster. A cluster of smaller tubercles develop on the posterior end of the head. Numerous minute tubercles form on scales between the nape and the dorsal fin and also on some scales between the dorsal and caudal fins. Tubercles do not form on body scales (apart from the ones mentioned above). Rows of tubercles develop on the primary dorsal ray and pectoral fin rays. Only a few minute tubercles occur in sexually active females (mainly on the snout).

Distal margin of dorsal fin slightly concave with 3 or 4 simple and 7 branched rays, anterior base of dorsal fin is inserted slightly posterior to the origin of pelvic fins. Anterior base of dorsal fin generally closer to base of caudal fin than tip of snout. Fins larger in males than females. Pectoral fin fan-shaped, with 13 or 14 rays, shorter than head length, almost reaching anterior base of pelvic fin in males, but only reaching slightly beyond halfway to anterior base of pelvic fin in females. Posterior edge of pelvic fin gently rounded with 8 rays, shorter than head length, reaching the anus in males and reach within two scale rows from the anus in females. Distal margin of anal fin almost straight, with three simple and five branched rays, origin closer to anterior base of pelvic fin than caudal fin base. Anus situated near the base of the anal fin. Caudal fin is forked, length shorter than head length.

Scales moderately large; lateral line complete, perforating 36–39 scales; 6 or 7 scale rows between lateral line and anterior base of dorsal fin; 5 or 7 scale rows between lateral line and anterior base of pelvic fin; and 4–6 scale rows between lateral line and anterior base of anal fin. Predorsal scales 17–21, smaller than flank scales, embedded in skin, with numerous small tubercles extending from nape to anterior base of dorsal fin. Fifteen to 18 circumpedunculer scales (mode 16 or 17). Naked patch between head and posterior base of pectoral fins, scales between posterior end of pectoral fins and anterior end of pelvic fins reduced and embedded. Scales between nape and anterior base of dorsal fin embedded. Axillary scales of pelvic fin not prominent or elongate.

Thirty-six to 38 vertebrae (mode 37), 19–21 precaudal vertebrae (mode 20), 16–18 caudal vertebrae (mode 17), 12 predorsal vertebrae and 20–22 preanal vertebrae (mode 21). Gill rakers sparse and conspicuous (7–9 on leading arch; observed in two individuals). Three rows of pharyngeal teeth, teeth pattern 2, 3, 5-5, 3, 2 (observed in 2 individuals); teeth are asymmetrical and hooked at their tips.

Colouration. Refer to Figure 3 for general live colouration. In fresh specimens, body olive to golden-tan dorsally and laterally, becoming lighter ventrally. Base of fins yellowish-orange with red patches in some specimens. Juveniles and subadults have well marked spots above lateral line, but spots are absent in adults. Alcohol preserved specimens are dark greyish dorsally and laterally and ash grey or whitish ventrally. Black spots present above lateral line in juveniles and sub-adults, but spots are absent in adults.

Reproduction. Unknown, but spawning possibly occurs around November-December, based on the observation that the males had large tubercles. Development of tubercles in redfins has been found to coincide with reproductive season (Skelton 1988; Cambray 1994), but this needs to be confirmed for *P. skeltoni*.

Distribution and habitat. *Pseudobarbus skeltoni* was collected from only two localities, one in the upper Riviersonderend River (a major tributary of the Breede River) and another in the Krom River (a tributary of the Molenaars River), in the Breede River system (Fig. 6). Some specimens from two lots collected by P.H. Skelton and P.H. Greenwood in 1975 (AMP 3463) and by Farquharson (AMP 1411) from the Wit River (Bainskloof) (Fig. 6) and deposited in the Albany Museum as *P. burchelli* are morphologically concordant with the new species described herein, and are thus reassigned to *P. skeltoni*. *Pseudobarbus skeltoni* has not been collected from the Wit River in recent surveys, suggesting that this population may have possibly been extirpated, because the river section where the specimens were originally collected in 1975 has now been invaded by alien African sharptooth catfish (*Clarias gariepinus* (Burchell, 1822)) and North American smallmouth black bass (*Micropterus dolomieu* (Lacepède, 1802)). The Riviersonderend population occurs in sympatry with *P. burchelli*, as was the case for the Wit River population.

Molecular data revealed a close relationships (sharing of haplotypes) between the Riviersonderend and Krom populations, suggesting that these populations may have been connected historically. It also suggests that *P. skeltoni* could have occurred in mainstem habitats, or at least used it as conduits for dispersal. Given the comprehensive sampling that was done by the authors across the south-western Cape Floristic Region between December 2008 and December 2009, the Riviersonderend and Krom populations probably represent the remaining natural range of the species. Based on the extensive surveys, genetic results and considering what happened to other threatened fishes of the Breede River system, *P. skeltoni* probably underwent an initial rapid decline after the introduction of alien fishes (particularly black bass species) due to predation and competition. The alien fishes are now dominant in the mainstem habitats, precluding the possibility for native fish species to breed or even survive as adults in these areas.

In the upper Riviersonderend (Fig. 7A), specimens were caught at the pool-riffle interphase using fyke nets, with a single individual caught in gill nets in the deep pools (> 2 m depth). The water was brown and peat stained, moderately flowing at the pool-riffle interphase, and static in the pools. Water temperature was 19.3°C, pH was 4.5, conductivity was 22 µS/cm and TDS 22 ppm. The river bed is dominated by large boulders and cobbles. The banks along the pool margins were covered with dense stands of Palmiet *Prionium serratum* (Fig. 7A). In the Krom River (Fig.7B), the water was clear and the fish were observed in both riffles and pools with moderately flowing water. Samples were collected at 485 m altitude, water temperature was 20.3 °C, pH was 4.4, and conductivity was 11 µS/cm. The river bed has large boulders, cobbles and gravel and little sand. The riparian zone is dominated by woody vegetation. Submerged rocks were covered with periphyton. The inferior mouth in juveniles and sub-adults suggest that younger age groups of *P. skeltoni* probably feed by picking prey or algae from rock surfaces, while the distinct terminal mouth in adults suggest a shift towards a more predatory lifestyle and possibly a water column feeder.
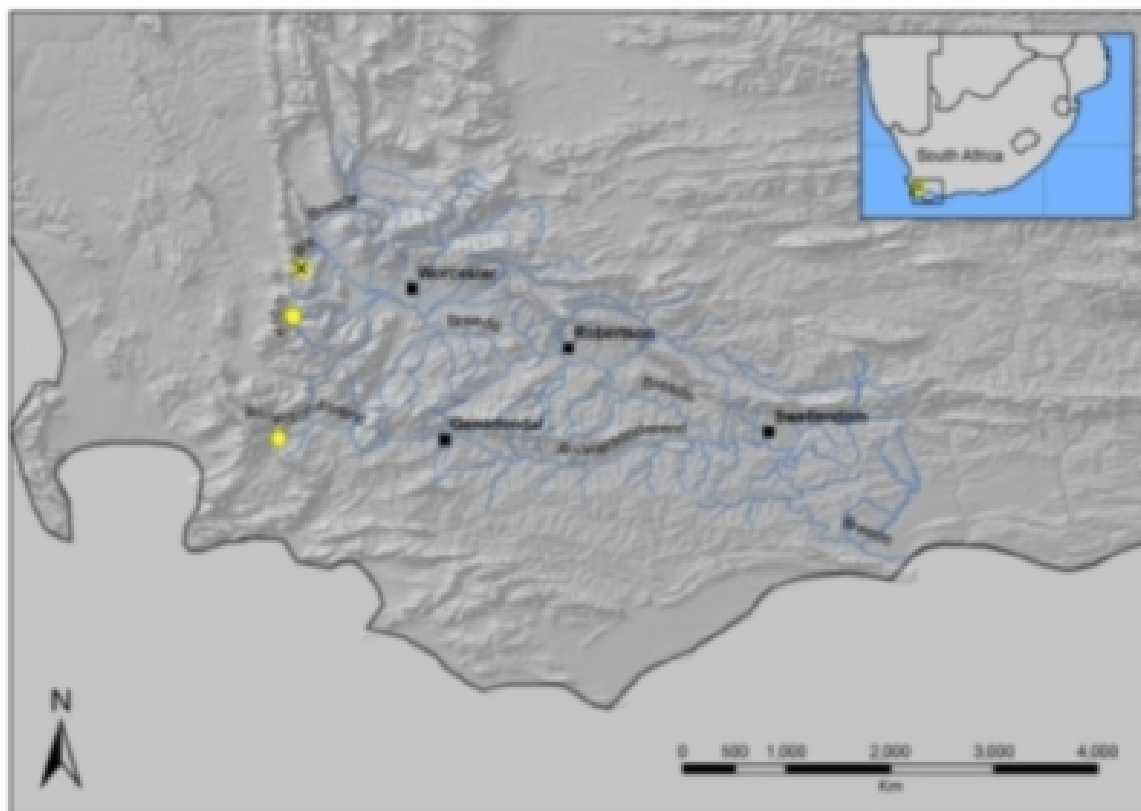
FIGURE 6. Map of the Breede River system in the south-western Cape Floristic Region of South Africa showing the distribution of *Psedobarbus skeltoni* **sp. nov.** Individuals of this species were previously collected from the Wit River in 1975, but have not been found in recent surveys. The upper Riviersonderend is the type locality.

FIGURE 7. Pictures showing localities and habitat where specimens of *Pseudobarbus skeltoni* were collected: (A) upper Riviersonderend (note the foot bridge of hiking trail crossing the river just below the gauging weir), and (B) Krom River.

Conservation. Ensuring the future survival of *P. skeltoni* presents a serious challenge to conservation managers due to its restricted distribution and small number of remaining individuals. The upper Riviersonderend population persists above Theewaterskloof Dam, which has large populations of North American black bass species (*Micropterus dolomieu* (Lacepède, 1802), *M. punctulatus* (Rafinesque, 1819) and *M. salmoides* (Lacepède,

1802)), the sharp-tooth catfish (*Clarias gariepinus* (Burchell, 1822)) and other alien fishes. The upper Riviersonderend currently has the largest specimens and the highest number of individuals of *P. skeltoni* (87 adult adults and over 200 subadults were caught in fyke nets in December 2009 and were released alive). The future survival of this population is uncertain, because there is no barrier to prevent upstream migration of non-native fishes from Theewaterskloof Dam. Snorkel counts indicate that the Krom population is represented by less than 120 adult individuals in a stream reach of less than 50 m. Here, the major threat is the presence of rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), that has invaded beyond popular fly-fishing streams lower down in the catchment.

Etymology. The specific name, *skeltoni*, is given in honour of Professor Paul H. Skelton in recognition of his lifelong service to taxonomic and systematic research on freshwater fishes in southern Africa, his contribution to the taxonomic revision and systematics of *Pseudobarbus* and specifically for his mentoring of students on this group of fishes.

Discussion

Specimens from recently collected material (herein described as *P. skeltoni*) clearly belong to the genus *Pseudobarbus* because they have a flexible primary dorsal ray, and display all the derived characters of this genus such as well developed, distinctively patterned nuptial tubercles in the males (Skelton 1988). A combination of meristic, morphometric and anatomical characters, as well as molecular data discriminate the new *Pseudobarbus* species from the two morphologically most similar species, *P. burgi* and *P. burchelli*.

Skelton (1988) noted broad intraspecific variation within *P. burchelli*, with specimens from samples collected in the Wit River exhibiting distinct characters (i.e., a higher modal count of preanal vertebrae, a higher number of pharyngeal teeth, different mouth form and longer gut) than other populations of *P. burchelli*. These specimens have been re-examined in the present study and are morphologically concordant with *P. skeltoni* **sp. nov.** The adults of *P. skeltoni* can be readily distinguished from both *P. burchelli* and *P. burgi* due to their possession of a distinct terminal mouth, while the latter two species have subterminal mouths. The juveniles, subadults and young adults of the new species, however, are less readily distinguished from *P. burchelli* and *P. burgi* because they also have subterminal mouth. Possession of retracted lips and presence of a cartilaginous plate are however unique features that readily distinguish both *P. burchelli* and *P. burgi* from *P. skeltoni* juveniles, subadults and young adults.

Existence of historically isolated lineages in *P. burgi* and *P. burchelli* (excluding the newly described species from the present study) has been reported from previous studies (Bloomer & Impson 2000; Swartz *et al*. 2009). Revision of these lineages is the subject of further research being undertaken at SAIAB. *Pseudobarbus skeltoni* **sp. nov.** is the sister species to all the other double barbed redfins. This is a significant discovery as it will help to shed more light on the evolution and biogeography of the CFR ichthyofauna.

It is possible that *P. skeltoni* had a wide distribution across the Breede River system, where it could have co-occurred with *Barbus andrewi* Barnard, 1937 (a large endemic cyprinid) in the mainstream environments. *Barbus andrewi*, currently listed as Endangered under the IUCN list of threatened species (Tweddle *et al*. 2009), has been extirpated from almost all mainstream habitats of the Breede River system due to invasion of these environments by alien fishes (e.g., *C. gariepinus*, *M. dolomieu*, *M. salmoides*, *M. punctulatus* and *Lepomis microchirus* Rafinesque, 1819 and others), habitat degradation, over abstraction of water, bulldozing and channelisation of rivers, sedimentation and pollution (Impson & Swartz, 2007). While *Barbus andrewi* has large populations persisting in Brandvlei and Sandrif dams in the Breede catchment as well as some riverine populations (Impson & Swartz 2007; Chakona *et al*. in preparation), the situation is dire for *P. skeltoni*, because the only two known populations of this species are highly fragmented and probably have very low numbers. Urgent conservation actions are needed to ensure the long-term survival of *P. skeltoni*. Eradication of aliens and building of weirs to prevent re-invasion are immediate measures that can prevent extinction of the two remaining populations of *P. skeltoni*. Similar actions could be used to rehabilitate the lower Witte River where *P. skeltoni* occurred before, which could create an opportunity for successful reintroduction.

Acknowledgements

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