

**RESEARCH ARTICLE**

# Reconstruction of the historical distribution ranges of imperilled stream fishes from a global endemic hotspot based on molecular data: Implications for conservation of threatened taxa

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**Abstract**

1. Understanding historical distribution patterns of freshwater fishes prior to human impacts is crucial for informing effective strategies for biodiversity conservation. However, incomplete information on species occurrence records, the existence of cryptic species and sensitivity to small sample sizes limit the application of historical records in natural history collections as well as conventional species distribution modelling algorithms to infer past distributions of species.
2. This study used molecular data as an alternative and objective approach to reconstruct the historical distribution ranges of four stream fishes from the Breede River system in the Cape Fold Ecoregion, a global hotspot of imperilled endemic freshwater biodiversity in southern Africa.
3. The study used 249 occurrence records and 208 mitochondrial cytochrome *b* sequences to reconstruct the potential historical ranges of four taxa: *Galaxias* sp. 'zebratus nebula', *Galaxias* sp. 'zebratus Riviersonderend', *Pseudobarbus* sp. 'burchelli Breede' and *Pseudobarbus skeltoni*.
4. All four taxa historically had broader distribution ranges across the Breede River system before human impacts, but they have suffered severe attrition as the main-stem populations have been extirpated.
5. The severe decline in the historical ranges of these four taxa is a result of multiple impacts, particularly hydrological modification, habitat degradation and the introduction of non-native species, which are also global challenges for freshwater ecosystems.
6. The approach presented in this study has great potential for reconstructing historical ranges of stream-dwelling taxa from disparate regions where fragmentation has resulted from human-mediated impacts. This information is crucial for identifying appropriate conservation strategies such as river rehabilitation and eradication of non-native species, as well as guiding reintroductions and informing assisted gene flow where these are deemed necessary interventions.

**KEYWORDS**

Africa, Cape Fold Ecoregion, conservation, freshwater fish, *Galaxias*, mitochondrial DNA, past distribution, *Pseudobarbus*

**1 | INTRODUCTION**

Knowledge of past and present distribution ranges of freshwater fishes is essential for identifying the causes of past extirpations, evaluating species resilience to human impacts, assessing extinction risk and identifying effective strategies for biodiversity management in a rapidly changing world (Dudgeon et al., 2006; Mace et al., 2008; Vera-Escalona, Senthivasan, Ruzzante, & Habit, 2018; Willis & Birks, 2006; Xenopoulos et al., 2005). However, for many regions, detailed information on species occurrences before human impacts is often either limited or completely lacking, or may have biases towards species of commercial interest – for example, as reported by Haidvogel et al. (2013) for the Austrian Danube and northern Russian rivers. A comparison of historical records in natural history collections with data from current surveys provides an alternative approach for documenting shifts and declines in species distributions (Shaffer, Fisher, & Davidson, 1998). The existence of cryptic species may, however, potentially lead to overestimation of historical distribution ranges because morphologically variable and indistinguishable species have often been included under a single widespread 'species' (Bickford et al., 2007). Advances in molecular techniques that allow extraction and sequencing of DNA from formalin-preserved specimens are promising in that these are likely to add value to natural history collections through the detection of cryptic species and possibly uncover species that are now extinct in the wild (Ruane & Austin, 2017). Unfortunately, in many developing countries, limited financial resources dedicated to scientific research are likely to impede the application of these modern techniques.

Other studies have applied species distribution modelling (SDM) approaches to infer historical geographical ranges of riverine and diadromous fishes (Labay et al., 2011; Lassalle & Rochard, 2009; Pont, Logez, Carrel, Rogers, & Haidvogel, 2015). Performance of these SDMs is influenced by the quality of presence–absence data and the accuracy of the measured predictor variables (Pont et al., 2015). Although natural history collections represent the most informative sources of species occurrence data, these records do not necessarily represent species distribution ranges because only voucher-linked (presence-only) data are represented, whereas absence data are usually not recorded. There is also a likelihood of spatial and taxonomic biases in these records (Graham, Ferrier, Huettman, Moritz, & Peterson, 2004; Newbold, 2010). The absence of accurate species distribution information, coupled with the lack of up-to-date and accurately measured predictor variables, presents challenges for the effective application of SDM approaches, especially in understudied regions (Carneiro, Lima, Machado, & Magnusson, 2016). Another limitation of SDM approaches is that predictions of expected distributions are largely

based on contemporary information and the assumption that present distribution reflects a species' inherent habitat preference, rather than accommodating an ecologically more realistic concept that these predictors, habitats and species distributions could have changed over time (see Holt, 2003, and references cited therein). Furthermore, the performance and reliability of SDM approaches are strongly influenced by sample size. For example, Wisz et al. (2008) detected inconsistent performance for several modelling algorithms with small sample sizes. Similarly, Soutan and Safi (2017) reported that the MAXENT SDM algorithm had poor predictive performance for narrow-range species with low sample sizes (<20 occurrence records).

Sensitivity to low sample size therefore limits the effective application of SDM approaches for narrow-range species that are represented by a few remnant populations. Examples of such species are endemic fishes from the Cape Fold Ecoregion (CFE) of South Africa, where recent studies have led to the description of a number of new species and uncovered the existence of several lineages with narrow distribution ranges (Chakona & Skelton, 2017; Chakona & Swartz, 2013; Chakona, Swartz, & Gouws, 2013; Chakona, Swartz, & Skelton, 2014; Swartz, Skelton, & Bloomer, 2009). Because many of these lineages and new species have only been identified and described recently, their distributions and ecological requirements are poorly known. The available information for these taxa is thus often insufficient for effective application of SDM tools to infer historical distribution ranges.

Molecular techniques provide an alternative approach for inferring past distributions of riverine fishes that were fragmented owing to human modifications (Cole et al., 2016). This is based on the premise that dispersal maintains population (and hence genetic and demographic) connectivity for highly mobile riverine taxa (Davis, Epps, Flitcroft, & Banks, 2018; Hughes, Huey, & Schmidt, 2013; Hughes, Schmidt, & Finn, 2009). Given that human impacts largely occurred during recent decades, remnant populations of mobile taxa that were fragmented by human activities are expected to exhibit genetic signatures of recent disruption of population connectivity that are characterized by high levels of admixture and shallow genetic divergence. Thus, given the spatial distribution of remnant populations and the patterns of genetic structuring, it can be possible to identify tributaries and river channels that a species would have either occupied or used as conduits for dispersal.

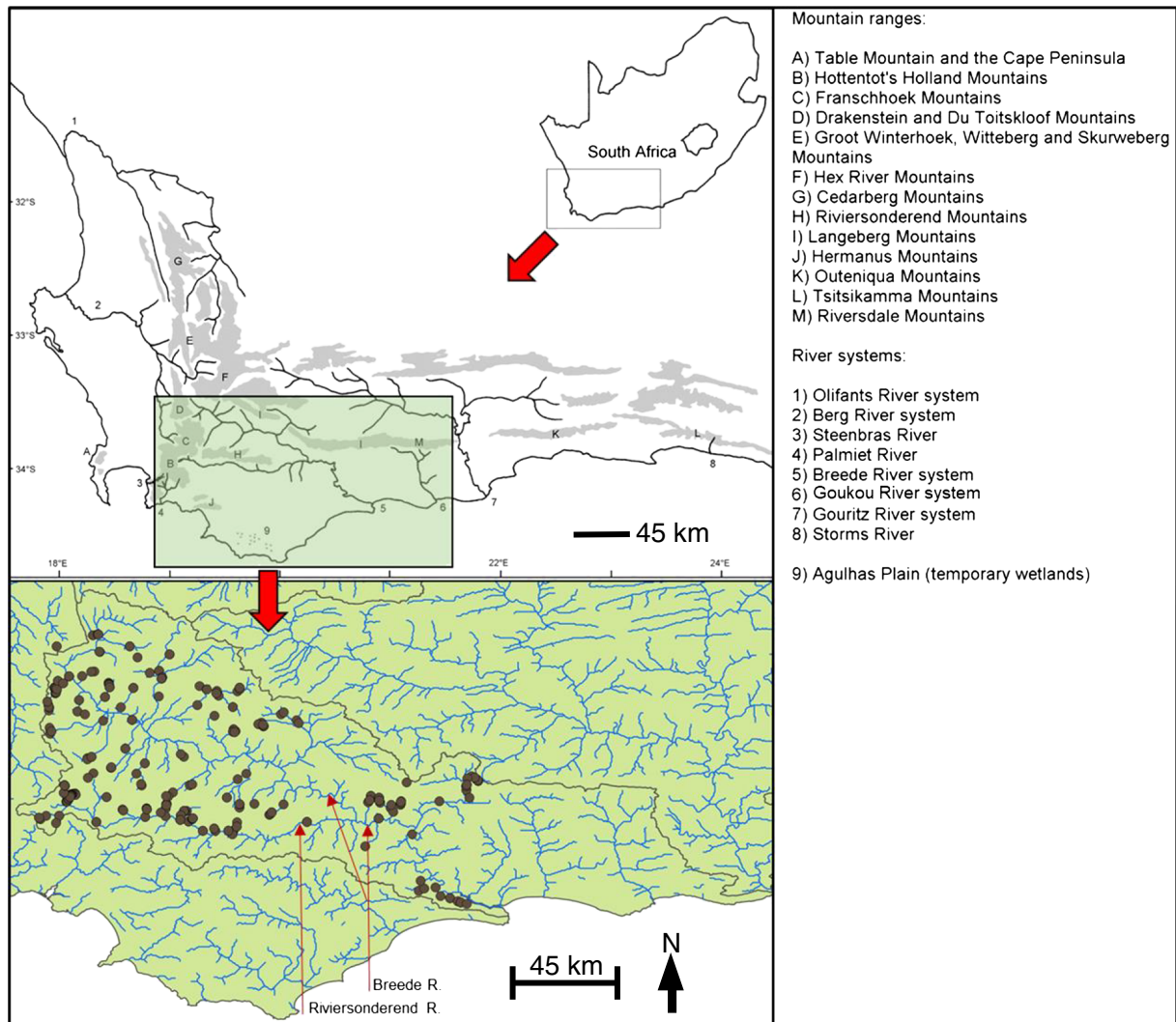
Molecular data therefore provide a more objective approach for reconstructing historical distributions of extant taxa, including narrow-range species that may be represented by small sample sizes. Provision of reliable and accurate information on historical ecological conditions and distributions of riverine fish assemblages is a fundamental requirement to avoid adoption of degraded states of freshwater

ecosystems and current species' distributions as benchmarks for assessing future environmental impacts (Stoddard, Larsen, Hawkins, Johnson, & Norris, 2006). However, for many regions, including southern Africa, freshwater fish distribution records are either incomplete or inaccurate (Bickford et al., 2007; Chakona, Kadye, Bere, Mazungula, & Vreven, 2018; Chakona, Malherbe, Gouws, & Swartz, 2015). This undermines attempts to manage aquatic biodiversity for future sustainability effectively because the magnitude of ecosystem change is likely to be underestimated as large-scale historical shifts are overlooked. This is particularly concerning, especially given that a considerable proportion (one-third) of freshwater species assessed by the IUCN are threatened with extinction (Darwall et al., 2018).

Recent studies have shown that the diversity and level of endemism of native fishes in the CFE, a global biodiversity hotspot located at the southern tip of Africa, was severely underestimated (Chakona et al., 2013, 2014, 2015; Chakona & Skelton, 2017; Chakona & Swartz, 2013; Swartz et al., 2009; Wishart, Hughes, Stewart, & Impson, 2006). The CFE, as is the case for other Mediterranean-

climate regions, is a hotspot of threatened freshwater biodiversity (see Hermoso & Clavero, 2011; Tweddle et al., 2009). The discovery of new species and historically isolated lineages further brought into question our understanding of freshwater fishes in the CFE, and called for a re-assessment of historical ranges, ecological requirements and the conservation status of native stream fishes in this region. The aim of the present study was to use spatial distribution records and genetic patterns described by Chakona et al. (2013) to reconstruct the potential historical distribution ranges of stream fishes from the CFE. This was done using four lineages that were identified within *Galaxias* and *Pseudobarbus* (including the recently described species *Pseudobarbus skeltoni* Chakona & Swartz, 2013) in the Breede River system (Figure 1) as case studies.

The Breede River system currently contains only five formally described native primary freshwater fishes: *Galaxias zebratus* Castelnau, 1861, *Pseudobarbus burchelli* (Smith, 1841), *P. skeltoni*, *Sandelia capensis* (Cuvier, 1831) and *Cheilobarbus capensis* (Smith, 1841) (Chakona & Swartz, 2013; Skelton, 2001; Skelton, Swartz, &

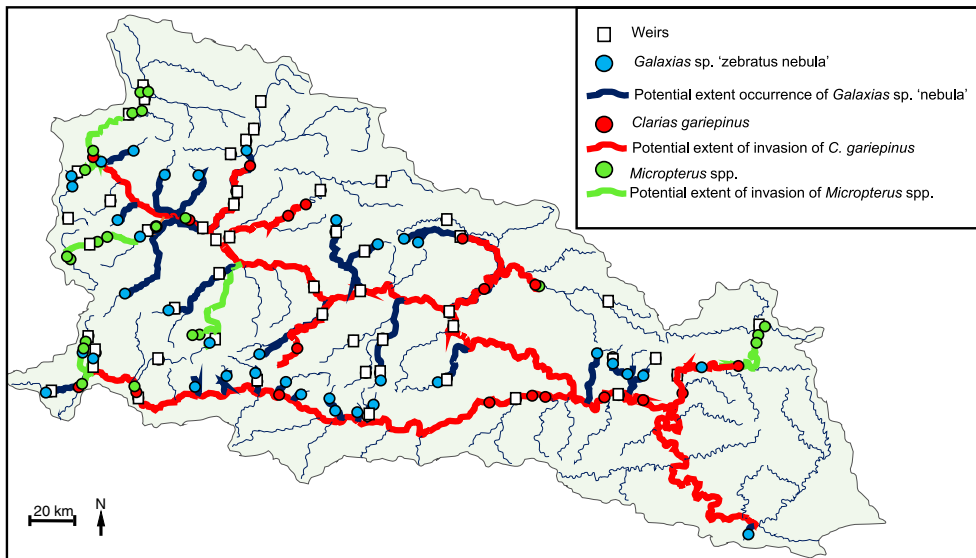


**FIGURE 1** The southern tip of the African continent showing the Cape Fold Mountains, some of the river systems associated with the Cape Fold Ecoregion (CFE), the Breede River system and the localities that were sampled between November 2008 and December 2010

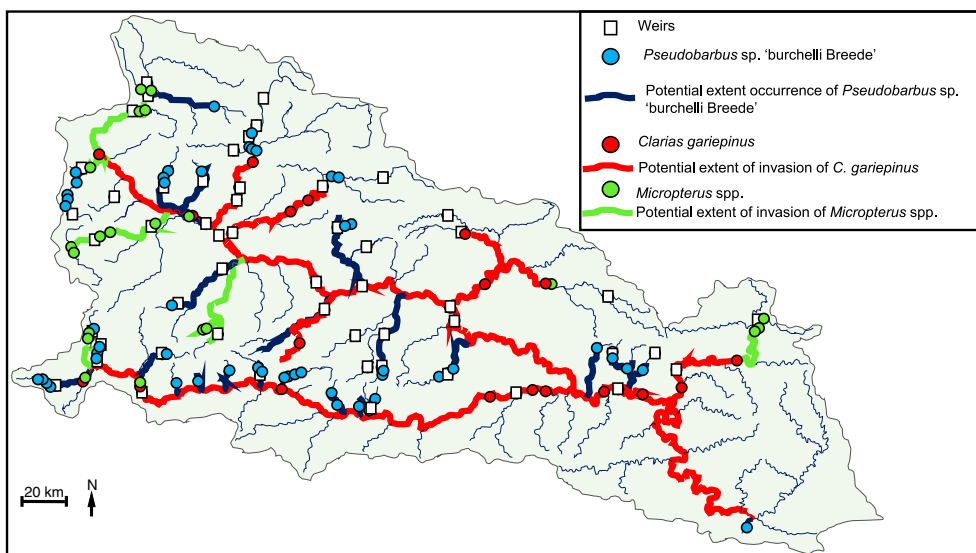
Vreven, 2018). Similar to many other river systems in the CFE, the Breede River system has a long history of hydrological and habitat modification, as well as biotic alteration through the introduction of invasive non-native fishes and plants. Consequently, native stream fishes of the Breede River and surrounding systems have undergone severe attrition, and almost all main-stem populations have been extirpated (Chakona et al., 2013; Clark, Impson, & Rall, 2009; Van der Walt, Weyl, Woodford, & Radloff, 2016). The remaining native fish populations persist in isolated headwater tributaries with little potential for connectivity owing to the construction of instream physical barriers, such as weirs and other water diversion structures, and the presence of non-native predators that have invaded the main-stem and lower foothill sections of the rivers (Figures 2–5).

It was hypothesized that if the contemporary fragmented distribution pattern of stream fishes in the Breede River system resulted

from human impacts (i.e. it does not reflect natural fragmentation patterns), then the fishes are expected to exhibit genetic patterns that are consistent with recent disruption of gene flow, characterized by low geographical structuring and sharing of haplotypes among isolated remnant populations. The potential historical distribution range for each species or lineage could thus be inferred or reconstructed by connecting remnant populations through the tributary and river networks (see Methods section) that would have been used as conduits for dispersal to facilitate connectivity. Contemporary connectivity of the isolated populations is, however, unlikely owing to the presence of instream physical barriers, such as weirs, together with the occurrence of non-native piscivorous fishes in the main-stem sections (Figures 2–5). The information on the past and present distribution ranges of native fishes considered in the present study is essential for the design and implementation of effective conservation strategies to ensure the persistence of these threatened taxa.



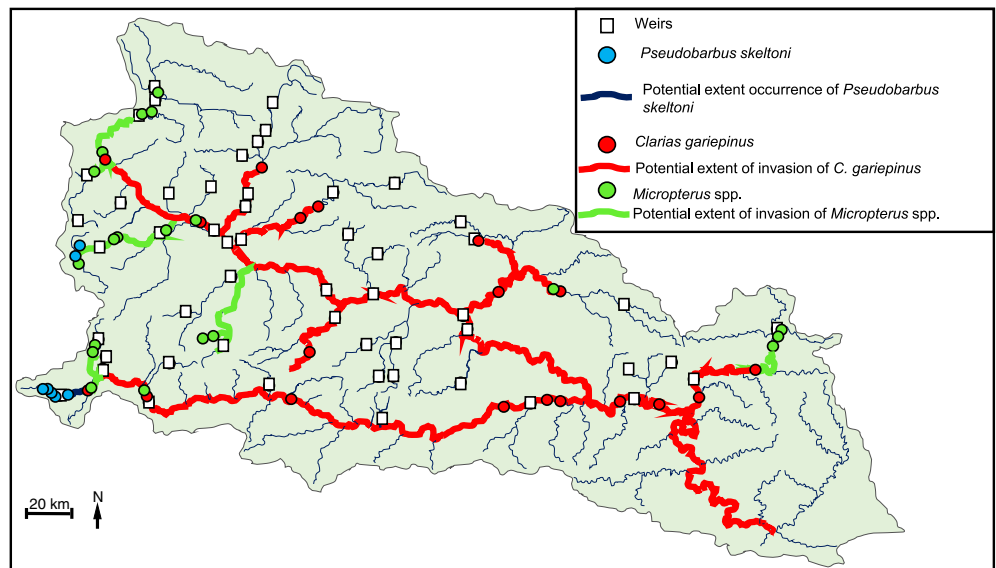
**FIGURE 2** Map of the Breede River system showing the location of artificial instream physical barriers (weirs and other water diversion structures), localities where the non-native fishes (*Clarias gariepinus* and *Micropterus* spp.) were recorded and their potential distribution range across the system, and the localities where *Galaxias* sp. 'zebratus nebula' was recorded



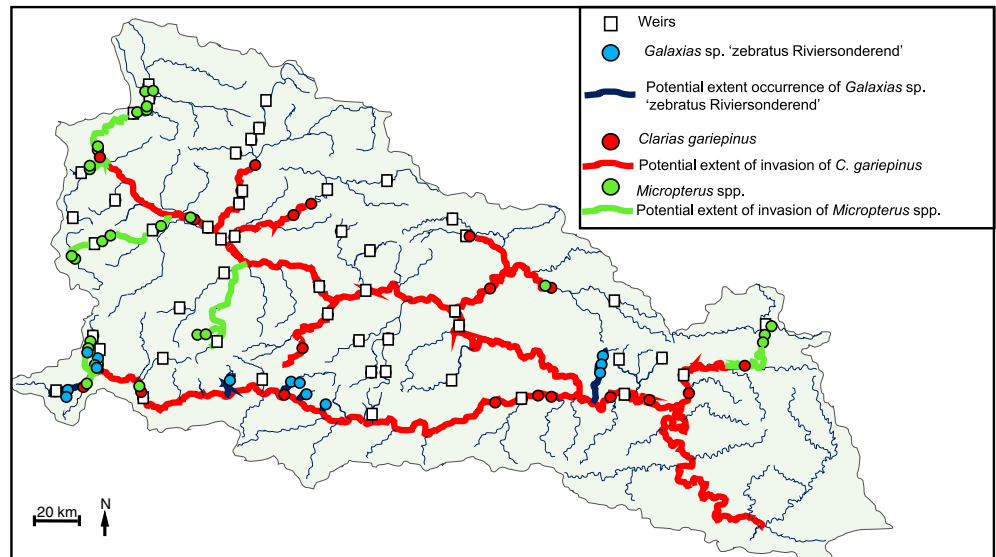
**FIGURE 3** Map of the Breede River system showing the location of artificial instream physical barriers (weirs and other water diversion structures), localities where the non-native fishes (*Clarias gariepinus* and *Micropterus* spp.) were recorded and their potential distribution range across the system, and the localities where *Pseudobarbus* sp. 'burchelli Breede' was recorded



**FIGURE 4** Map of the Breede River system showing the location of artificial instream physical barriers (weirs and other water diversion structures), localities where the non-native fishes (*Clarias gariepinus* and *Micropterus* spp.) were recorded and their potential distribution range across the system, and the localities where *Pseudobarbus skeltoni* was recorded



**FIGURE 5** Map of the Breede River system showing the location of artificial instream physical barriers (weirs and other water diversion structures), localities where the non-native fishes (*Clarias gariepinus* and *Micropterus* spp.) were recorded and their potential distribution range across the system, and the localities where *Galaxias* sp. 'zebratus Riviersonderend' was recorded



## 2 | METHODS

### 2.1 | Data collection

The present study used occurrence records and genetic data from Chakona et al. (2013) to reconstruct the potential historical ranges of four taxa from the Breede River system: *Galaxias* sp. 'zebratus nebula', *Galaxias* sp. 'zebratus Riviersonderend', *Pseudobarbus* sp. 'burchelli Breede' and *P. skeltoni* (see Chakona et al., 2013; Chakona & Swartz, 2013). Chakona et al. (2013) provided data from four comprehensive fish surveys in the Breede River system that were undertaken between November 2008 and December 2010. In total, 249 localities were sampled across the main-stem sections and multiple tributaries of the Breede and Riviersonderend rivers (Figure 1). At each locality, fish were sampled across all available habitats using a combination of

sampling methods, including a backpack electroshocker, seine net, snorkelling with a hand net and fyke nets. Details about sample processing, preservation, DNA extraction, amplification and sequencing are presented in Chakona et al. (2013). Tissue samples, voucher specimens and all the linked data have been deposited in the National Fish Collection at the National Research Foundation – South African Institute for Aquatic Biodiversity in Grahamstown. Genetic sequences were submitted to GenBank (see Chakona et al., 2013). The present study used 208 mitochondrial cytochrome *b* sequences: *Galaxias* sp. 'zebratus nebula' (79), *Galaxias* sp. 'zebratus Riviersonderend' (22), *Pseudobarbus* sp. 'burchelli Breede' (95) and *P. skeltoni* (12). In addition, locations of instream weirs and water abstraction points were recorded using a GPS. These data were supplemented with data on gauging weir localities from the provincial Department of Water and Sanitation.

## 2.2 | Criteria for reconstructing historical ranges

Many of the newly identified lineages of stream fishes in the Breede River system (and elsewhere in the CFE) are represented by a few remnant and highly fragmented populations (Chakona et al., 2013). These small sample sizes precluded the use of conventional SDM approaches to reconstruct past distribution ranges of these taxa (Soulтан & Safi, 2017; Wisz et al., 2008). This article presents an alternative and more direct method for reconstructing potential historical ranges of stream fishes using molecular data. This approach is based on the assumption that remnant populations of a historically panmictic taxon that was fragmented by human impacts are expected to show genetic signatures that are consistent with recent disruption of gene flow, reflected by: (i) shallow or no genetic structuring across the Breede system; (ii) considerable sharing of haplotypes among localities; (iii) low genetic distance (expressed as percentage divergence) among localities; (iv) no association between genetic and geographical distances; and (v) little genetic differentiation (measured using  $\Phi_{ST}$  values) because of mixing of haplotypes from different populations (Hughes et al., 2009, 2013; Figure 6). The pattern of genetic variation and the present geographical distribution of remnant populations of each taxon was used to reconstruct potential 'minimum' historical distribution ranges by connecting populations with river sections that each taxon is likely to have occupied or at least used as conduits for dispersal (Figure 6). The discovery of historically isolated lineages within the freshwater fishes of the Breede River system precludes the use of old distribution records because it is uncertain which of the different lineages are represented in historical records. The present study therefore used occurrence (and absence) data collected between 2008 and 2009, as all specimens were verified using genetic analysis (Chakona et al., 2013).

## 3 | RESULTS

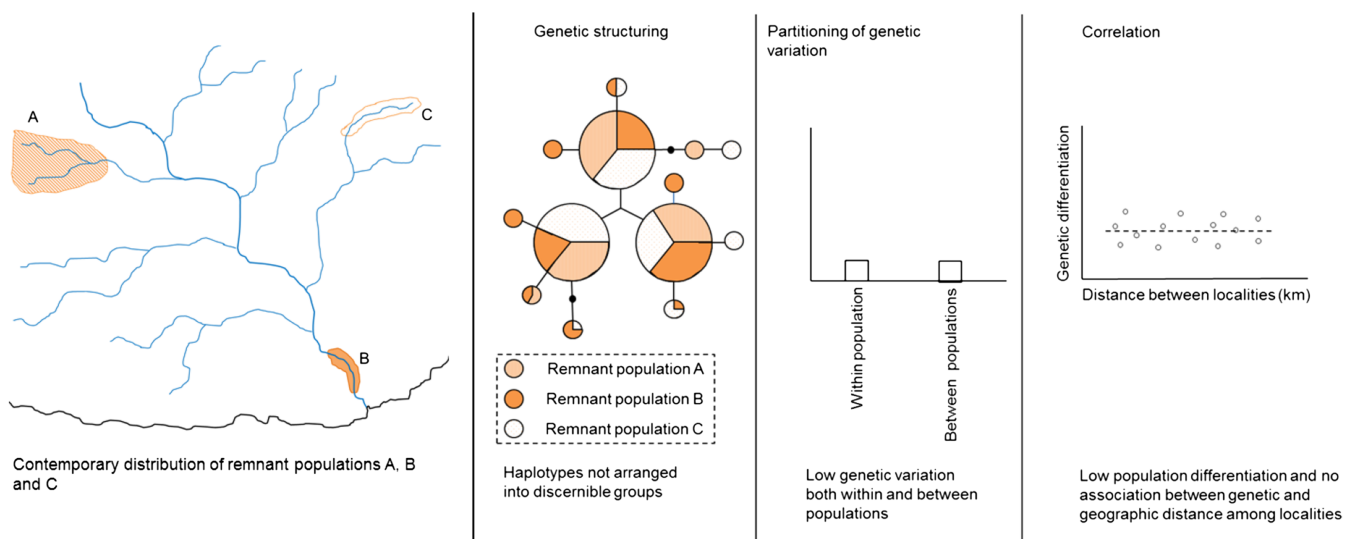
### 3.1 | Reconstructed potential historical ranges

Genetic patterns of remnant populations for all the taxa assessed in the present study were consistent with expected signatures of panmixia (Table 1). This supports the hypothesis that remnant populations of these taxa were historically connected, but connectivity was recently disrupted by human-mediated impacts. For each taxon, distribution maps are presented showing the known distribution as recorded from the surveys of 2008–2010, together with the reconstructed historical range based on extant distribution and genetic patterns of the remnant populations.

The current distributions of stream fishes in the Breede River varied by taxon. *Galaxias* sp. 'zebratus nebula' occurred in 37 tributaries and at one locality in the main-stem Breede River (Figure 7a). *Pseudobarbus* sp. 'burchelli Breede' was found in 36 tributaries (Figure 8a) and *Galaxias* sp. 'zebratus Riviersonderend' occurred in 10 tributaries (Figure 9a), while *P. skeltoni* was recorded from only three tributaries (Figure 10a).

The potential historical range of *Galaxias* sp. 'zebratus nebula' encompassed much of the Breede River system (Figure 7). However, the range of this lineage has suffered severe decline, and is almost extirpated from the main-stem section of the Breede River (Figures 2 and 7). Individuals of this lineage were collected from only one locality in the main-stem Breede River in 2009, albeit in very low numbers.

Similar to *Galaxias* sp. 'zebratus nebula', the potential historical range of *Pseudobarbus* sp. 'burchelli Breede' encompassed much of the Breede River system (Figure 8); however, this species has suffered a severe decline in distribution as populations in the main-



**FIGURE 6** Schematic representation showing remnant populations and the expected genetic patterns for a species where fragmentation and disruption of gene flow resulted from human impacts. The potential historical distribution range for the species can thus be inferred or reconstructed by connecting remnant populations A, B and C through the tributary and river networks that would have been used as conduits for dispersal to facilitate connectivity (see Figures 7–10)

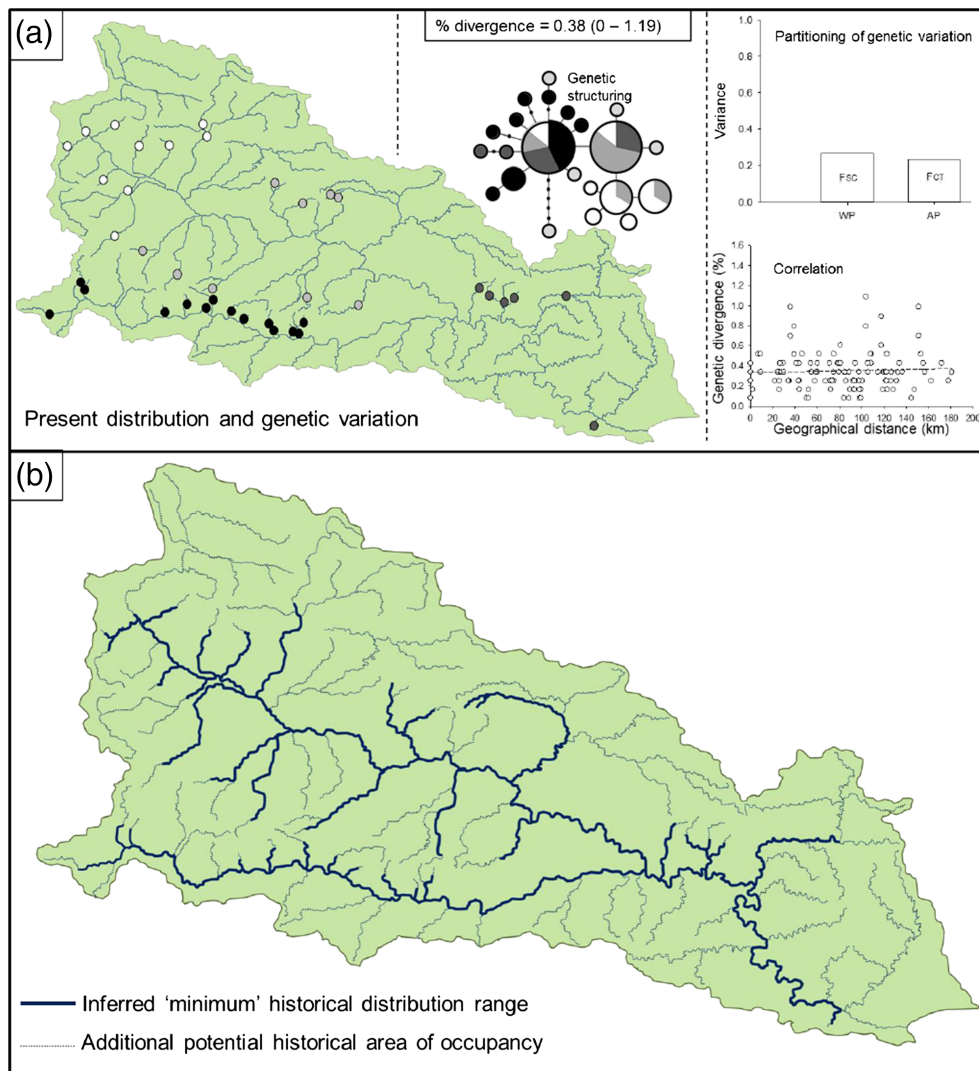
**TABLE 1** Summary of the genetic patterns exhibited by the remnant populations show that none of the four taxa assessed in the present study met the criteria for naturally fragmented population structuring. This indicates that fragmentation resulted from recent disruption of population connectivity and gene flow

Taxon	Genetic structuring	Partitioning of genetic variation	Genetic divergence	Correlation between genetic differentiation and geographic distance	Overall comment
<i>Galaxias</i> sp. 'zebratus nebula'	High degree of haplotype sharing among remnant populations.	No discernible pattern of partitioning of genetic variation.	Shallow genetic divergence between populations (0.0–1.19%).	No association between genetic differentiation and geographic distance.	Genetic patterns of remnant populations for this taxon were consistent with expected signatures of panmixia. This supports the hypothesis that remnant populations of this taxon were historically connected, but connectivity was recently disrupted by human-mediated impacts.
<i>Pseudobarbus</i> sp. 'burchelli Breede'	High degree of haplotype sharing among remnant populations.	No discernible pattern of partitioning of genetic variation.	Shallow genetic divergence between populations (0.0–0.91%).	No association between genetic differentiation and geographic distance.	Genetic patterns of remnant populations for this taxon were consistent with expected signatures of panmixia. This supports the hypothesis that remnant populations of this taxon were historically connected, but connectivity was recently disrupted by human-mediated impacts.
<i>Pseudobarbus skeltoni</i>	Two haplotypes were shared between the two remnant populations.	Low sample size precluded estimation of the degree of within and between population variation.	Shallow genetic divergence between populations (0.0–0.45%).	Low sample size precluded calculation of $\Phi_{ST}$ values.	Genetic patterns of remnant populations for this taxon were consistent with expected signatures of panmixia. This supports the hypothesis that remnant populations of this taxon were historically connected, but connectivity was recently disrupted by human-mediated impacts.

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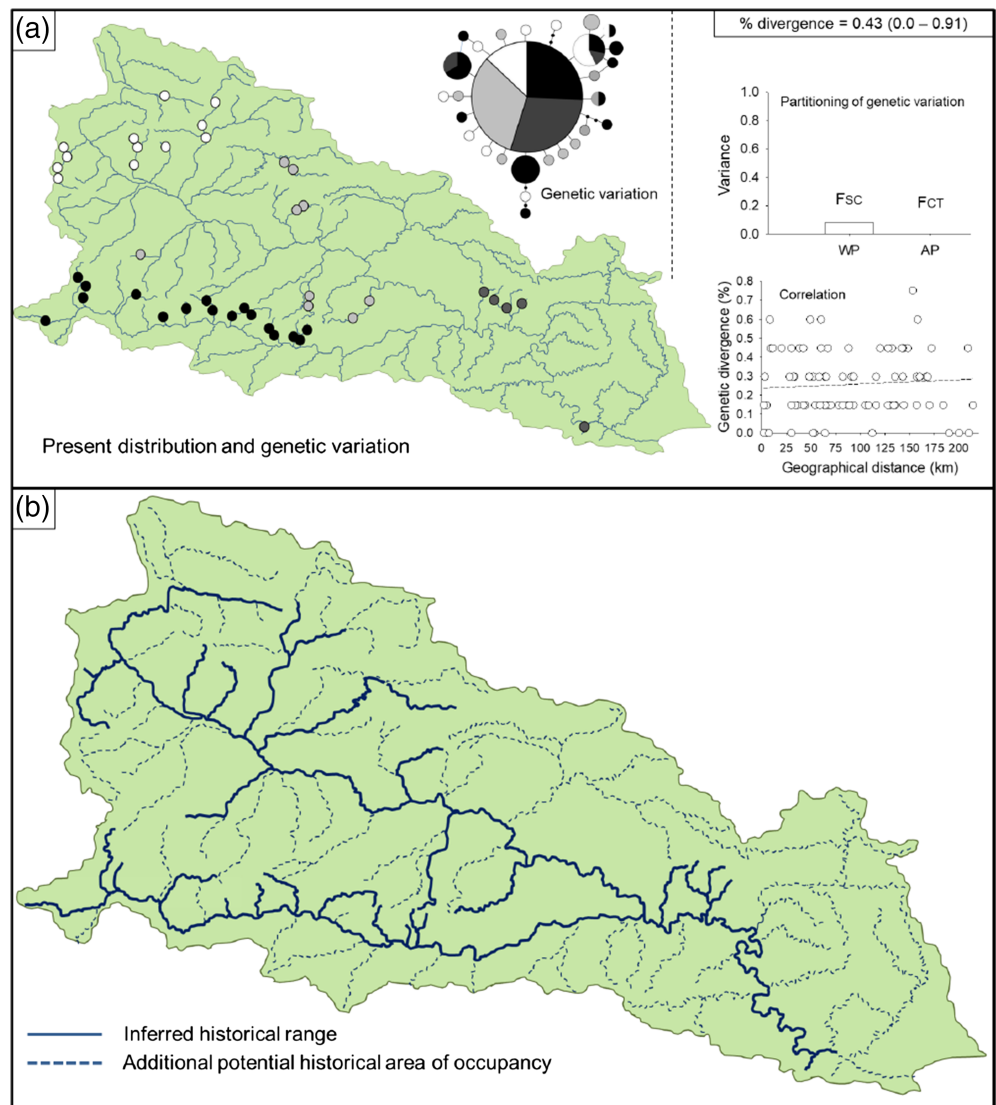
TABLE 1 (Continued)

Taxon	Genetic structuring	Partitioning of genetic variation	Genetic divergence	Correlation between genetic differentiation and geographic distance	Overall comment
<i>Galaxias</i> sp. 'zebratus Riviersonderend'	High degree of haplotype sharing among remnant populations.	No discernible pattern of partitioning of genetic variation.	Shallow genetic divergence between populations (0.0–0.79%)	No association between genetic differentiation and geographic distance.	Genetic patterns of remnant populations for this taxon were consistent with expected signatures of panmixia. This supports the hypothesis that remnant populations of this taxon were historically connected, but connectivity was recently disrupted by human-mediated impacts.



**FIGURE 7** Map of the Breede River system showing: (a) the contemporary distribution and the genetic patterns of *Galaxias* sp. 'zebratus nebula'; and (b) the reconstructed 'minimum' historical distribution range of this lineage before anthropogenic fragmentation

**FIGURE 8** Map of the Breede River system showing: (a) the contemporary distribution and the genetic patterns of *Pseudobarbus* sp. 'burchelli Breede'; and (b) the reconstructed 'minimum' historical distribution range of this lineage before anthropogenic fragmentation



stem sections of the Breede and Riviersonderend rivers and lower foothill sections of several tributaries have been extirpated (Figure 3, 8).

The reconstructed potential historical range of *P. skeltoni* largely encompassed the main-stem sections of the Breede and Riviersonderend rivers (Figure 9). This species has disappeared from much of its potential historical range as it is currently represented by only three known remnant populations, one in the upper Riviersonderend River and another in the Krom and Tierkloof rivers (both tributaries of the Molenaars) in the upper Breede River (Figures 4 and 9).

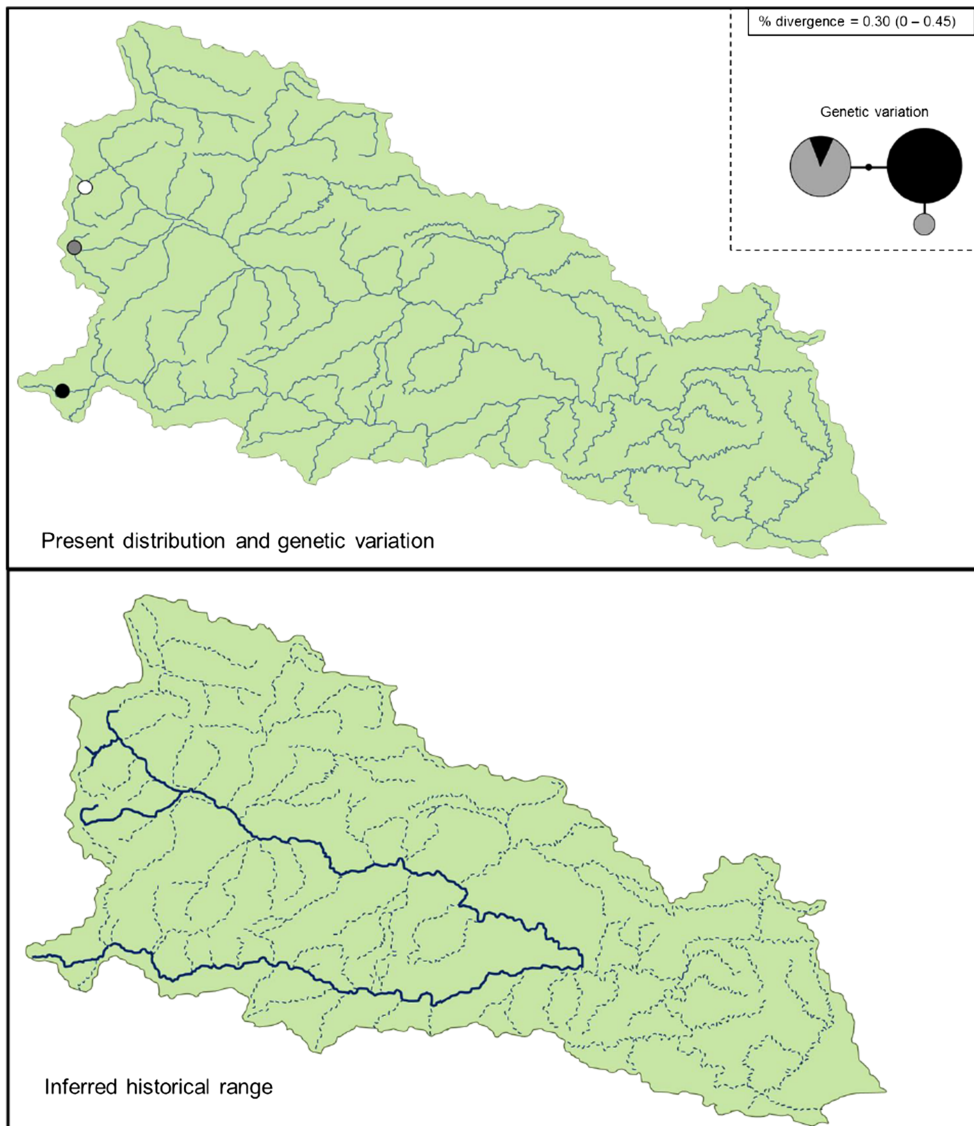
*Galaxias* sp. 'zebratus Riviersonderend' was inferred to have been largely confined to the Riviersonderend River, with the exception of one population that occurred in the Wolfkloof River, a tributary of the lower Breede (Figure 10). This lineage is now highly fragmented compared with its potential historical range (Figures 5 and 10).

## 4 | DISCUSSION

### 4.1 | Historical distribution ranges

The shallow genetic divergence, lack of population structuring, lack of significant isolation by distance and the sharing of haplotypes among spatially distinct remnant populations of *Galaxias* 'zebratus nebula', *Galaxias* 'zebratus Riviersonderend', *Pseudobarbus* 'burchelli Breede' and *P. skeltoni* are consistent with genetic signatures of panmictic riverine populations connected through the river networks as described by Hughes et al. (2009, 2013). However, contemporary gene flow (or population connectivity) for these fishes is unlikely owing to the presence of dams and weirs that are likely to form effective barriers to fish movement (Rahel, 2013; Weyl, Ellender, Woodford, & Jordaan, 2013). The Breede River system has several dams and weirs, which have resulted in considerable hydrological modification of this system





**FIGURE 9** Map of the Breede River system showing: (a) the contemporary distribution and the genetic patterns of *Pseudobarbus skeltoni*. The open circle indicates the locally extinct population of this species in the lower Witte River. Panel (b) shows the reconstructed 'minimum' historical distribution range of *Pseudobarbus skeltoni* before anthropogenic fragmentation

through excessive or complete abstraction of water. For many of the tributaries, surface water completely disappears in river sections below weirs and water abstraction points during the dry season. The presence of non-native piscivores in the main-stem and foothill sections of the rivers is also likely to prevent successful dispersal to maintain contemporary population connectivity for the native fishes. There is limited literature on the invasion status within the Breede River system, but surveys conducted for the present study indicate that invasive fishes, including *Clarias gariepinus*, *Micropterus dolomieu*, *Micropterus salmoides* and *Lepomis macrochirus*, are now dominant in the main-stem sections of the Breede and Riviersonderend rivers, as well as in several tributaries across the system. Results from a provincial River Health Programme survey presented evidence for the extensive spread and establishment of non-native fishes in the Breede River system, coupled with contraction of distribution ranges of native fishes (River Health Programme, 2011).

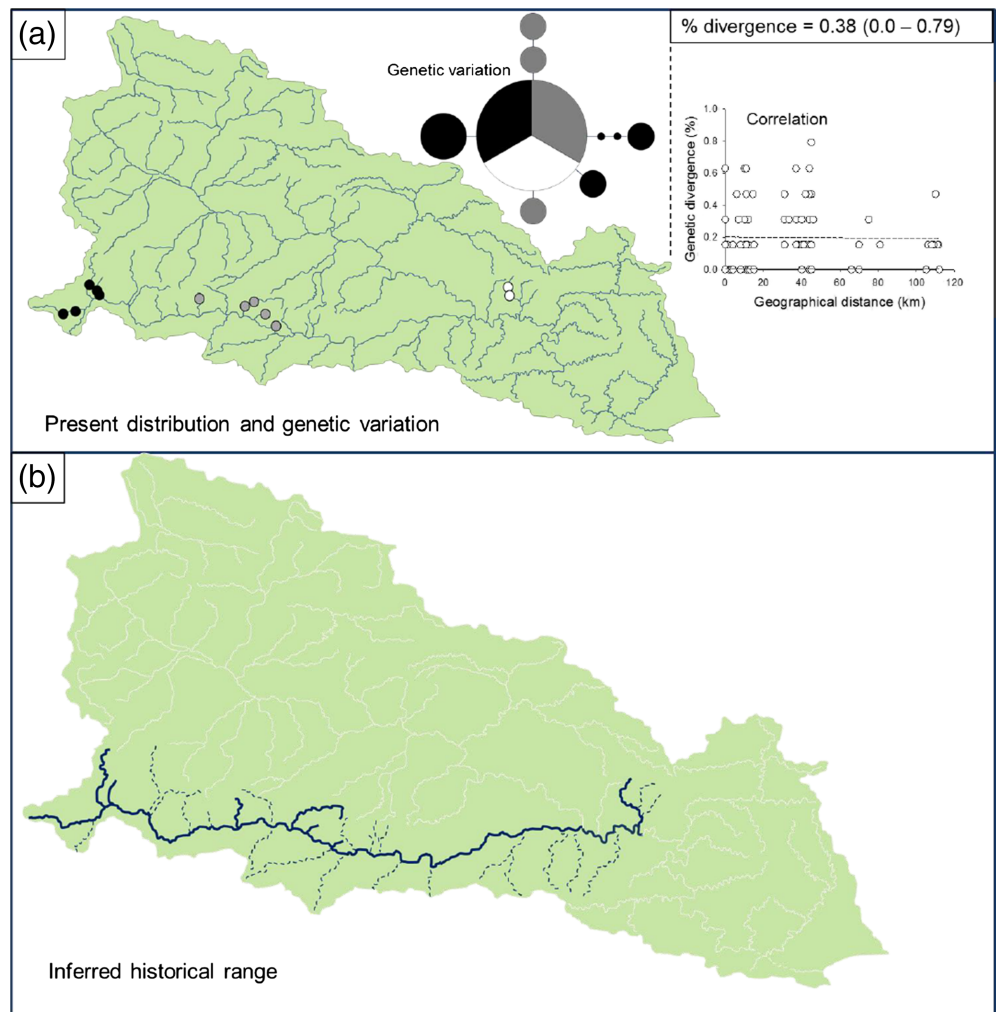
The genetic patterns of the native fishes considered in the present study therefore indicate that, before human impacts, the populations were historically connected or possibly continuously

distributed throughout the tributary networks and main-stem sections of the Breede River system. All four taxa, however, suffered substantial attrition and the remnant populations of these fishes represent a small proportion of their inferred historical ranges. Given the comprehensive sampling approach of Chakona et al. (2013), these distributions probably closely represent the remaining natural ranges of these taxa.

## 4.2 | Causes of decline in historical ranges

Previous studies identified habitat degradation and invasion by non-native species as the greatest threats to the freshwater biodiversity of the CFE (Tweddle et al., 2009). Hydrological modification through the construction of weirs, dams and other water abstraction structures also represents a major threat to stream fishes in the Breede River system and the broader CFE (River Health Programme, 2011). Observations during field surveys indicated that, for almost all of the streams, the river reaches downstream of

**FIGURE 10** Map of the Breede River system showing: (a) the contemporary distribution and the genetic patterns of *Galaxias* sp. 'zebratus Riviersonderend'; and (b) the reconstructed 'minimum' historical distribution range of this lineage before anthropogenic fragmentation



weirs and water diversion structures dry up completely during the dry season, and only reconnect with the rest of the river system after the winter rains. These dams and weirs were constructed to provide irrigation water to support the development of extensive agricultural activities in the Breede Valley, as well as to provide drinking water to surrounding towns and communities. Most of the remnant populations of native species in the Breede River system were found upstream of weirs or water abstraction structures in the mountain tributaries, and, in most instances, they were absent from the downstream sections of many of the impoundments or water abstraction points. This suggests that the construction of instream physical barriers and complete water abstraction has played a major role in causing the contraction of geographical ranges of stream fishes in the Breede River system. Chakona and Swartz (2012) reported that *Pseudobarbus* sp. 'burchelli Breede' and *Sandelia capensis* were absent from tributaries where weirs were built at altitudes above the upper distribution limits of these fishes. Surveys also indicated that remnant populations of native fishes only occurred in very short stretches of rivers upstream of weirs, indicating that the weirs were built just below the upper limit of these fishes.

Although weirs and their associated hydrological impacts may have contributed to large-scale decline in the historical distribution ranges of native fishes in the Breede River system, they have certainly prevented range expansion of non-native fish species into many of the tributaries. The spread and establishment of non-native piscivores has been implicated in many studies as one of the major causes for large-scale decline in the distribution ranges of native fishes in many regions worldwide (Collares-Pereira, Cowx, Rodrigues, Rogado, & da Costa, 1999; Ellender et al., 2018; Ellender, Wasserman, Chakona, Skelton, & Weyl, 2017; Moyle, Crain, Whitener, & Mount, 2003; Shelton, Samways, & Day, 2014; Van der Walt et al., 2016; Woodford & McIntosh, 2010). Nine alien and invasive species – *Cyprinus carpio*, *L. macrochirus*, *Tinca tinca*, *Pangasius sanitwongsei*, *Oncorhynchus mykiss*, *M. salmoides*, *M. dolomieu* and *Micropterus punctulatus* – were recorded from the Breede River system during extensive surveys conducted between November 2008 and December 2010 as part of the present study. Extralimital species, defined as species native to South Africa but which have been introduced outside their native range, were also detected. Two extralimital species, *Clarias gariepinus* and *Tilapia sarrmanii*, were found to be widely distributed in the Breede River system during the surveys.

*Clarias gariepinus* is a global invader (Weyl, Daga, Ellender, & Vitule, 2016) which is successfully established across much of the main-stem sections of the Breede and Riviersonderend rivers. This species has also penetrated into the lower- to mid-reaches of several tributaries in this system. The successful invasion of this species into headwater streams, which are the remaining refugia for threatened native species, was also reported by Ellender, Weyl, and Swartz (2011) and Ellender, Woodford, and Weyl (2015) in the eastern CFE. There are serious concerns because *C. gariepinus* represents an emerging invader that is capable of breaching some of the barriers that have successfully prevented the spread of other non-native fishes, particularly *Micropterus* spp. Black bass, *Micropterus* spp., have also become established in the main-stem sections and a number of tributaries in the Breede River system. Rainbow trout (*O. mykiss*) was limited to upper sections of the streams. Shelton, Samways, and Day (2014) investigated the impacts of *O. mykiss* in headwater streams of the Breede River system and provided evidence for localized displacement of native fish populations. The highly threatened *P. skeltoni* is particularly vulnerable to the impacts of trout in one of its three remaining localities (Chakona, Jordaan, Kadye, & Van der Walt, 2017; Weyl, Ellender, Wasserman & Woodford, 2015). Studies indicate that, once established, sustained effort is required to reduce the population size and manage the ecological impacts of *O. mykiss* in invaded river reaches (Shelton et al., 2017).

Native fishes were absent from nearly all localities where non-native fishes were recorded, with the exception of only two localities in the main-stem Breede River, where *Galaxias* sp. 'zebratus nebula' and *Galaxias* sp. 'zebratus Breede' were recorded (albeit in very low numbers). This is consistent with findings from other studies where non-native piscivores caused localized extirpations of native fish (Ellender et al., 2018; Shelton, Day, & Impson, 2014; Van der Walt et al., 2016). Given the severe impact of predatory non-native fishes, localized removal of these species to allow recolonization or translocation of native species should form part of a regional conservation strategy. Elsewhere in the CFE, eradication of non-native *Micropterus* spp. resulted in rapid recolonization and establishment of native fishes in the rehabilitated section of the Rondegat River (Van der Walt et al., 2019; Weyl, Finlayson, Impson, Woodford, & Steinkjer, 2014). This indicates that, before the introduction of non-native predators, propagules of native fish would wash out from the upper sections of rivers every year to maintain population connectivity through the tributary and main-stem river networks.

The lower foothill sections of tributaries and mainstream sections of the Breede and Riviersonderend rivers have also been affected by other impacts, including repeated bulldozing of the river channel, which leads to increased turbidity, sedimentation and bank erosion (Chakona & Swartz, 2012). Other impacts include diffuse and point source pollution from agricultural and urban sources. The impacts of pollutants, such as agrochemicals, are often exacerbated by reduced surface flows. Pollution and water abstraction can act synergistically in their impacts on aquatic ecosystems. Thus, the decline in the historical ranges of native fishes in the Breede River system resulted from a combination of factors that include hydrological modification, building

of instream physical barriers, complete water abstraction, habitat degradation and the introduction and spread of non-native species.

### 4.3 | Future threats and conservation recommendations

Population fragmentation is considered a major threat to aquatic biodiversity, because loss of population connectivity and disruption of gene flow is likely to increase the risk of extinction caused by inbreeding depression and loss of within-population genetic diversity (Bessert & Ortí, 2008; Pavlova et al., 2017). This can affect the long-term evolutionary potential of species to adapt to future environmental changes (Reed, 2005). Remnant populations of stream fishes in the CFE are also vulnerable to the projected impacts of global climate change, particularly increases in water temperature and reductions in river flows and the concomitant increase in human demand for water (Dallas & Rivers-Moore, 2014; Shelton et al., 2018). These threats are consistent with those reported for riverine ecosystems in other Mediterranean-climate regions such as the Murray–Darling Basin in south-eastern Australia (Bond, Thomson, Reich, & Stein, 2011; Pavlova et al., 2017; Pittock & Finlayson, 2011), the Iberian Peninsula (Hermoso & Clavero, 2011; Maceda-Veiga, 2013) and California in the USA (Moyle, Kiernan, Crain, & Quiñones, 2013).

The approach described in the present study has several practical applications, including guiding rehabilitation and reintroduction plans for regions where demographic connectivity of remnant populations was disrupted by human-mediated impacts. It is important, however, to note that, in some cases, eradication of non-native species from large river systems or the demolition of impoundments to re-establish connectivity of fragmented populations may not be feasible or achievable options (Mantel, Rivers-Moore, & Ramulifho, 2017; Rahel, 2013). Under such circumstances, assisted gene flow through small-scale translocations may be needed to rescue remnant populations that could be at risk of losing genetic diversity caused by genetic drift, as reported for the endangered *Macquaria australasica* in the Murray–Darling River system in south-eastern Australia (Pavlova et al., 2017). Thus, information on patterns of genetic structure for remnant populations has several applications, including the reconstruction of historical distribution patterns as reported in the present study, and is also relevant when planning for translocation and reintroductions where those interventions are deemed necessary. Over the years, the cost of DNA sequencing has decreased significantly, which makes the approach used in the present study accessible and widely applicable, including in many developing countries where allocation of financial resources dedicated for scientific research is usually limited. Future studies should investigate the genetic consequences of population fragmentation in stream fishes of the Breede River and the broader CFE to inform appropriate and effective management and conservation measures to prevent deterioration of genetic diversity, which could result in loss of fitness in small isolated populations.

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