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Naming the other cousin: A new goldie barb (Cyprinidae: Smiliogastrininae) from the northeast escarpment in South Africa, with proposed taxonomic rearrangement of the goldie barb group in southern Africa

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Abstract

A growing body of evidence indicates that the global diversity of freshwater fishes has not been fully documented. Studies of freshwater fishes that were previously thought to be morphologically variable have revealed the existence of deeply divergent lineages, with many distinct species. In southern Africa a number of *Enteromius* species exhibit either exceedingly wide or divided distribution patterns that should be rare for freshwater fishes with limited dispersal opportunities between river systems. One such species is the sidespot barb, *Enteromius neefi*. As currently defined, *E. neefi* has a disjunct distribution that is divided between rivers in the northeast escarpment in South Africa and Eswatini, and tributaries of the Upper Zambezi in Zambia and southern Congo in the Democratic Republic of Congo, with a large geographic gap between these two populations. With the use of molecular and morphological methods, the level of divergence between the two populations was examined, and a new species was described from the Steelpoort River in the Limpopo River system of South Africa. Findings from this study provide further evidence for a number of taxonomic problems within the goldie barbs of southern Africa, and some taxonomic rearrangements are proposed for this group.

KEYWORDS
color pattern, Cypriniformes, integrative taxonomy, systematics

1 | INTRODUCTION

Understanding the systematics of fishes in mega-diverse orders such as the Cypriniformes has improved markedly in recent years due to molecular phylogenetic analyses (Mayden et al., 2008, 2009; Saitoh et al., 2011), and interfamilial relationships are becoming well established (ShunPing et al., 2008; Wang et al., 2012; Yang et al., 2015). The family Cyprinidae consists of freshwater fishes widely distributed throughout North America, Eurasia, and Africa (Skelton, 2001). Morphological similarity is common among cyprinids, which led early taxonomists, relying on morphological characters alone, to group together species in outsized genera (Skelton et al., 2018). One such genus was *Barbus* (Daudin 1805), which Myers (1960) called a "monstrous aggregation," consisting of more than

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800 species spread across Africa, Asia, and Europe (Hayes & Armbruster, 2017). However, phylogenetic analyses revealed that *Barbus* included many different and nonrelated lineages and groups, indicating that the aggregation of large numbers of freshwater species spread across three continents into a single genus made little sense (Skelton et al., 2018).

In the late 1980s a number of taxonomic and karyological studies that investigated the diversity and relationships of African cyprinids provided evidence that species placed in *Barbus* showed three different ploidy levels, resulting in the revalidation of many genera that were at that time considered synonyms of *Barbus*. The genera *Luciobarbus* (Heckel 1843), *Cheilobarbus* (Smith 1841), and *Pseudobarbus* (Smith 1841), which were considered to be synonyms of *Barbus*, as well as the recently described genera *Amatolacypris* Skelton et al., 2018, *Namaquacypris* Skelton et al., 2018, and *Sedercypris* Skelton et al., 2018, are tetraploids ($2n = c.$ 100 chromosomes). All hexaploid ($2n = c.$ 150 chromosomes) species have been placed in the genus *Labeobarbus* Rüppel 1835, whereas the African diploid species ($2n = c.$ 50 chromosomes) were initially designated as "*Barbus*" to indicate that they are not closely related to the Eurasian tetraploid *Barbus* s.s. (Berrebi et al., 1996; Golubtsov & Krysanov, 1993; Guégan et al., 1995; Oellermann & Skelton, 1990; Skelton, 1988; Skelton et al., 2018; Tsigenopoulos et al., 2002). Following the study of Yang et al. (2015), the African diploid species have been assigned to the genus *Enteromius* Cope 1867 in the subfamily Smiliogastrinae (Tan & Armbruster, 2018). This new designation was initially contested (Ren & Mayden, 2016; Schmidt & Bart, 2015; Stiassny et al., 2016; Stiassny & Sakharova, 2016) but is now widely accepted as the use of "*Barbus*" would still link the small African species to the true Eurasian *Barbus*, when in fact the African and Eurasian taxa belong to two different subfamilies, Smiliogastrinae and Barbinae, respectively (Skelton 2015, 2016; Yang et al., 2015; Skelton et al., 2018; Tan & Armbruster, 2018). The resurrection of *Enteromius* concerns only those African diploid species not already placed in the genera *Barboides* Brüning 1929, *Barbopsis* Di Caporiacco 1926, *Caecobarbus* Boulenger 1929, *Clypeobarbus* Fowler 1936, and *Prolabeops* Schultz 1941. The phylogenetic placement of *Barbopsis* is still unknown, and *Barboides*, *Caecobarbus*, *Clypeobarbus*, *Prolabeops*, and the tetraploid genus *Pseudobarbus* are nested within *Enteromius* (Hayes & Armbruster, 2017; Ren & Mayden, 2016; Schedel et al., 2022), which renders *Enteromius* polyphyletic and illustrates the need for further taxonomic changes.

Currently, *Enteromius* consists of ~226 valid species distributed across Africa (Fricke et al., 2023; Hayes & Armbruster, 2017), with new species continuously being described (e.g., Kambikambi et al., 2021; Katemo Manda et al., 2020), revalidated (e.g., Englmaier et al., 2020; Maetens et al., 2020; Schmidt et al., 2018), or awaiting formal description (e.g., Popoola et al., 2022). Forty-two *Enteromius* species occur in the southern African region as defined by Skelton (2001), 23 of which are endemic to South Africa (Froese & Pauly, 2022; Kambikambi et al., 2021; Skelton, 2001). Skelton (2001) placed the southern African barbs in three groups based on differences in the primary dorsal-fin ray morphology: the soft-rayed barbs with a smooth and flexible primary dorsal-fin ray, the sawfin barbs with a spinous and serrated primary dorsal-fin ray, and the spinefin barbs with a smooth and spinous primary dorsal-fin ray. Within the soft-rayed barbs, two additional groups were identified, the goldie and chubbyhead barbs, based on a distinctive breeding colouration and unique morphological characteristics. Currently, only three species belong to the goldie barb group: the shortfin barb *Enteromius brevipinnis* (Jubb 1966), the sidespot barb *Enteromius neefi* (Greenwood, 1962), and the goldie barb *Enteromius pallidus* (Smith 1841). The three species in the goldie barb group are characterized by relatively small (<70 mm standard length [SL]) compact bodies, two pairs of barbels, and a bright golden color attained by males during the breeding season. Despite possessing similar characteristics, *Enteromius greenwoodi* (Poll, 1967), *Enteromius lineomaculatus* (Boulenger 1903), *Enteromius thamalakanensis* (Fowler 1935), and *Enteromius viviparus* (Weber 1897) have not been included under the goldie barb group, but no justification has been provided for their exclusion (Skelton, 2001).

E. neefi was described based on 17 specimens collected in 1960 from the Kabompo River, a major tributary of the Upper Zambezi River system, in Zambia (Greenwood, 1962). A combination of characteristic markings distinguish *E. neefi* from *E. brevipinnis* and *E. pallidus*: a spot at the base of the anal and pectoral fins, variable number of large dark spots along the body, and thin wavy parallel lines along the top and bottom of each scale row of the flank, extending ventrally beyond the lateral line scale row (Greenwood, 1962; Jubb, 1968; Skelton, 2001). Initially the Upper Zambezi and headwaters of the Lualaba (southern Congo) were the only known distribution range for the species, until specimens with similar characteristics were recorded from the Orighstad and Steelpoort rivers, Limpopo River system in South Africa (Jubb, 1968). These specimens were assigned to *E. neefi*, mainly based on possession of wavy parallel lines that are the key distinguishing feature for this species. Subsequently more populations were identified in the Letaba, Mutale, Levuhu, Sabie, Crocodile, Makondo, and Mfolozi rivers. This resulted in the species having a disjunct distribution pattern, with the two known populations separated by a large geographic gap of ~1000 km between the Upper Zambezi River in the north and the northeast escarpment of South Africa (Skelton, 2001). Disjunct distribution patterns have been recorded for other southern Africa freshwater fish species, including *E. pallidus*, *Mesobola brevianalis* (Boulenger 1908), and *Amphilius natalensis* (Boulenger 1917). These species, initially thought to represent widely distributed species, but following application of integrative taxonomic research, were shown to represent deeply divergent lineages, resulting in the recent description of new species (Chakona et al., 2015; Mazungula & Chakona, 2021; Riddin et al., 2016).

The aim of the present study was to use molecular *COI* (cytochrome oxidase subunit I) and morphological data within an integrative taxonomy perspective to delimit species boundaries within *E. neefi*. Furthermore, the phylogenetic status of the "goldie" barb group was explored by acquiring topotypic sequences for southern African species with two pairs of barbels and males that attain bright golden colouration during the breeding season. Potential taxonomic rearrangements are proposed, and conservation implications are highlighted.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Description of the new *Enteromius* species is based on 15 specimens collected during field surveys in the Steelpoort River (Limpopo River system), South Africa, in May 2012 and May 2021. Comparative topotype specimens of *E. neefi* were collected during field surveys between 2011 and 2019 from the Kabompo River in Zambia, and the type specimens were measured by the author Albert Chakona at the National History Museum, London. A total of 11 new sequences were generated for the two *E. neefi* populations, *E. brevipinnis*, *E. greenwoodi*, *E. thamalakanensis*, and *E. viviparus*. Twenty-five sequences were used from BOLD (Barcode of Life Data) and a single sequence from GenBank. The approaches used for sample collection and processing were approved by the NRF-SAIAB Animal Ethics Committee (reference no.: 2014/03).

2.2 | Molecular data

2.2.1 | Extraction, amplification, and sequencing

DNA was extracted from preserved tissues using the salting-out protocol of Sunnucks and Hales (1996), and DNA concentrations were quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Inc.). A fragment of the mitochondrial COI gene was amplified by PCR using universal fish DNA barcoding primer sets: FishF1 and FishR1 (Ward et al., 2005) and VF2-T1 and VR1-T1 (Ivanova et al., 2007). PCRs were performed with a Veriti 96-well thermal cycler (Applied Biosystems, Foster City, CA, USA). Each reaction mixture (25 μL) contained 100–200 ng of template DNA, 12 μL of Taq DNA Polymerase 2× Master Mix RED (Ampliqon PCR Enzymes & Reagents), 0.5 μL of each primer (20 pmol), and 7 μL of molecular-grade water. PCR amplification was performed using the following profile: 3 min at 95°C, followed by 12 cycles of 30 s at 95°C, 40 s at 62°C, decreasing by 0.5°C per cycle to 56.5°C, and 50 s at 72°C. This was followed by 25 cycles of 30 s at 95°C, 30 s at 56°C and 50 s at 72°C, with a final extension of 72°C for 7 min. PCR products were purified using the ExoSAP method with a reaction mixture containing 5 μL of PCR product, 0.5 μL of exonuclease I, and 1 μL of FastAP (Applied Biosystems). Cycle sequencing was performed using the BigDye Cycle Sequencing Kit (Applied Biosystems) and sequenced at SAIAB using an ABI 3730xl DNA Analyzer (Applied Biosystems). Sequences were edited and trimmed using Geneious Prime 2021.2.2.

2.2.2 | Phylogenetic analysis and species delimitation

To assess the phylogenetic affinities of the two *E. neefi* populations and verify the distinctiveness of the new species, all COI sequences of African Smiliogastrinae available in BOLD and GenBank and new sequences from this study were used to generate a phylogenetic tree (Figure S1). Sequences were aligned using MAFFT, version 7.450 (Katoh & Misawa, 2002; Katoh & Standley, 2013), and inspected using MEGA 11 (Tamura et al., 2021) for the presence of stop codons. Identical sequences were removed prior to phylogenetic analysis. Bayesian analysis was performed using MrBayes, version 3.2.6 (Huelsenbeck & Ronquist, 2001), partitioning the dataset by codon position and employing reversible-jump Markov chain Monte Carlo (RJ-MCMC) sampling, with time-reversible substitution models and γ-distributed rate heterogeneity (Huelsenbeck et al., 2004). The RJ-MCMC method precludes the a priori selection of substitution models for each partition and instead allows models to be sampled in proportion to their posterior probability (Huelsenbeck et al., 2004). Two parallel analyses of four Markov chains and 5 million generations were run, sampling trees every 1000 generations and discarding the first 25% of trees as burn-in. Resulting trees were visualized in FigTree, version 1.4.4 (Rambaut, 2009). To assess the convergence between the two runs, the average standard deviation of split frequencies was monitored in MrBayes to ensure it was <0.05. In addition, Effective Sample Size (ESS) values and the potential-scale reduction factor for all parameters were examined using MrBayes and found to approach >100 and 1.0, respectively. From this tree the clade containing the two *E. neefi* populations was retained for detailed analysis.

Four molecular-based species delimitation methods were used to identify operational taxonomic units (OTU) and explore species boundaries between taxa belonging to the retained lineage. The first two methods, "automatic barcode gap discovery" (ABGD) and "assemble species by automatic partitioning" (ASAP), are genetic distance–based methods that rely on the analysis of single-locus sequence alignments to define species partitions based on pair-wise genetic distances (Puillandre et al., 2012; Puillandre et al., 2021). Two coalescent methods, the "Bayesian implementation of the Poisson tree processes" (bPTP) (Zhang et al., 2013) and the "general mixed Yule coalescent" (GMYC) (Fujisiwa & Barraclough, 2013), were performed. The bPTP relies on single-locus molecular data to delimit species based on the number of nucleotide substitutions between haplotypes. In the GMYC method, species are delimited based on branch lengths, and it requires an utrametric tree to define intraspecific and interspecific threshold patterns. (Fujisiwa & Barraclough, 2013). The ultrametric tree used for the GMYC analysis was constructed in BEAST2 (Bouckaert et al., 2019) using the Yule model prior with an optimized relaxed clock. The assumption for all these methods is that interspecific variability will be substantially higher than intraspecific differentiation.

2.3 | Morphological data

Following Chakona et al. (2014), 15 morphological and 16 meristic characters were obtained for 16 specimens of *E. neefi* from the Kabompo River, including the type series. For the Limpopo population, 15 specimens from the Steelpoort River (Limpopo River system) were included. Ten specimens of the Limpopo population and 13 specimens of the Kabompo population were radiographed to facilitate the

counting of skeletal features. Measurements were taken using digital calipers to the nearest 0.1 mm. Principal component analysis (PCA) was performed using PAST (Hammer et al., 1999) on raw meristic data and normalized morphometric data (Lleonart et al., 2000) to explore the variables that might assist in distinguishing both species. The normalization procedure allows for size-free comparison between specimens. Invariant characters (see Table 3) were excluded from the analysis.

3 | RESULTS

Genetic and morphological data support the recognition of the Steelpoort *E. neefi* population as a new goldie barb species and here described as *Enteromius niggie* sp. nov. from the Limpopo River system of South Africa.

3.1 | Molecular phylogenetic analyses

A Bayesian phylogenetic tree of all available COI sequences of African Smiliogastrins revealed that *E. niggie* sp. nov. and *E. neefi* clustered within a major clade containing eight morphologically identified species, *Enteromius atkinsoni* (Bailey 1969), *Enteromius macinensis* (Daget 1954), *E. viviparus*, *E. pallidus*, *E. brevipinnis*, *E. thamalakanensis*, *E. lineomaculatus*, and *E. greenwoodi*, as well as two possible candidate species *Enteromius* sp. "Cuebe" and *Enteromius* sp. "Bie" from multiple drainages in sub-Saharan Africa (Figure 1; Table 1; Figure S1). Furthermore, two species, *E. brevipinnis* and *E. viviparus*, were each split into at least two polyphyletic lineages.

Detailed analysis using four species delimitation methods based on 38 COI sequences representing *E. niggie* sp. nov., *E. neefi*, and the other eight taxa mentioned earlier recovered 19 OTUs or candidate species (Figure 1). These OTUs were grouped into three well-supported clades (Figure 1). Clade A contained *E. macinensis* from Burkina Faso as well as two OTUs that were morphologically identified as *E. atkinsoni* from East Africa. The OTU-containing specimens from Malawi and Mozambique are substantially differentiated (3.3% divergence) from the topotype of *E. atkinsoni* from the Rufiji River system (Figure 1). Clade B comprised two OTUs from coastal East Africa, lower Zambezi, and the Incomati systems that are currently identified as *Enteromius* cf. *viviparus*. These two OTUs are substantially differentiated (9.1%–9.8% divergence) from and are distantly related to the topotypes of *E. viviparus* from the Mdloti River system, in South Africa (Figure 1). Clade C contained 14 OTUs, including *E. niggie* sp. nov. and three species currently assigned to the goldie group: *E. pallidus*, *E. neefi*, and *E. brevipinnis*. Topotypes of *E. viviparus*, *E. thamalakanensis*, and *E. greenwoodi* as well as specimens that were morphologically identified as *E. lineomaculatus* were recovered within this group. Three OTUs were recovered for specimens that were morphologically identified as *E. brevipinnis*, whereas two OTUs were identified within *E. thamalakanensis*, and a similar pattern for *E. greenwoodi*. Interestingly the disjunctly distributed species *E. niggie* sp. nov. and *E. neefi* were found to be distantly related and deeply divergent (3.5%) from each other (Figure 1).

3.2 | Morphological data

PCA performed on 15 morphometric characters of *E. niggie* sp. nov., and *E. neefi* exhibited a broad overlap between the two species, indicating that they could not be separated based on these characters (Figure 2). The first principal component axis (PCI) accounted for 24.3%, PC2 21.2%, and PC3 15.4% of the variation observed between the lineages (Table 2). The first PCA axis (PCI) was included as size differences were accounted for in the normalization procedure. PCI was mainly defined by differences in pectoral- to pelvic-fin length. PCII contrasted differences in caudal peduncle length. PCIII highlighted differences in pelvic- to anal-fin length.

PCA performed on 10 meristic characters showed incomplete separation between the two species (Figure 3). PCI accounted for 49.84%, PCII 19.52%, and PCIII 12.69% of the variation between lineages. Factor loadings are presented in Table 2. PCI was defined by differences in the number of scales along the lateral line. PCII contrasted with differences in the number of pectoral-fin rays. PCII highlighted differences in the number of both total and caudal vertebrae. Despite the considerable overlap in morphometric and meristic characters between *E. niggie* sp. nov. and *E. neefi*, there are consistent qualitative color pattern differences between these two disjunctly distributed and genetically divergent species that warrant their separation as distinct taxonomic entities. These characteristics include the patterns of wavy parallel lines on the flank and the pattern of bold spots on the dorsal midline (Figure 4). All 16 examined specimens of *E. neefi* had the wavy parallel lines extending below the lateral line, and bold spots were present on the dorsal midline, whereas in the 15 specimens of *E. niggie* sp. nov. from the Limpopo, these are absent (Figure 4).

For diagnosis of the new species and comparison with *E. neefi*, the meristic and morphometric measurements obtained in the present study were used. For comparisons with the other species of interest, information from the original descriptions and other key references was used (Martin & Chakona, 2019; Poll, 1967; Skelton, 2001).

3.3 | Taxonomic accounts

***Enteromius niggie* sp. nov. [niggie: 'nəχi]** (g/ch from Afrikaans/Dutch) is pronounced with a hard guttural sound, made at the back of the throat.

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(Figures 5 and 6; Table 3).

3.4 | Proposed common names

Southern sidespot barb; Suidelike sykol ghieliemientjie (Afrikaans).

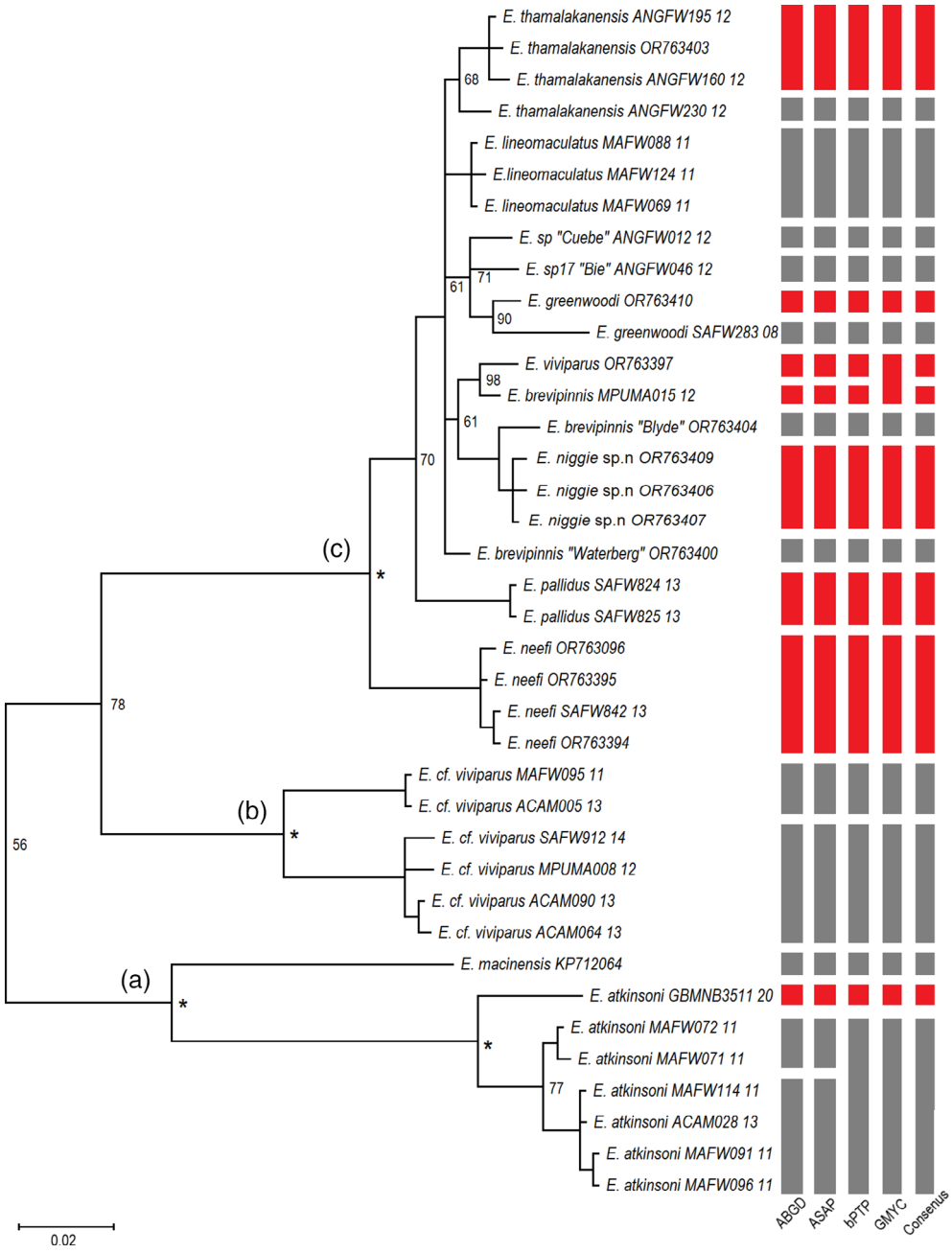

FIGURE 1 Bayesian phylogenetic tree showing the phylogenetic relationships within the goldie barb group. The lineage is extracted from the broader analysis of available COI (cytochrome oxidase subunit I) sequences incorporating 95 species currently assigned to the genus *Enteromius* (Figure S1). Bayesian posterior probabilities are given on the branches as percentages; asterisk indicates PP >99. Bars show the OTUs (operational taxonomic units) identified by each of the four species delimitation methods. Red OTUs contain sequences of topotypic specimens.

3.5 | Holotype

SAIAB 236359, field number: NPEJ21-B080, male, 41.10 mm SL, Steelpoort River, Limpopo River system, −24.80238, 30.11740, collected by Hoffman A., May 2021 (Figures 5b and 6b).

3.6 | Paratypes

SAIAB 236360, nine unsexed, 33.35–42.21 mm SL, same collector and locality as holotype. SAIAB 186470, five unsexed, 35.76–44.36 mm SL, Steelpoort River, Limpopo River system, −24.72907, 30.18468, collected by Palmer R., May 14, 2012.

3.7 | Additional material

SAIAB 26620, 1 unsexed, 48.00 mm SL, Mohlapitse River, Limpopo River System, −24.04999, 30.03333, collected by J. Engelbrecht, April 1, 1986. SAIAB 27269, 1 unsexed, 39.15 mm SL, Letaba River, Limpopo River system, collected by D. Curle, September 22, 1986. SAIAB 49504, 6 unsexed, 35.40–45.10 mm SL, tributary of the Levuvhu River, Limpopo River system, −23.06667, 30.25000, collected by M. Angliss, April 6, 1995. SAIAB 61031, 20 unsexed, 20.00–39.10 mm SL, Mutale River, Limpopo River system, −22.73444, 30.65861, collected by R. Bills, D. Naran, B. Van der Waal, November 13, 1999. SAIAB 61044, 1 unsexed, 28.10 mm SL, Mutale River, Limpopo River system, −22.70000, 30.64305, collected by R. Bills,

TABLE 1 Species names, reference numbers for BOLD/GenBank sequences, and locality details of sequences used for the present study.

Species	Sequence ID	Catalogue number	Country	River	System	Latitude	Longitude
Enteromius atkinsoni	MAFW071-11	SAIAB 119079	Malawi	Ruo	Zambezi	−16.04	35.79
E. atkinsoni	MAFW072-11	SAIAB 119079	Malawi	Ruo	Zambezi	−16.04	35.79
E. atkinsoni	ACAM028-13	SAIAB 190277	Mozambique	Chipembe Dam	Montepuez	−13.20	38.62
E. atkinsoni	MAFW114-11	SAIAB 118777	Malawi	Shire	Zambezi	−15.06	35.22
E. atkinsoni	MAFW096-11	SAIAB 185654	Malawi	Phalombe	Lake Chilwa	−15.81	35.65
E. atkinsoni	MAFW091-11	SAIAB 185654	Malawi	Phalombe	Lake Chilwa	−15.81	35.65
E. atkinsoni	GBMNB3511-20	NA	Tanzania	Great Ruaha	Rufiji	−07.63	36.89
Enteromius cf. *viviparus*	MPUMA008-12	SAIAB 194032	South Africa	Klein-Sand	Incomati	−24.66	31.09
Enteromius cf. *viviparus*	SAFW912-14	SAIAB 081022	Mozambique	Zambezi	Zambezi	−15.60	32.72
Enteromius cf. *viviparus*	ACAM064-13	SAIAB 190265	Mozambique	Tshidi	Zambezi	−15.62	33.67
Enteromius cf. *viviparus*	ACAM090-13	SAIAB 190291	Mozambique	Tshidi	Zambezi	−15.69	33.67
Enteromius cf. *viviparus*	ACAM005-13	SAIAB 190235	Mozambique	Muhukwa	Montepuez	−13.43	38.61
Enteromius cf. *viviparus*	MAFW095-11	SAIAB 185652	Malawi	Phalombe	Lake Chilwa	−15.81	35.65
Enteromius macinensis	KP712064	NA	Burkina Faso	NA	NA	NA	NA
Enteromius brevipinnis	MPUMA015-12	SAIAB 194050	South Africa	Mac-Mac	Incomati	−25.02	31.00
E. brevipinnis	OR763400[a]	SAIAB 206418	South Africa	Grootspruit	Limpopo	−24.53	27.87
E. brevipinnis	OR763404[a]	SAIAB 194786	South Africa	Blyde	Limpopo	−24.90	30.75
Enteromius viviparus	OR763397[a]	SAIAB 235471	South Africa	Umdloti	Umdloti	−29.64	31.09
Enteromius neefi	OR763394[a]	SAIAB 210153	Zambia	Kabompo	Zambezi	−11.89	25.25
E. neefi	OR763396[a]	SAIAB 210153	Zambia	Kabompo	Zambezi	−11.89	25.25
E. neefi	OR763395[a]	SAIAB 210153	Zambia	Kabompo	Zambezi	−11.89	25.25
E. neefi	SAFW842-13	SAIAB 082862	Democratic Republic of the Congo	Kando	Congo	−10.80	25.98
Enteromius pallidus	SAFW824-13	SAIAB 186173	South Africa	Baakens	Baakens	−33.96	25.51
E. pallidus	SAFW825-13	SAIAB 186173	South Africa	Baakens	Baakens	−33.96	25.51
Enteromius lineomaculatus	MAFW088-11	SAIAB 185653	Malawi	Phalombe	Lake Chilwa	−15.81	35.64
E. lineomaculatus	MAFW069-11	SAIAB 119069	Malawi	Ruo	Zambezi	−16.10	35.69
E. lineomaculatus	MAFW124-11	SAIAB N/A	Malawi	Nkatha Bay	Lake Malawi	−11.62	34.23
Enteromius thamalakanensis	ANGFW230-12	SAIAB 187035	Namibia	Okavango	Okavango	−18.12	21.58
E. thamalakanensis	ANGFW160-12	SAIAB 186818	Angola	Luassingua	Okavango	−14.59	18.17
E. thamalakanensis	ANGFW195-12	SAIAB 186874	Angola	Cuito	Okavango	−15.14	19.19
E. thamalakanensis	OR763403[a]	SAIAB 202855	Botswana	Okavango	Okavango	−19.21	22.75
Enteromius greenwoodi	OR763410[a]	SAIAB 85020	Angola	Cuanza	Cuanza	−12.03	17.63
E. greenwoodi	SAFW283-08	SAIAB 84816	Angola	Cuanza	Cuanza	−9.80	15.46
Enteromius sp. *"Cubango"*	ANGFW046-12	SAIAB 186686	Angola	Cubango	Okavango	−13.59	16.88
Enteromius sp. *"Cuebe"*	ANGFW012-12	SAIAB 186640	Angola	Cuebe	Okavango	−14.94	17.72
E. niggie sp. nov.	OR763406[a]	SAIAB 236360	South Africa	Steelpoort	Limpopo	−24.80	30.12
E. niggie sp. nov.	OR763409[a]	SAIAB 236359	South Africa	Steelpoort	Limpopo	−24.80	30.12
E. niggie sp. nov.	OR763407[a]	SAIAB 236360	South Africa	Steelpoort	Limpopo	−24.80	30.12

Abbreviation: BOLD, Barcode of Life Data; NA, not available.
[a]Novel sequences generated from this study.

D. Naran, B. Van der Waal, 13 November 1999. SAIAB 63443, 4 unsexed, 40.70–48.40 mm SL, Wyliespoort, tributary of Mutamba River, Limpopo River system, −22.91670, 29.92811, collected by J. Engelbrecht, September 20, 2000. SAIAB 190517, 44 unsexed, 27.50–41.2 mm SL, Orighstad River, Limpopo River system, −24.89027, 30.58833, collected by E. Swartz, L. da Costa, October 22, 2005. SAIAB 190556, 10 unsexed, 20.60–35.70 mm SL, Sterkspruit River, Limpopo River system, −25.156944, 30.558611, collected by E. Swartz, L. da Costa, October 23, 2005. SAIAB 203320, 1 unsexed, 31.00 mm SL, Thabina River, Limpopo River system,

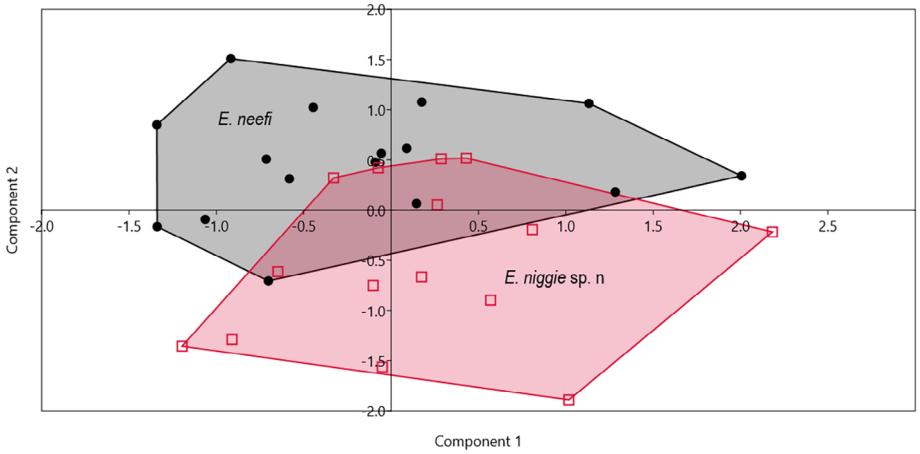

FIGURE 2 Scatterplot of PC1 against PC2 for a PCA (principal component analysis) carried out on 16 normalized morphometric characters for 31 specimens of *Enteromius niggie* sp. nov. and *Enteromius neefi*.

TABLE 2 Factor loadings for the first three principal component axes on 16 morphometric and 10 meristic characters from 31 specimens of *Enteromius niggie* sp. nov. and *Enteromius neefi*.

Character	PC1	PC2	PC3
Morphometrics	24.35%	21.16%	15.36%
Head length	−0.128	−0.106	0.046
Body depth	−0.108	0.546	−0.146
Predorsal length	−0.126	0.174	0.060
Dorsal-fin base	0.098	−0.197	0.246
Pectoral- to pelvic-fin length	**0.838**	0.320	−0.237
Pelvic- to anal-fin length	0.213	0.274	**0.784**
Anal-fin base	0.006	−0.246	0.289
Caudal peduncle length	0.366	**−0.599**	−0.160
Caudal peduncle depth	0.056	0.051	−0.002
Head depth	0.013	−0.069	0.237
Snout length	−0.050	−0.050	0.026
Orbit diameter	−0.026	−0.002	0.080
Inter-orbit width	−0.108	0.108	−0.084
Post-orbit length	−0.214	0.057	−0.250
Anterior barbel length	−0.128	−0.106	0.046
Posterior barbel length	−0.108	0.546	−0.146
Meristics	49.84%	19.52%	12.69%
Lateral line scales	**0.816**	−0.505	−0.034
Circumpeduncular scales	0.032	0.064	0.003
Predorsal scales	0.393	0.268	−0.387
Pectoral-fin rays	0.326	**0.756**	−0.134
Pelvic-fin rays	−0.069	−0.257	−0.119
Total vertebrae	0.122	0.122	**0.641**
Predorsal vertebrae	0.174	0.047	0.319
Pre-caudal vertebrae	0.002	0.017	0.026
Pre-anal vertebrae	0.002	0.017	0.026
Caudal vertebrae	0.148	0.116	**0.551**

Note: The most important factor loadings are in bold font.

−24.02706, 30.18103, collected by A. Chakona, L. Dlamini, March 25, 2016. SAIAB 76348, 29 unsexed, 27.3–40.7 mm SL, Buffelskloof-spruit, tributary of Crocodile River, Incomati River system, −25.40833, 30.46917, collected by R. Bills, J. Engelbrecht, May 19, 2005. SAIAB 27262, 2 unsexed, 33.00–40.12 mm SL, Mlumati River, Incomati River system, −25.79999, 31.10000, collected by D. Curle, July 29, 1986. SAIAB 70742, 3 unsexed, 34.70–57.10 mm SL, Houtbosloop, tributary of Crocodile River, Incomati River system, −25.42222, 30.74333, collected by R. Boycott, R. Bills, J. Engelbrecht, April 29, 2003. SAIAB 67330, 1 unsexed, 33.50 mm SL, tributary of Mkhondvo River, Maputo River system, −26.96861, 31.02139, collected by J. Msibi, M. Fakudze, R. Boycott, N. Khumalo, November 6, 2002. SAIAB 76071, 1 unsexed, 27 mm SL, Besterspruit, Mfolozi River system, −27.75056, 30.83222, collected by B. Grant, August 6, 2005. SAIAB 76077, 3 unsexed, 35.00–45.00 mm SL, tributary of Lenjane River, Mfolozi River system, −27.90083, 31.07472, collected by B. Grant, June 8, 2005.

3.8 | Diagnosis

E. niggie sp. nov. belongs to the goldie barb group in southern Africa, which is characterized by species with a soft primary dorsal-fin ray, a relatively short compact body (<70 mm SL), the presence of two pairs of well-developed barbels, 24–30 lateral line scales, and a bright golden breeding colouration in males. Along with *E. niggie* the goldie barb group includes the species *E. pallidus* (Smith 1841), *E. brevipinnis* (Jubb 1966), *E. neefi* s.s. (Greenwood, 1962), *E. thamalakanensis* (Fowler 1935), *E. greenwoodi* (Poll, 1967), *E. lineomaculatus "Malawi"* (Boulenger 1903), and *E. viviparus* (Weber 1897). *E. niggie* and *E. neefi* can be readily distinguished from all the aforementioned species by the presence of distinctive pigmentation along the margins of flank scales that are expressed as wavy parallel lines (Figure 4a,b). Further, *E. niggie* can be distinguished from *E. neefi* by the lack of wavy parallel lines below the lateral line (Figure 4c) and by the lack of dark bold and rounded spots on the dorsal midline of the body (Figure 4d).

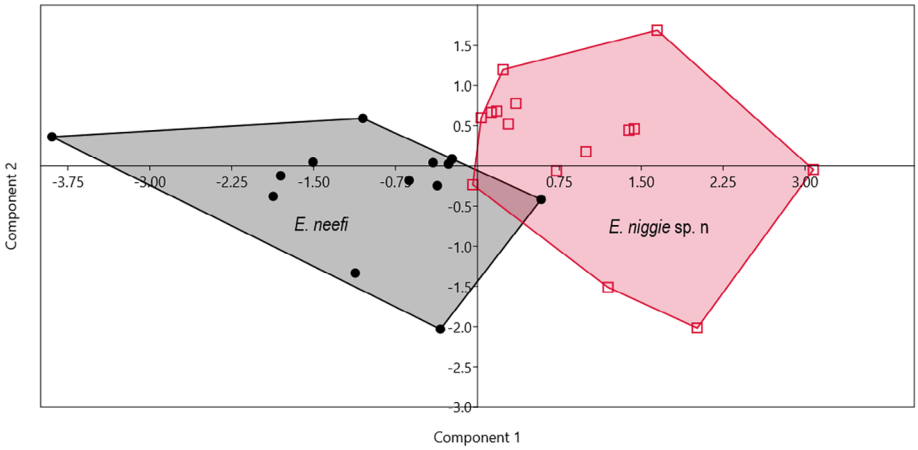

FIGURE 3 Scatterplot of PC1 against PC2 for a PCA (principal component analysis) carried out on 9 meristic characters for 31 specimens of *E. niggie* sp. nov. and *Enteromius neefi*.

FIGURE 4 Lateral view of (a) *Enteromius neefi* (34.6 mm SL) and (b) *Enteromius niggie* sp. nov. (39. 7 mm SL) showing the presence and absence of the wavy parallel lines below the lateral line. Dorsal view of (c) *E. neefi* (34.1 mm SL) and (d) *E. niggie* sp. nov. (34.6 mm SL) showing the presence and absence of the dark rounded spots on the dorsal surface.

FIGURE 5 Alcohol preserved colouration of *Enteromius niggie* sp. nov. (a) NPEJ21-B081 37.9 mm SL (SAIAB 236360) and (b) NPEJ-B080, 41.1 mm SL, holotype (SAIAB 236359).

FIGURE 6 General body features and live colouration of *Enteromius niggie* sp. nov. (a) Male during breeding season, field ID NPEJ21-B081 37.9 mm SL (SAIAB360). (b) Male during non-breeding season, field ID NPEJ21-B080, 41.1 mm SL, holotype (SAIAB 236359).

TABLE 3 Morphometric and meristic data for *Enteromius niggie* sp. nov. and *E. neefi*.

	E. niggie sp. nov.		*E. neefi*		
Character	**Holotype**	**Paratypes**	**Holotype**	**Paratypes**	**Other specimens**
Number of specimens	1	14	1	8	7
Standard length (SL) (mm)	41.1	33.6–44.4	32.2	25.0–37.0	24.4–40.0
Head length (HL) (mm)	10.9	8.4–10.4	8.5	6.9–9.3	6.5–9.9
Caudal peduncle length (CPL) (mm)	9.6	8.5–12.6	7.6	5.7–8.6	6.0–9.5
Percentage of SL (%)					
HL	26.5	21.3–26.1	26.4	24.9–27.9	22.7–26.8
Predorsal length	52.4	48.9–53.8	49.7	51.5–54.8	49.9–53.3
Dorsal-fin base	15.4	12.0–17.2	15.2	10.8–14.5	11.9–15.6
Dorsal-fin height	25.2	22.4–28.1	NA	24.9–28.7	20.0–25.9
Pectoral-fin length	19.9	17.6–21.0	NA	NA	14.8–20.1
Pelvic-fin length	19.0	17.0–19.5	NA	NA	14.4–18.0
Pectoral- to pelvic-fin length	24.9	18.7–28.3	23.6	18.8–24.1	21.0–26.7
Pelvic- to anal-fin length	17.9	14.6–21.9	17.4	14.3–19.6	14.5–21.1
Anal-fin base	8.3	7.5–10.8	6.8	6.4–7.7	5.7–8.7
Anal-fin height	18.9	15.1–19.6	NA	16.2–22.0	14.1–18.4
Body depth	26.9	23.2–28.0	27.6	27.5–30.0	24.8–29.2
Body width	15.8	11.8–15.1	NA	NA	12.9–17.1
CPL	23.4	22.1–26.7	23.6	21.9–25.0	20.0–25.7
Percentage of HL (%)					
Head depth	80.9	77.0–88.0	75.3	74.1–77.5	74.9–91.7
Orbit	32.1	30.8–37.7	36.5	34.4–38.4	29.8–40.8
Inter-orbit	25.7	22.8–34.3	31.8	29.5–35.6	21.2–30.9
Snout length	26.6	20.8–30.8	24.7	21.8–26.9	21.6–25.1
Post-orbit	43.3	30.5–45.5	45.9	42.7–47.4	37.8–46.5
Anterior barbell	23.8	19.8–36.4	31.8	20.3–37.1	12.0–34.2
Posterior barbell	40.0	38.1–51.1	38.8	37.1–49.4	14.9–58.7
Percentage of CPL (%)					
Caudal peduncle depth	54.3	42.4–66.7	61.8	52.3–64.1	51.6–63.3
Meristics					
Unbranched dorsal-fin rays	iii	iii	iii	iii	iii
Branched dorsal-fin rays	8	8	8	8	8
Unbranched anal-fin rays	iii	iii	iii	iii	iii
Branched anal-fin rays	5	5	5	5	5
Pectoral-fin rays	13	12 (8–14)	NA	NA	11 (10–11)
Pelvic-fin rays	8	8 (8–9)	NA	NA	8 (8–9)
Lateral line scales (LL)	30	27 (27–30)	27	27 (26–28)	26 (24–28)
Scale rows between LL and dorsal fin	5	5	5	5	5
Scale rows between LL and pelvic fin	3	3	3	3	3
Scale rows between LL and anal fin	3	3	3	3	3
Circumpeduncular scales	12	12	11	12 (11–12)	12 (11–12)
Predorsal scale rows	12	11 (11–12)	11	12	12 (11–12)
Total vertebrae	32	33 (32–34)	32	32	32
Pre-caudal vertebrae	18	18	18	18	18
Caudal vertebrae	14	15 (14–16)	14	14	14
Predorsal vertebrae	9	9 (8–10)	9	9 (8–9)	8 (8–9)
Pre-anal vertebrae	19	19 (19–20)	19	19	19

3.9 | Description

Proportional measurements and meristic characters are presented in Table 3.

Body fusiform, moderately compressed laterally. Dorsal profile convex from tip of snout to origin of dorsal fin; body depth greatest between dorsal-fin and pelvic-fin origins, tapering from posterior margin of dorsal-fin base to base of caudal fin. Ventral profile slightly concave from tip of snout to anal-fin origin, slightly convex from posterior end of anal-fin base to base of caudal fin.

Head relatively small, head length equal to body depth. Eye relatively large and round, located dorsolaterally, closer to tip of snout than distal margin of operculum. Snout slightly rounded, shorter than post-orbital length; equal to or less than eye diameter; nuptial tubercles absent. Mouth inferior with two pairs of barbels; rostral (anterior) barbels relatively long, extending past the anterior end of eye, length similar to eye diameter; maxillary (posterior) barbels 2.0 times longer than rostral barbels, reaching posterior edge of eye.

Dorsal fin with three unbranched and eight branched rays, last unbranched ray flexible; distal margin almost straight; origin centered vertically with origin of pelvic fins. Pectoral fin with 1 unbranched and 11 branched rays; posterior edge gently rounded, not reaching pelvic-fin origin. Pelvic fin with 1 unbranched and 7 branched rays; posterior edge gently rounded, almost reaching anus; origin closer to anal-fin origin than pectoral-fin origin. Anal fin with three unbranched and five branched rays; distal margin almost straight; origin inserted midway between origin of pelvic fin and base of caudal fin. Caudal fin bifurcate; with one unbranched ray and eight branched rays on each lobe.

Scales moderately large, radiately striated. Lateral line complete, with 27–30 (mode 28) perforated scales in lateral scale series; 5 scale rows between dorsal-fin origin and lateral line; 3 scale rows between pelvic-fin origin and lateral line; 3 scale rows between lateral line and anal-fin origin; 12 circumpeduncular scale rows; 11–12 (mode 12) predorsal scale rows.

3.10 | Colouration

Alcohol preserved colouration (Figure 5). Dorsal and upper half of flank grayish brown, whitish below lateral line scales onto abdomen. Characteristic markings more apparent upon preservation and include a dark spot at the bases of the anal and caudal fins, variable number of rounded dark spots, not exceeding size of single scale along the midline in some specimens. Dark background pigmentation band running along the mid-lateral flank from caudal fin toward but not reaching the head. Wavy parallel lines along scale margins of flank, darker above lateral line to dorsal fin, not present below the lateral line scales. Dorsal portion of head dark gray. Preoperculum off-white. Operculum off-white with small scattered pigmentation markings. Fins uniform white.

Live colouration (Figure 6). Body olive green on dorsal surface and upper flanks, gold around the midline and silver toward and on the belly. Males turn bright golden during breeding season. Characteristic spots on flank inconspicuous. Three wavy parallel lines visible on dorsal surface extending from nape to caudal fin. Dorsal surface of head olive green, preoperculum silver, operculum iridescent gold. Fins are pale olive.

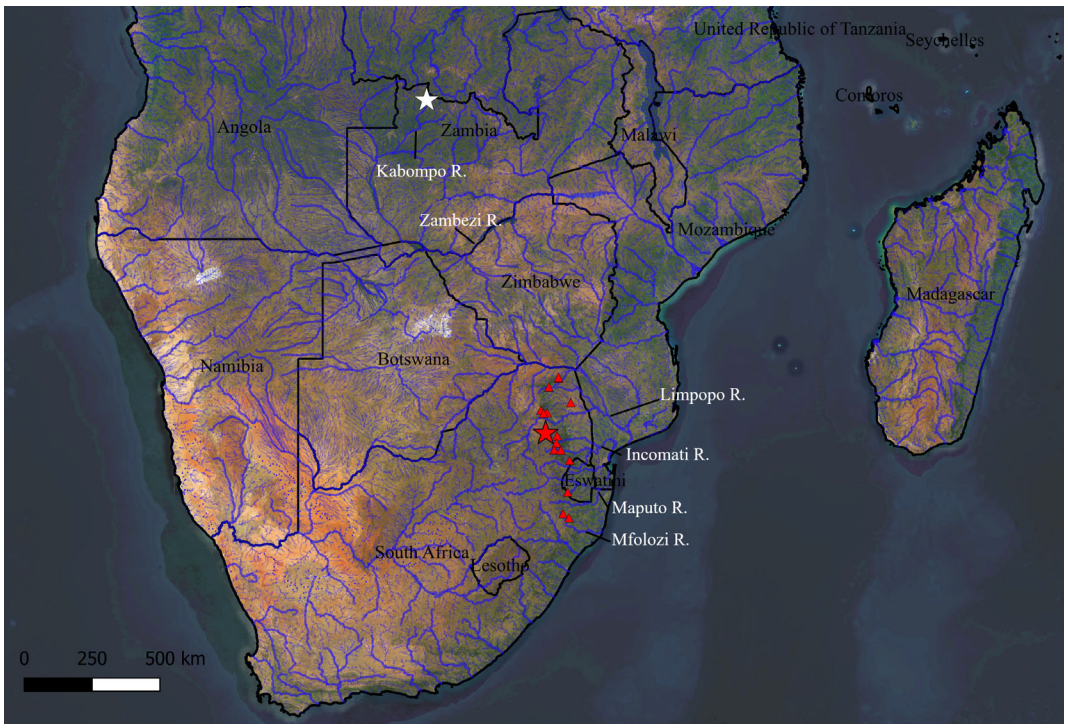

FIGURE 7 Map showing the sampling localities of *Enteromius neefi* (white star) and *Enteromius niggie* (red star). Red triangles represent examined nontype material, showing the likely distribution of *E. niggie*.

3.11 | Distribution

E. niggie is known from escarpment streams of northeastern South Africa and Eswatini, notably in the headwaters of the Limpopo, Incomati, Maputo, and Mfolozi River systems (Figure 7).

3.12 | Etymology

When describing the species *E. neefi*, Greenwood (1962) used the Afrikaans word *neef*, which means "male cousin," a humorous acknowledgment to Graham Bell-Cross (1927–1998) who collected the types of *E. neefi* and often called Greenwood by the Afrikaans word *oom* which means "uncle" (Paul Skelton, personal communication). Therefore, in keeping with Afrikaans familial terms, the use of *nig* [**ˈnəχ**] to name the new species, which means "female cousin," is a symbolic representation of the historical association between these two species, which were considered to represent disjunct populations of the same species.

3.13 | Conservation

The southern sidespot barb was listed as "near threatened" in the most recent IUCN assessment (Roux & Hoffman, 2017). Threats to the survival of this species are mainly associated with farming and logging activities and the presence of alien predatory fish. Agricultural and timber production cause habitat degradation through a combination of water regulation and extraction, which results in sedimentation. Pollution resulting from these industries further increases habitat degradation. Alien predatory fish, specifically largemouth bass *Micropterus salmoides* (Lacepède 1802), and to some extent trout *Oncorhynchus mykiss* (Walbaum 1792), are present in much of the catchments within the distribution range of *E. niggie*. Studies will be required to assess the viability of constructing barriers, which could establish refugia where habitat degradation and the presence of alien predators are limited.

4 | DISCUSSION

There has been considerable confusion regarding the taxonomic distinctiveness of species in the goldie barb group as proposed by Skelton (2001) as some researchers suggested that *E. neefi*, *E. brevipinnis*, and *E. pallidus* may possibly constitute a single widely distributed species (Chakona et al., 2015; Engelbrecht et al., 2002; Engelbrecht & van der Bank, 1996). In the pre-molecular era this confusion was warranted as these species are morphologically similar in many ways. Early molecular work on the goldie barbs by Engelbrecht and van der Bank (1996) and Engelbrecht et al. (2002) employed allozymes to infer relationships among species in the group. Deep genetic divergences were found within *E. brevipinnis* from the northeast escarpment of South Africa, suggesting that this species consists of two lineages, one from the Incomati system and another from the Limpopo system (Waterberg region). Another DNA-based study by Chakona et al. (2015) revealed deep genetic divergence between a "northwestern" (Orange-Vaal system) population and the "southern" (Cape Fold) population of *E. pallidus* based on mitochondrial *cytb* sequence data. Subsequent detailed morphological examination provided evidence that *E. pallidus* s.s. is restricted to the eastern Cape Fold Ecoregion (Martin & Chakona, 2019), indicating that the northern or inland populations that were previously included under this species either represent an undescribed species or belong to one of the known species or species complexes within the goldie barb group. Future surveys will specifically target to collect tissue samples and voucher specimens to determine that taxonomic status of these populations.

Based on molecular evidence from this study, hidden diversity is identified and taxonomic rearrangement of the goldie barb group is proposed. Traditionally, this group included only three species from southern Africa: *E. pallidus*, *E. brevipinnis*, and *E. neefi* (Skelton, 2001). However, molecular data from the present study indicate that *E. viviparus*, *E. thamalakanensis*, *E. greenwoodi*, and *E. niggie*, along with the candidate species *E.* sp. "Bie" and *E.* sp. "Cuebe," also belong to this group. These species are likely to possess the distinctive characteristics that are unique to members of this group. The present study has provided a resolution on the identity of specimens from the northeast escarpment of South Africa that, for a long time, had been attributed to the sidespot barb, *E. neefi*, due to overlap in scale counts and superficial similarities in color pattern. Integration of genetic data and detailed examination of specimens representing the northern and southern populations of *E. neefi* sensu lato provided evidence supporting the recognition of these populations as distinct taxonomic entities, *E. neefi* s.s. confined to the Kabompo and Lualaba rivers, and *E. niggie* endemic to rivers in the northeast escarpment in South Africa and Eswatini.

There is also evidence for additional undocumented diversity within the goldie barb group. Specimens currently attributed to *E. lineomaculatus* in southern Africa are genetically divergent and distantly related to the topotypes of this species in East Africa (Figure 1; Supplementary Information). This indicates that *E. lineomaculatus* s.s., described from northern Tanzania, does not occur in this region, and what is currently referred to as *E. lineomaculatus* in southern Africa represents a species complex. Similarly, two or more distinct lineages were identified within *E. brevipinnis*, *E. greenwoodi*, and *E. thamalakanensis*. Two genetic lineages were found within both *E. greenwoodi* and *E. thamalakanensis*. In each of these cases the distinct groups occur in the same river system and point to hidden diversity within these taxa. Within *E. brevipinnis*, three different genetic lineages were found, supporting the pattern found by Engelbrecht and Van der Bank (1996) who suggested the presence of multiple lineages, each of them occurring in different rivers and not closely related to each other. *E. brevipinnis* from the type locality (Sabie River) is sister to *E. viviparus*, and the *E. brevipinnis* "Blyde" lineage is sister to *E. niggie*, and both are closely related to *E. brevipinnis* "Waterberg." Although no detailed morphometric study has been done on the different *E. brevipinnis* lineages, there are preliminary indications of color

pattern differences that, along with the genetic differences, point to multiple species within what is currently considered a single species. There is ongoing work assessing the taxonomic distinctiveness of the lineages recovered within the *E. brevipinnis* complex.

Additional undocumented diversity was identified within the other soft-rayed species that were included for comparison in the present study. These include *E. atkinsoni* in clade A, a species that was described from the Rufiji River system that comprises at least two genetically distinct OTUs with strong geographic affinities. The topotype samples for this species are genetically differentiated from samples from the Ruo, Phalombe, Shire, and Montepuez rivers in Malawi and Mozambique. The latter lineages are likely to represent distinct taxonomic entities, but detailed morphological examination is required to identify diagnostic characters that separate these OTUs. Similarly, specimens from the Incomati, Zambezi, Montepuez, and Phalombe rivers that are currently attributed to *E. viviparus* were found to represent two genetically divergent OTUs that are distantly related to *E. viviparus* topotypes from the Mdloti River system. The clade (clade B) containing these two OTUs was recovered as the sister lineage to the goldie barb clade (clade C). Further taxonomic evaluation is required to establish the degree of morphological divergence between these OTUs and their distinctiveness from *E. viviparus* s.s. Although it is likely that clades A and B may belong to the goldie barb group, currently no information is available regarding the breeding colouration of males for *E. atkinsoni* and *Enteromius* cf. *viviparus*. Further, the relationships between the three clades were unclear (PP 56–78) and may need additional genetic markers in future to fully determine, but a more expanded goldie barb clade is likely.

AUTHOR CONTRIBUTIONS

Albert Chakona, Pedro H. N. Bragança, and Martinus Scheepers conceptualized the study and generated the data. Martinus Scheepers analysed the data and prepared drafts of the manuscript. Albert Chakona and Pedro H. N. Bragança contributed to substantial revisions of the manuscript.

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SUPPORTING INFORMATION

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