

Conservation of evolutionary patterns and processes in the Maloti minnow, (*Pseudobarbus quathlambae*, Cyprinidae, Smiliogastrini) a narrow-range stream fish imperiled by water transfer scheme developments in the Lesotho Highlands

Ernst R. Swartz¹, Pedro H. N. Bragança¹, Johannes L. Rall¹, Skhumbuzo P. Kubheka², Paulette Bloomer³, Paul H. Skelton¹, Albert Chakona^{1,4,*}

¹South African Institute for Aquatic Biodiversity, Private Bag 1015, Makhanda (Grahamstown), 6140, South Africa

²Ezemvelo KZN Wildlife, 1 Peter Brown Drive, Montrose 3202, South Africa

³Department of Genetics, University of Pretoria, 0002, Pretoria, South Africa.

⁴Department of Ichthyology and Fisheries Science, Rhodes University, Makhanda (Grahamstown), 6140, South Africa

*Corresponding author: a.chakona@saiab.nrf.ac.za

Postal address: South African Institute for Aquatic Biodiversity, Private Bag 1015, Makhanda (Grahamstown), 6140, South Africa

Abstract

Pseudobarbus quathlambae (Barnard, 1938) is a cyprinid minnow with a disjunct distribution divided into tributaries of the Upper Orange River system in the Lesotho Highlands and the Mzimkhulu River system in KwaZulu Natal Province (KZN), South Africa. Recent records in the Mzimkhulu River system extended the species' geographic range, and this could represent the last remnant population of this species in South Africa. However, its future existence is in doubt, because of continued threats to all the remnant populations by non-native fishes, and more recently by the building of large, interconnected dams as part of the Lesotho Highlands Water Project (LHWP). One of these dams has directly affected the Mohale population in central Lesotho, which was the largest of the seven extant populations. Analysis of mitochondrial control region sequences (534 bp) revealed considerable genetic differentiation (2.3–6.4%) and geographic structuring among the remnant populations. The phylogenetic analysis identified three lineages within *P. quathlambae*: (i) one in KZN, (ii) a second one in eastern Lesotho, and (iii) a third one in Mohale, central Lesotho. The deep genetic divergence and pronounced geographic structuring among the populations suggests a long period of isolation, indicating they represent unique evolutionary units.

Keywords: conservation, Drakensberg, endemism, redbin, southern Africa

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Introduction

Water is arguably the most limiting resource in South Africa's drive to be a fast growing developmental state (Blignaut & van Heerden, 2009). Recognising this, South Africa entered into agreements to develop large reservoirs in the highlands of the Kingdom of Lesotho and to import the water through a network of inter-basin transfer schemes to eventually reach the Gauteng Province, which is a large and critical economic hub in Africa. When completed, the Lesotho Highlands Water Project (LHWP) will be the largest water development scheme in Africa, with a total of five mega reservoirs and at least four Inter-basin Transfer tunnels. Given the magnitude of this engineering infrastructure development, the ecological impact on aquatic biodiversity, such as hydrological modification and translocation of non-native species, particularly on the narrow-range endemic Maloti minnow, *Pseudobarbus quathlambae* (Barnard, 1938a), is inevitable.

Pseudobarbus quathlambae has been one of the flagship species for conservation in southern Africa, particularly in Lesotho as it is the only endemic vertebrate in this country (Skelton, 2000). The species was first discovered in the Mkhomazana River (Mkhomazi River system) at the foot of the Drakensberg Mountains in the KwaZulu-Natal Province of South Africa (Barnard, 1938a; Jubb, 1966; **Fig. 1**) where it was reportedly abundant in 1938 (Pike & Tedder, 1973). However, predation and competition from trout (*Salmo trutta* Linnaeus, 1758 and *Oncorhynchus mykiss* (Walbaum, 1792)) introduced into the Mkhomazana River around 1910 – 1920, and again in 1926 and 1927 caused the extinction of this population and therefore the species was feared extinct as well (Jubb, 1966; Pike & Karssing, 1995). In 1970 the species was rediscovered in the Tsoelikane River (Sethlabathebe National Park, eastern Lesotho) (Pike & Tedder, 1973). With the aim of promoting survival of the species in the wild, these authors introduced 56 individuals of *P. quathlambae* above the Tsoelikane waterfall in 1973 (**Fig. 1**). This was done to ensure the survival of the species in the upper reaches of the Tsoelikane River in face of the threat represented by the non-native rainbow trout which were abundant downstream. Additional populations of *P. quathlambae* were discovered in the upper Senqu and Moremoholo rivers in 1975 (Rondorf, 1976), followed by the Mohale River population (Senqunyani catchment) in 1987 (Cambray & Meyer, 1987), the Sani River population in 1988 (Skelton, 2000) and the Matsoku River population in 2000 (see **Fig. 1**). Waterfalls protect the Mohale, Senqu, Moremoholo and the translocated Tsoelikane populations from invasion by trout, whereas the Matsoku, Sani and the original Tsoelikane populations co-occur with trout. More recently, *P. quathlambae* was rediscovered in South Africa, in the Mzimkhulu River, which is adjacent to the Mkhomazi River where the species has gone extinct (Kubheka et al., 2017). The discovery of the Maloti minnow in the Mzimkhulu River suggests that this species was historically widespread in the Drakensberg streams prior to the introduction of trout (Kubheka et al., 2017; Skelton, 1988). The discovered population has a highly restricted distribution range which makes it highly susceptible to impacts such as introduction of non-native piscivores, habitat degradation and alteration of flow regimes through excessive water abstraction.

The distribution of *P. quathlambae* represents an anomaly within the genus. It is the only redfin species that is not associated with the Cape Fold Ecoregion as it is isolated in the Drakensberg-Maloti Highlands and the Southern Temperate freshwater ecoregions (Abell et al., 2008; Skelton, 1974, 1988). This geographic isolation is reflected in its phylogenetic relationships, because it represents the earliest diverging lineage within the genus and genetically it is the most divergent *Pseudobarbus* species (Swartz et al., 2009). Compared to the other redfins that generally occur in altitudes below 1000 meters above sea level (asl), *P. quathlambae* populations in the Lesotho Highlands occur at much higher altitudes of about 1950 to 2760 meters asl (Rall, 1993; Rondorf, 1976). At these altitudes, *P. quathlambae* is exposed to extreme daily temperature shifts in the shallow low order streams that often freeze in winter. In contrast, the extinct Mkhomazana population occurred in a less extreme environment at a lower altitude around the type locality at approximately 1615 meters asl (Barnard, 1938a), while the recently discovered population in the Mzimkhulu River system is known from altitudes between 1000 and 1200 metres asl (Kubheka et al., 2017). All the Lesotho Highlands populations of *P. quathlambae* occur in clear water rivers flowing over Drakensberg basalt, except for the Tsoelikane River that flows over sandstone of the Clarens

Formation (Keyser, 1998). The streams where the Maloti minnow was collected in the Mzimkhulu River system had clear to moderately milky water and the river flow over sandstones and mudstones.

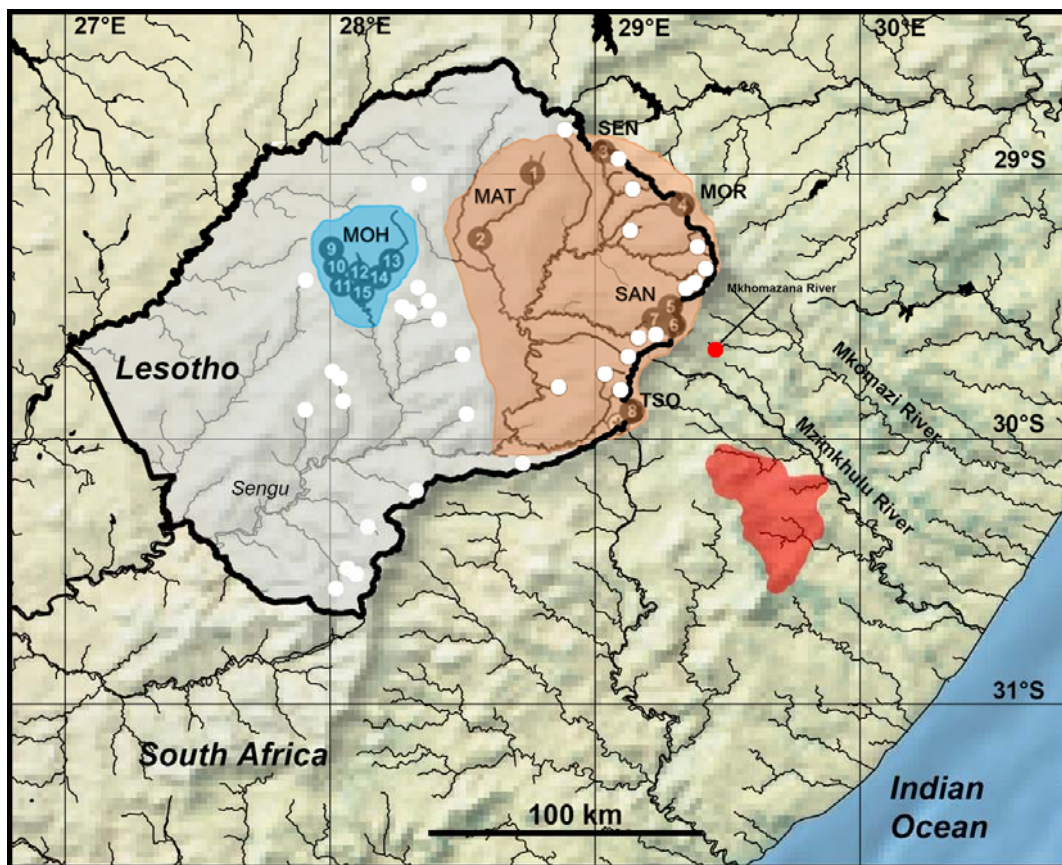


Fig. 1. Maps showing the extant populations of *Pseudobarbus quathlambae* in rivers draining the Lesotho Highlands (solid numbered circles), the type locality in the Mkhomazi River system and a recently discovered population in the Mzimkhulu River system (exact locality not shown due to conservation sensitivities) in KwaZulu Natal (KZN) Province of South Africa. Open circles show localities where Skelton *et al.* (2001) failed to record *P. quathlambae*. Matsoku (MAT), Senqu (SEN), Moremoholo (MOR), Sani (SAN), Tsoelikane (TSO) and Mohale (MOH) are the only remaining populations of this species. The open circles with asterisks show where known extinctions have occurred (Skelton *et al.* 2001; Pike & Karssing 1995; Jubb 1966).

The geographic range of the Mohale population, isolated above the 20m high Semonkoang waterfall, historically represented 70 % of the extent of occurrence of the species (Skelton *et al.*, 2001). A large impoundment called Mohale Dam was built in this catchment in 2003, and an Inter-Basin Transfer (IBT) tunnel was constructed to link it and the Katse Dam as part of the LHWP. The IBT facilitated the spread of non-native and extra-limital species from Katse Dam into the upper Mohale catchment. Recently, Shelton *et al.* (2016) conducted a survey in the Mohale catchment and reported that this population of Maloti minnows was nearing extinction as only five individuals were recorded from a total of 32 sampled sites, compared to more than 250 individuals recorded from the same localities in 1995 (Steyn *et al.*, 1996).

Additional impoundments, the Polihali and Tsoelikane, are proposed to be built in the upper and lower Senqu River catchment (**Fig. 1**). This second phase of the LHWP commenced in 2021 with the construction of the Polihali Dam which is expected to be completed in 2025 and will increase water supply

to South Africa by 780 million cubic meters per year. All impoundments of the LHWP will be linked by a series of IBT tunnels that will deliver water to the Vaal River to reach the development hub of Gauteng Province, which is the largest economic block in Africa. There are concerns about the impact of: (i) the loss of riverine habitat through conversion to lentic habitats, (ii) spread of non-native and extra-limital fishes through the IBTs and their likely establishment through creation of favourable lentic habitats, and, (iii) possible mixing of potentially historically isolated populations of *P. quathlambae* due to breaking of geographical barriers through creation of the IBTs.

A fundamental requirement for sound biodiversity conservation and management is to maintain the long-term evolutionary potential of species by allowing the processes that shaped current intraspecific diversity to continue into the future (Crandall et al., 2000; Moritz, 1999, 2002). The objective of the present study was to assess the degree of genetic differentiation and geographic structuring between and within extant *P. quathlambae* populations in KZN and in the Lesotho Highlands by sequencing and analysing the mtDNA control region. We acknowledge that, as a single locus marker, the mtDNA control region does not adequately reflect species level evolutionary history (Heled & Drummond, 2008; Moritz & Cicero, 2004). However, mitochondrial genes are the currently available molecular markers that are more suitable for studying inter and intraspecific processes in polyploid vertebrates, including the redfin minnows of the genus *Pseudobarbus* which are tetraploid (Naran et al., 2006; Oellermann & Skelton, 1990; Tsigenopoulos et al., 2002). Despite being based on a single locus marker, our results provide important insights that are crucial for formulating science-based conservation management preventing the loss of unique evolutionary units within the Maloti minnow.

Materials and Methods

Surveys and sampling

In response to the threat posed by the LHWP, a major survey was done from 27 September to 6 October 2000 to assess the distribution and conservation status of *P. quathlambae* in the Lesotho Highlands (Skelton et al., 2001). This survey collected samples of this species from the Matsoku, Senqu, Moremoholo, Sani, Tsoelikane and Senqunyani rivers. Another major survey was undertaken from 27 June to 30 July 2002 in the Jordane, Bokong and Senqunyani rivers by Rall et al. (2002). Localities for these surveys were selected based on the habitat requirements of this species (Rall, 1993). A strong emphasis was placed on localities in rivers where geographic gradients are less than 1:40 (P. H. Skelton & S. Mashapa, unpublished) and where waterfalls have potentially prevented invasion by trout (Skelton et al., 2001). A helicopter was used to reach the remote localities and a total of 39 rivers and 47 localities were surveyed in Lesotho's Senqu River system (called Orange/Gariep in South Africa).

Specimens were collected using Deca electro-shockers during the surveys of Skelton et al. (2001) and Rall et al. (2002). Muscle or whole fish samples were stored in liquid nitrogen in the field and transferred to a –70 °C freezer upon returning to the laboratory or whole fish samples were placed in ethanol (Department of Genetics, University of Pretoria). In addition, whole fish samples and samples from which muscle tissue was dissected, were fixed in formalin and deposited in the National Fish Collection Facility at the South African Institute for Aquatic Biodiversity (NRF-SAIAB) as voucher specimens (SAIAB 62656, 63392, 63394, 63399, 63408-9, 63414 and 63416-17).

Samples were also collected in 2017 from one locality representing the recently discovered population in the Mzimkhulu River system in the KwaZulu-Natal Province of South Africa (Kubheka et al., 2017; **Fig. 1**). A SAMUS electro-shocker was used for sample collection during the recent survey of Kubheka et al. (2017). A subsample of the captured fish was anaesthetized with clove oil and a small piece of muscle tissue was dissected and preserved in 95% ethanol for genetic analysis. The source specimens were fixed in formalin and deposited at NRF-SAIAB (SAIAB 204589). The 2017 surveys were carried out following the evaluation and approval of the sampling protocols by the South African Institute for Aquatic

Biodiversity (NRF-SAIAB) ethics committee (Ref: 2014/03). Permits to carry out this research were obtained from Ezemvelo KZN Wildlife.

DNA extraction, amplification and sequencing

Total DNA was isolated from frozen specimens or ethanol preserved tissue using the chemical digestion and phenol/chloroform extraction protocol (Sambrook et al., 1989), or the salting out method (Sunnucks and Hales, 1996). The mitochondrial control region was amplified using the primers L16560 and H677 developed by Swartz et al. (2007) and following the PCR protocol described in this same study. PCR products were purified with Exosap (Applied Biosystems), cycle-sequenced using BigDye Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and sequenced using an ABI 377 or ABI 3730xl genetic analyser (Applied Biosystems). These sequences were uploaded to GenBank in 2004 under accession numbers AY791701 to AY791807. For the recently collected samples from KZN, DNA was extracted from preserved tissue using the salting out method (Sunnucks and Hales, 1996). The mitochondrial control region was amplified using the same set of primers and protocol as described above. PCR products were purified with Exosap (Applied Biosystems), cycle-sequenced using BigDye Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and sequenced at NRF-SAIAB using an ABI 3730xl DNA Analyzer (Applied Biosystems). The seven newly generated sequences were submitted to GenBank, (accession numbers: OP413900 – OP413906; Appendix S1).

Data analyses

Gaps in the aligned sequences were excluded prior to data analyses. To delineate the relationships among unique haplotypes, Bayesian phylogenetic tree was inferred based on the selected model of sequence evolution. JModeltest (Darriba et al., 2012) inferred the TPM1uf+I+G as the best model of sequence evolution based on the Akaike's Information Criteria (AIC) (Burnham & Anderson 2002). We included 16 sequences from other *Pseudobarbus* species to show relationships of *P. quathlambae* with congeners from the Cape Fold Ecoregion (CFE) (see Appendix S1). *Sedercypris calidus* (Barnard, 1938b) was used as outgroup. Bayesian Inference was performed using the Bayesian Markov Chain Monte Carlo (BMCMC) algorithm implemented in MrBayes 3.1.2 (Ronquist et al., 2012). Each analysis was run across four chains for ten million generations and sampled every 1000th generation to obtain 10000 sampled trees. The burn-in value was determined by plotting the average standard deviation of split frequencies, tree length and log-likelihood scores against generation time using the program TRACER ver 1.6 (Rambaut et al., 2014). The first 2500 trees were discarded as burn-in and the remaining trees were used to calculate Bayesian posterior probabilities.

Model-corrected genetic distances between unique lineages identified within *P. quathlambae* and among these lineages and other species within the genus *Pseudobarbus* were calculated using PAUP* (Swofford, 2003). Haplotype and nucleotide diversity were calculated for each lineage identified within *P. quathlambae* using DnaSP v5 (Librado & Rozas, 2009).

A haplotype network was inferred, using the Median Joining Network method in PopART 1.7 (Leigh & Bryant, 2015), including all *P. quathlambae* sequences (n = 114). The haplotypes were assigned to their respective populations within one of the three main geographical areas: (1) Mohale, in central Lesotho; (2) eastern Lesotho; (3) and KwaZulu-Natal Province (KZN) in South Africa. Within the Mohale region there are seven populations: the Jordane, Jordane below Hatiti, Senqunyani, Bokong, Sequenyani above Bokong confluence, lower Jordane and Mohale; within the Eastern Lesotho there are five populations, Matsoku, Maremoholo, Sani, Senqu and Tsoelikane; and, the KZN region is represented by the Mzimkhulu River population (Appendix S1).

Results

Surveys

Samples were successfully collected and knowledge regarding the range of the six extant populations was improved, but no new *P. quathlambae* populations were discovered in Lesotho (**Fig. 1**). The persistence of the translocated population above the Tsoelikane waterfall was confirmed. Only a single *P. quathlambae* individual was found below this waterfall amongst a population of rainbow trout. The Tsoelikane population has therefore, for all practical reasons, been extirpated from their natural range. Recent surveys of the Mkhomazana River, a tributary of the Mkhomazi River system, failed to find any specimens of *P. quathlambae*, confirming previous assertions that this population is likely extinct. The recently discovered records in the Mzimkhulu River system extended the known historical geographic range of the Maloti minnow, and this could represent the last remnant population of this species in South Africa (Kubheka et al., 2017; **Fig.1**).

Phylogenetic estimation, lineage delineation and haplotype network

The alignment of the 114 D-loop sequences of *P. quathlambae*, comprising 107 sequences of the Lesotho Highlands population downloaded from GenBank and seven newly generated sequences for the recently discovered population in the Mzimkhulu River system had a total of 534 bp. This region had a total of 34 variable sites which defined 23 haplotypes for *P. quathlambae* (**Fig. 2**). In the phylogenetic and haplotype network analyses, *P. quathlambae* haplotypes clustered in three distinct lineages (**Figs. 2, 3**). One lineage is restricted to the Mzimkhulu River in KZN, whereas samples from the Lesotho Highlands were split into two lineages. The eastern Lesotho contained haplotypes from the Tsoelikane, Sani, Moremoholo, Senqu and Matsoku rivers which drain the eastern and northern sections of Lesotho. The Mohale lineage contained haplotypes from the Mohale, Jorodane, Bokong and Senqunyani rivers from central Lesotho.

The Mzimkhulu River population, represented by a unique haplotype, can be distinguished from the eastern Lesotho cluster by the possession of 19 unique nucleotides, whereas the eastern Lesotho cluster, is more genetically diverse, including 14 distinct haplotypes within the five populations sampled. Geographically, the distribution of the eastern Lesotho haplotypes indicate ongoing connectivity between all populations, except the Tsoelikane population that includes three haplotypes not shared with the other eastern Lesotho populations (**Fig. 3**). The Mohale cluster, represented by eight haplotypes within the five sampled populations, can be distinguished from the eastern Lesotho cluster by the possession of 10 different nucleotide substitutions. Similar to the pattern seen in the eastern Lesotho cluster, within the Mohale cluster the haplotype network indicates an ongoing connectivity between its different populations, with only one haplotype (from Bokong) not shared between populations (**Fig. 3**).

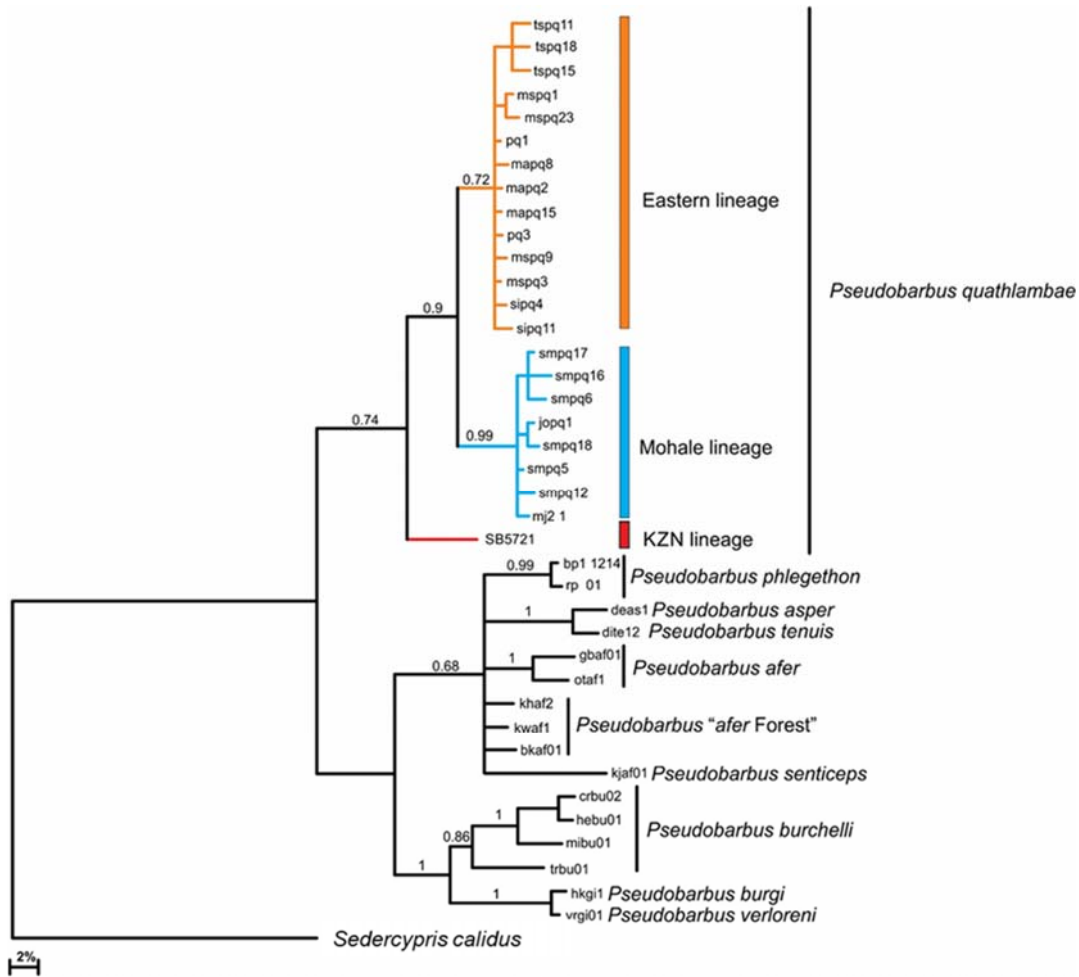


Fig. 2. Bayesian phylogenetic tree based on unique haplotypes of the mtDNA control region sequences showing the candidate species or operational taxonomic units (OTUs) identified within *Pseudobarbus quathlambae* from the Lesotho Highlands and the Mzimkhulu River system in KwaZulu Natal province in South Africa. The colours of the lineages correspond to the colours depicted in Fig 1 showing the distribution ranges for each lineage.

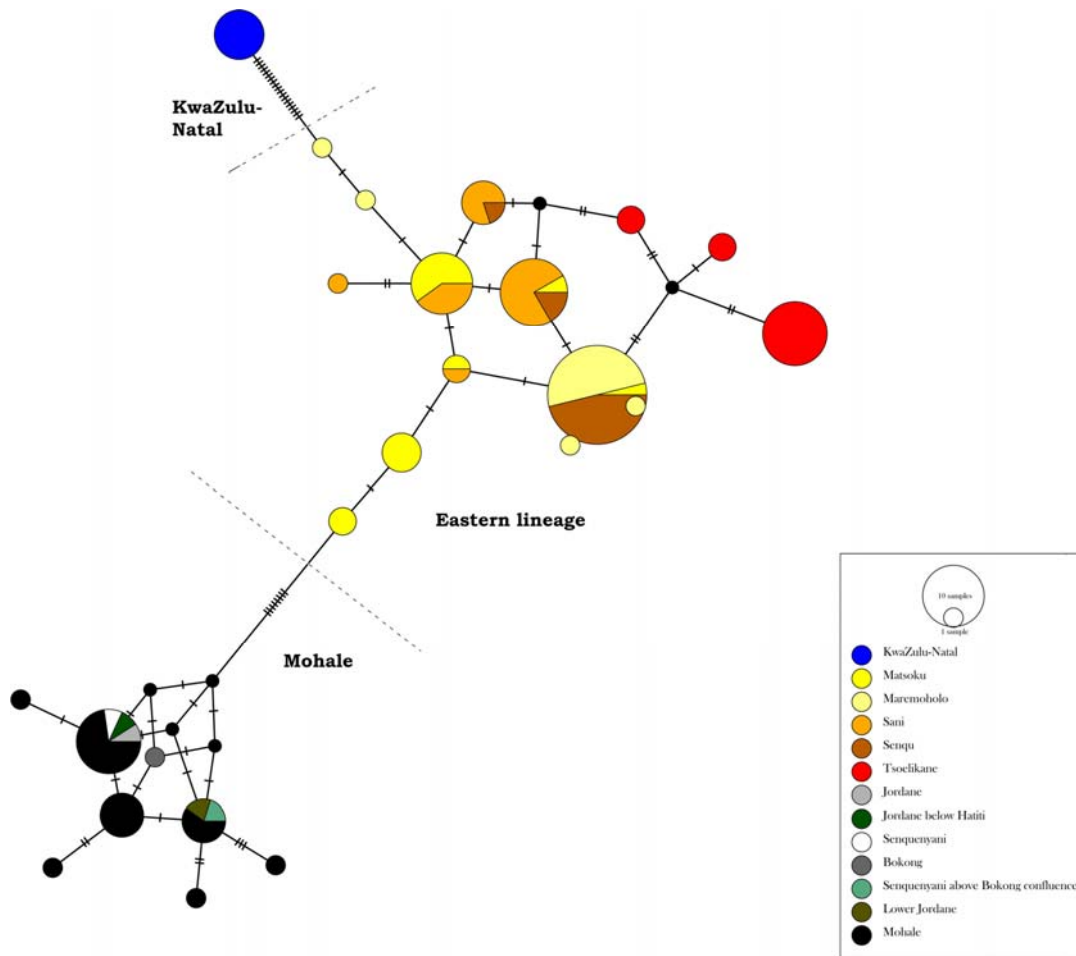


Fig. 3. Median Joining Network for 114 sequences of *Pseudobarbus quathlambae* generated using PopART 1.7 (Leigh and Bryant, 2015).

Genetic divergences, diversity indices and population differentiation

We found considerable genetic divergence among the three lineages (2.3 – 6.1%), with comparatively shallow intra-lineage divergences (0.0 – 1.8%) (Table 1). The divergence values among them are consistent with those recorded between morphologically defined congeneric species from the CFE, for example between the single barbeled redfins, *P. tenuis* (Barnard, 1938b) and *P. asper* (Boulenger, 1911), and at least five-fold higher than the divergence between the double barbeled redfins, *P. burgi* (Boulenger, 1911) and *P. verloreani* Chakona, Swartz & Skelton 2014 (Table 1). Nucleotide diversity (π) and haplotype diversity (Hd) were higher in the eastern Lesotho lineage ($N = 81$; $Nh = 14$; $\pi = 0.0048$; $Hd = 0.834$) and in the Mohale lineage ($N = 26$; $Nh = 8$; $\pi = 0.0030$; $Hd = 0.769$) compared to the KZN lineage ($N = 7$; $Nh = 1$; $\pi = 0$; $Hd = 0$). However, due to the fact that all specimens from KZN were collected from a single locality, it is not possible to make robust conclusions on the generic diversity of this population. Within the eastern Lesotho lineage, these diversity indices were highest in the Matsoku and Sani River populations, whereas in the Mohale lineage, the Mohale River population had higher nucleotide and haplotype diversity compared to the other populations (Table 2). The ϕ_{ST} values between the Mohale population and the rest of the eastern Lesotho populations were considerably large and significant, indicating a high degree of differentiation among them (Table 2). The Tsoelikane population was the most differentiated among the eastern Lesotho populations as indicated by the high ϕ_{ST} values (Table 2).

Table 1 Genetic divergences (%) between the KwaZulu Natal (KZN), eastern Lesotho and Mohale lineages of *P. quathlambae* and comparison with congeners from the Cape Fold ecoregion (CFE) of South Africa

	KZN	Eastern Lesotho	Mohale	<i>afer</i>	<i>senticeps</i>	<i>asper</i>	<i>tenuis</i>	<i>phlegethon</i>	<i>burgi</i>	<i>verloreni</i>
KZN	0.00									
Eastern Lesotho	4.3–6.1	0.0–1.8								
Mohale	5.1–6.4	2.3–4.7	0.0–1.3							
<i>P. afer</i>	11.2–11.4	9.3–11.6	10.1–12.1	0.0–2.3						
<i>P. senticeps</i>	14.0	11.3–13.5	12.3–14.5	7.2–8.1	0.0					
<i>P. asper</i>	13.4	10.3–12.0	11.7–12.9	6.4–7.0	7.4	0.0				
<i>P. tenuis</i>	13.0	8.9–10.6	9.8–10.9	7.3–7.4	7.7	1.7	0.0			
<i>P. phlegethon</i>	12.1–12.5	8.4–11.1	10.4–12.5	5.7–6.1	6.6–6.9	6.4–6.8	6.5–6.8	0.0–0.2		
<i>P. burgi</i>	11.6	9.6–11.7	12.8–14.3	11.3–11.7	13.0	13.2	12.0	13.2–13.7	0.0	
<i>P. verloreni</i>	10.6	9.1–11.5	11.7–14.0	10.3–10.7	11.9	13.0	11.8	12.1–12.5	0.4	0.0

Table 2 Pairwise population ϕ_{ST} , nucleotide (π) and haplotype diversity (Hd) values for *P. quathlambae* from six populations in the Lesotho Highlands

		ϕ_{ST}					Genetic indices		
		1	2	3	4	5	N	π	Hd
Eastern lineage							81	0.0048	0.834
Mohale lineage							26	0.0030	0.769
1	Matsoku population						15	0.0030	0.790
2	Senqu population	0.435					15	0.0011	0.362
3	Moremoholo population	0.398	– 0.019				18	0.0017	0.331
4	Sani population	0.259	0.417	0.426			19	0.0022	0.719
5	Tsolikane population	0.728	0.738	0.718	0.738		15	0.0030	0.457
6	Mohale population	0.889	0.920	0.915	0.906	0.896	20	0.0032	0.784

For the F-statistics, only the comparisons between the Senqu and Moremoholo populations were not significant ($P > 0.05$). All the other ϕ_{ST} values were highly significant ($P = 0.000$)

Discussion

The present study clarified a long standing question of whether *P. quathlambae* ever actually occurred in South Africa. This uncertainty was raised following successive failures to sample specimens of this species from the type locality in the Mkomazana River, a tributary of the Mkomazi River system, in KZN (Crass, 1960, 1964; Pike & Karssing, 1995), resulting in some individuals (e.g. Crass, 1977, 1985) expressing doubts about the original locality of the specimens that were used by Barnard (1938a) for the description of this species. Crass (1977) proposed that the original specimens were likely collected from the Lesotho Highlands where extant populations were known to occur in the Tsoelikane, upper Senqu, Moremoholo, Mohale and the Sani rivers (Cambrey & Meyer, 1987; Pike & Tedder, 1973; Rondorf, 1976; Skelton, 2000). However, Skelton (1988) contested this hypothesis and asserted that the Mkomazana River was the type locality of *P. quathlambae*, surmising that predation by trout was the likely cause for the extirpation of this species from the type locality. The recent discovery of *P. quathlambae* in the Mzimkhulu River system which is adjacent to the Mkomazana River system in KZN presented an opportunity to test for evidence of either historical isolation of this recently discovered population or recent human mediated translocation of individuals from one of the Lesotho Highlands populations.

Our results revealed three distinct lineages with strong geographic affinities within *P. quathlambae*. The first lineage contained a unique haplotype restricted to the Mzimkhulu River system in KZN. The other two lineages, the eastern Lesotho and Mohale, comprised haplotypes restricted to tributaries of the upper Orange River system in the Lesotho Highlands. The presence of distinct lineages, geographically restricted haplotypes, and relatively deep genetic divergences between the KZN and Lesotho Highlands lineages refute Crass's (1977, 1985) proposition that this species did not naturally occur

in South Africa. These results support previous suggestions that historically this species had a widespread distribution in the Drakensberg streams of KZN, but these populations were extirpated following the introduction of trout (Jubb, 1966, 1983; Skelton, 1987).

Given that the Maloti minnow was originally described from specimens that were collected from the Mkhomazana River in KZN, the Mzimkhulu lineage is likely to represent *P. quathlambae* sensu stricto, while the Mohale and eastern lineages in the Lesotho Highlands represent new species that were previously unrecognised by science. The authors have initiated detailed morphological and osteological examination, including efforts to generate sequences from the syntypes of *P. quathlambae* from the Mkhomazana River, in order to determine the taxonomic distinctiveness of the lineages identified within the Maloti minnow.

Biogeographical patterns of Pseudobarbus quathlambae

The occurrence of *P. quathlambae* in the eastward draining Mzimkhulu and Mkomaz river systems which discharge into the Indian Ocean, as well as in the headwater tributaries of the westward draining Orange River system which discharges into the Atlantic Ocean is intriguing, because it is the only species that has this distribution pattern in the region (see Skelton, 2001). Biogeographic patterns of freshwater fishes are mainly linked to changes in river channel networks which may result in range expansion, followed by vicariance and speciation (Bishop, 1995; Burridge et al., 2006, 2007; Camelier et al., 2018).

In southern Africa, geological evidence indicates that the present day elevation of much of the subcontinent resulted from a relatively recent uplift that occurred during the Pliocene (Partridge, 1998). This uplift rejuvenated head-ward erosion processes which was more pronounced for the eastward draining rivers in the KwaZulu Natal Province of South Africa (King, 1944; Partridge, 1998). In particular, erosion of the weak Upper Ecca shales of the Drakensberg resulted in complex drainage reorganisations and captures of several headwater tributaries of the Orange River system by a number of eastward draining coastal rivers (King, 1944). Some of the most striking examples include the capture of the headwaters of the Khubedu River (headwaters of the Orange) by the Tugela River which led to the formation of the Tugela Falls, and the capture of the headwaters of the Slang by the Pongola and Pivaan rivers (King, 1944). Projections indicate that a large proportion of the upper Orange drainage will be captured by the eastward draining Umzimvubu River (King, 1944). The presence of depositional landforms adjacent to the Mkhomazana River in the lower sections of Sani Pass has been also interpreted as evidence of active head-ward erosion which has also been observed for a number of other river systems associated with the Drakensberg (Meiklejohn et al., 1999). Thus, river capture events could have potentially played a role in transferring some populations of *P. quathlambae* from headwater tributaries of the Orange River system to the headwaters of the Mzimkhulu and Mkomaz river systems. *Pseudobarbus quathlambae* is the only species that occurs in high altitude tributaries of the upper Orange River in the Lesotho Highlands, which, generally, increases susceptibility to transfer through river capture events (Albert et al., 2018).

Comparison of genetic patterns in the Lesotho Highlands lineages

Despite being part of the same river system, we found substantial genetic differentiation between the Mohale and the five eastern Lesotho populations, which were separated into two distinct lineages. This pattern in which the eastern Lesotho and the Mohale populations show clear signatures of historical isolation is expected when considering the large geographic distance and the lack of suitable habitat for this species between these distributional regions. The haplotype network also provided evidence of genetic structuring within the eastern Lesotho lineage as the Tsoelikane population did not share haplotypes with the other four north-eastern Lesotho populations (i.e. the Sani, Moremoholo, Senqu and Matsoku). However, the low differentiation between the Tsoelikane and the north-eastern Lesotho populations suggests that this isolation has been much more recent compared to the separation between the Mohale and the eastern Lesotho lineages. Genetic patterns among the north-eastern Lesotho populations are complex.

There were several haplotypes that were restricted to certain localities, but all the localities also shared haplotypes, suggesting more recent connectivity among these populations.

Conservation of Pseudobarbus quathlambae

The most important evolutionary processes that must be allowed to continue within *P. quathlambae* are relatively simple to manage. The differentiation between the Mohale and eastern lineages requires that they remain isolated, provided that population size remains large. The same applies to the Tsoelikane population which is differentiated from the other populations within the eastern lineage. Securing the survival of the Mohale lineage will be difficult due to the hydrological impact of the Mohale Dam and the introduction of alien *Labeobarbus aeneus* (Burchell, 1822) (Shelton et al., 2016). Until recently, this lineage contributed significantly to the overall genetic diversity of the species and was the largest population, but Shelton et al. (2016) indicated that this population had crashed. Persistence of this lineage is now dependent on the survival of translocated populations in the Maletsunyane and Jordane rivers, and the conservation efforts being undertaken by Lesotho Highlands Developmental Authority (LHDA). Based on the findings of the present study and the survey of Skelton et al. (2001), several measures, including translocation to sanctuary sites, were undertaken to conserve the Mohale lineage (Rall et al., 2002). Even though the Tsoelikane population is not as divergent from the remaining north-eastern Lesotho populations as the Mohale lineage, it should be managed in a similar way, since the underlying evolutionary process is isolation. Eradication of trout downstream will allow *P. quathlambae* to re-occupy its former natural distribution range in this catchment. As part of an integrated conservation management plan for *P. quathlambae*, a translocation program was designed on the basis of research carried out for the Lesotho Highlands Developmental Authority Contract 1041. In 2002, after impact assessments were completed, several sanctuaries were established in the highlands of Lesotho to ensure the survival of the Mohale population.

Similarly, the recently discovered lineage of *P. quathlambae* in KZN also needs to be managed as a separate conservation unit as it is distinct from the other extant populations from the Lesotho Highlands. Immediate conservation measures are required to secure this lineage from potential invasion by non-native fishes particularly the trout that are already in the system. Surveys are also urgently required to establish the extent of occurrence, population sizes, genetic diversity patterns, the biological and ecological attributes and identify effective management strategies to ensure persistence of this taxon into the future.

Conclusion

The present study represents the first comprehensive examination of the genetic diversity and evolutionary processes within the highly threatened Maloti minnow. Here we provide baseline information on molecular variation within the species before the construction of the Mohale Dam which was completed and commissioned in 2003. It also illustrates the importance of evaluating intraspecific genetic diversity as part of environmental impact assessments when major infrastructure developments are planned. This provided the basis for identifying and establishing evidence-based conservation management strategies to prevent extinction of vulnerable species and preserve genetic diversity that facilitates a species' long-term evolutionary potential. Findings from the present study highlight the increasing necessity to expedite implementation of appropriate conservation and management interventions to prevent the potential extinction of the genetically unique and indispensable Mohale and KZN populations.

Contributions

ERS, AC, PHNB, PHS conceptualised the study; ERS, AC, JLR, SPK conducted field work; ERS, AC, PHNB analysed the data; ERS, AC drafted the manuscript; all authors revised and edited the manuscript.

Statements and Declarations

We declare that there are no competing interests relative to the research and the content of this manuscript, and no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

Data availability

All specimens used in this study are housed at the NRF-SAIAB ichthyological collection, which is a national facility and open to the public. Specimens for research can be requested by contacting the institution. All sequences resulting from this study were deposited in GenBank and will be made available following the acceptance of the manuscript.

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