Received: 2 March 2017 | Revised: 13 August 2017 | Accepted: 4 September 2017

DOI: 10.1002/aqc.2850

WILEY

RESEARCH ARTICLE

The status and distribution of a newly identified endemic galaxiid in the eastern Cape Fold Ecoregion, of South Africa

Gamuchirai Chakona[1,2,3] | **Ernst R. Swartz**[1] | **Albert Chakona**[1]

[1] South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa

[2] Department of Ichthyology and Fisheries Science (DIFS), Rhodes University, Grahamstown, South Africa

[3] Department of Environmental Science, Rhodes University, Grahamstown, South Africa

Correspondence
Gamuchirai Chakona, South African Institute for Aquatic Biodiversity (SAIAB), Private Bag 1015, Grahamstown, 6140, South Africa.
Email: matarutseg@yahoo.com

Abstract

1. DNA-based studies have uncovered cryptic species and lineages within almost all freshwater fishes studied thus far from the Cape Fold Ecoregion (CFE) of South Africa. These studies have changed the way the CFE is viewed, as almost all stream fishes that were previously considered to be of low conservation priority, because they were perceived to have broad geographical ranges, contain multiple historically isolated lineages, many of which are narrow-range endemics.
2. As stream fishes of the CFE are of conservation concern owing to threats mainly posed by habitat degradation, invasion by alien species and hydrological modification, re-evaluation of the distribution and conservation status of newly identified unique lineages is required to inform the development and implementation of effective conservation and management strategies.
3. The present study conducted an IUCN Red List conservation assessment of a newly identified lineage of the *Galaxias zebratus* species complex (hereafter referred to as *Galaxias* sp. 'Joubertina') to identify key threats and provide recommendations to conservation authorities on appropriate measures to reduce extinction risk.
4. The lineage met the qualifying threshold for the Endangered category because of its very restricted geographic range, few remaining secure populations, small known population sizes and the intensity of threats to most of the populations. Only six populations remain, one of which could be an 'extralimital' population potentially established through an inter-basin water transfer scheme.
5. *Galaxias* sp. 'Joubertina' is threatened by invasive piscivores, habitat degradation and excessive water abstraction. These impacts have fragmented remnant populations, raising concerns about potential long-term adverse impacts on genetic diversity and evolutionary potential of this lineage.
6. Immediate conservation measures should protect remnant populations from further impacts, while long-term measures should aim to restore historical connectivity to reduce the potential deleterious effects of inbreeding in the small isolated populations.

KEYWORDS

Africa, biodiversity hotspot, climate change, conservation, Galaxiidae, habitat degradation, non-native species, risk assessment, stream fish

1 | INTRODUCTION

Globally, freshwater biodiversity is under severe threat owing to widespread land-use changes and associated habitat degradation, over-exploitation of water, pollution, flow modification and invasion by non-native species (Dudgeon et al., 2006; Magurran, 2009). These threats to freshwater biodiversity are interactive, but the greatest threat globally has been assessed as the introduction and spread of invasive non-native fishes (Cowx, 2002). All these impacts have collectively caused a sharp increase in the number of global freshwater fish extinctions (Harrison & Stiassny, 1999; Helfman, 2007; Hinton-Taylor et al., 2009).

The Cape Floristic Region (CFR), which refers to the phytogeographic region located at the southern tip of Africa, has been traditionally used as a synonym for the Cape Fold Ecoregion (CFE), a freshwater ecoregion which encompasses rivers associated with the Cape Fold Belt which runs parallel to the south and west coast of South Africa (Abell et al., 2008). This region is a world-renowned biodiversity hotspot that harbours several narrow-range endemic plants and animals (Goldblatt & Manning, 2002; Linder et al., 2010; Wishart & Day, 2002). Until only recently, the diversity of primary freshwater fishes in the CFE was thought to be low, as the region was understood to have only 18 nominal species (Skelton, 2001). However, Linder et al. (2010) estimated that there are at least 43 historically isolated lineages hidden within the 18 nominal freshwater fishes of the CFE, a revelation that has important implications for conservation management, given that many of the currently recognized primary freshwater fishes in this region are already listed in threatened categories of the IUCN (Tweddle et al., 2009). DNA-based studies and continuing taxonomic revision of stream fishes of the CFE are showing that many species previously thought to be wide-ranging are complexes comprising several narrow-range genetic lineages or species (Bloomer & Impson, 2000; Chakona & Skelton, 2017; Chakona & Swartz, 2013; Chakona, Swartz, & Gouws, 2013; Chakona, Swartz, & Skelton, 2014; Swartz, Skelton, & Bloomer, 2007, 2009; Wishart, Hughes, Stewart, & Impson, 2006). Fishes of the CFE are mainly threatened by introduced piscivorous fishes, and habitat modification through over-abstraction of water, channelization and bulldozing (Clark, Impson, & Rall, 2009; Nel et al., 2007; Rouget, Richardson, Cowling, Lloyd, & Lombard, 2003; Skelton, Cambray, & Lombard, 1995; Tweddle et al., 2009). Following Ellender and Weyl (2014), the term 'non-native species' in this paper refers to both alien (i.e. species that are not indigenous to South Africa) and 'extralimital' (i.e. indigenous species that have been introduced into other river systems outside their natural distribution ranges). Tweddle et al. (2009) suggested that the introduction of non-native fishes is the most serious of these threats. Non-native fishes introduced to South Africa have been identified as the main cause of the decline and localized extinctions of native fishes across the CFE (Clark et al., 2009; Shelton, Samways, & Day, 2015). Almost all mainstem populations of native fishes in the CFE have been extirpated, and remnant populations now typically survive in tributary streams as highly fragmented populations, mostly above barriers such as waterfalls or weirs that prevent upstream movement of non-native fishes (Chakona, Swartz, & Gouws, 2013; Clark et al., 2009). Occurrence of hidden diversity (Chakona & Skelton, 2017; Chakona & Swartz, 2013; Chakona, Swartz, & Gouws, 2013; Chakona et al., 2014; Swartz, Chakona, Skelton, & Bloomer, 2014; Swartz et al., 2007, 2009;) in these already threatened species calls for re-evaluation of the ecology and biology, conservation status and geographical distribution of stream fishes in the CFE (Kadye, Chakona, & Jordaan, 2016). This is critical as the different lineages may differ in their vulnerability to ecological impacts and may consequently require different management strategies for conservation actions to be successful.

The Cape *Galaxias*, *Galaxias zebratus* Castelnau 1861, was previously thought to be the only species of the family Galaxiidae that occurred in Africa. *Galaxias zebratus*, as previously understood, had the widest geographical range of stream fishes restricted to the CFE, spanning across more than 20 isolated river systems from the Olifants on the west coast to the Gamtoos in the eastern CFE (Cambray, Bok, & Smith, 1995; Skelton, 2001). However, molecular studies have shown that *G. zebratus* is a species complex, comprising at least 10 deeply divergent lineages (Chakona, Swartz, & Gouws, 2013; Waters & Cambray, 1997; Wishart et al., 2006). There are serious conservation concerns, because only one of these lineages has a wide distribution range across the CFE, while the rest of the lineages have much narrower ranges and potentially smaller population sizes (Chakona, Swartz, & Gouws, 2013; Chakona, Swartz, Gouws, & Bloomer, 2013). For example, *Galaxias* sp. 'Joubertina', one of the newly identified unique lineages within the *G. zebratus* species complex, is a narrow-range endemic comprising highly fragmented populations confined to tributaries of the Krom and Gamtoos River systems in the eastern CFE (Cambray et al., 1995; Chakona, Swartz, & Chakona, 2015). Future survival of *Galaxias* sp. 'Joubertina' is uncertain because remnant populations of this lineage face increasing human impacts owing to excessive water abstraction, habitat degradation and invasion by non-native American black bass *Micropterus* spp Lacepède 1802, blue gill sunfish *Lepomis macrochirus* Rafinesque 1819, and the extralimital African sharp tooth catfish *Clarias gariepinus* (Burchell 1822).

Given the multiple threats to remnant populations of *Galaxias* sp. 'Joubertina', accurate mapping and assessment of the conservation status of this lineage is urgently needed, even before it is formally described, to guide the development and implementation of appropriate conservation and management strategies. The purpose of the present study was to: (i) conduct an IUCN Red List conservation assessment of *Galaxias* sp. 'Joubertina'; (ii) ascertain the factors that pose the greatest extinction risk to *Galaxias* sp. 'Joubertina'; and (iii) make recommendations to conservation authorities on the effective and appropriate management initiatives to secure the remaining populations of *Galaxias* sp. 'Joubertina'. The present study will provide a template for assessing conservation status of other recently identified unique genetic lineages of stream fishes in the CFE (Chakona, Swartz, & Gouws, 2013) to expedite the development of appropriate conservation and management strategies to protect aquatic biodiversity in this global endemic hotspot.

2 | MATERIALS AND METHODS

2.1 | Sampling

Field surveys were carried out in January 2011, March 2011 and April 2012 during low-flow conditions. In total, 22 tributaries (nine in the Krom and 13 in the Gamtoos river systems) and 72 localities (30 in the Krom and 42 in the Gamtoos) were sampled (Figure 1) using a 3 m seine net, snorkelling with a hand net, electric fishing or a combination of these methods. At least three sites were surveyed per tributary to establish the lower and upper limits of the lineage in each tributary. At each site, all habitats present were sampled, and all

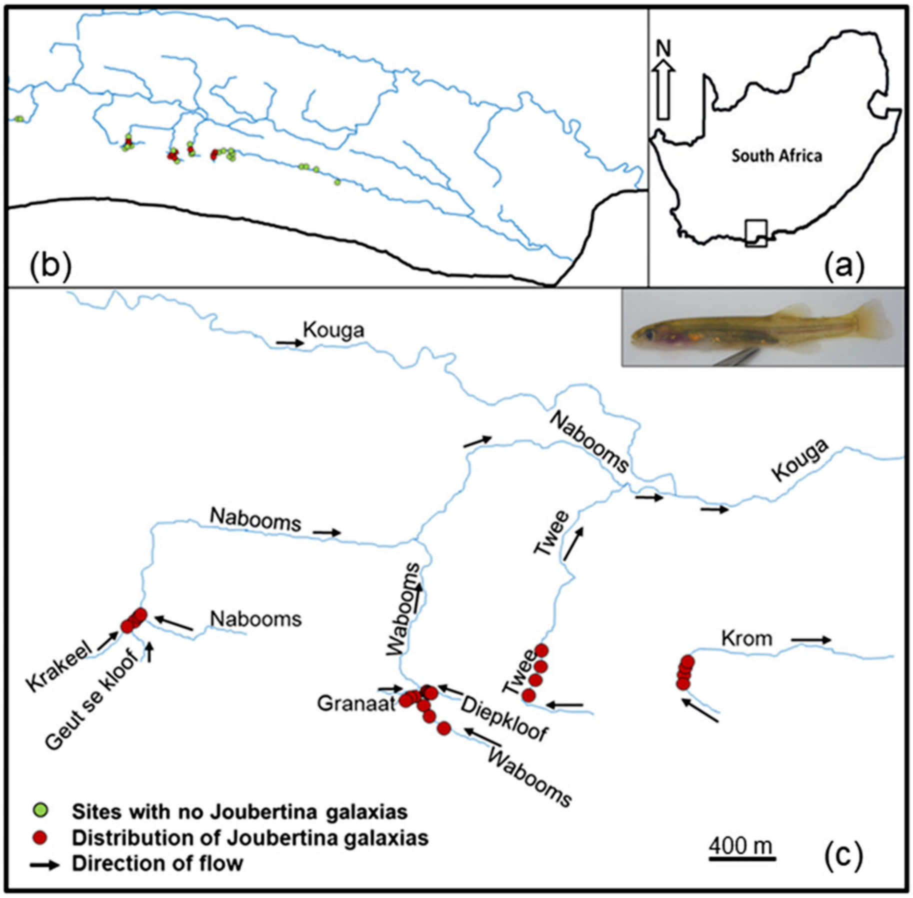

FIGURE 1 **(a)** Map showing location of the study area; **(b)** sampling sites that were visited to map the distribution of *Galaxias* sp. 'Joubertina'; and **(c)** the present localities where *Galaxias* sp. 'Joubertina' was collected in the Krom and Gamtoos river systems. Arrows in plate c indicate the direction of river flow

fishes captured were identified and recorded. Occurrence of non-native fishes was recorded as absent (0) or present (1). Geographic coordinates and site elevation were obtained in the field using a GPS.

Temperature, pH, conductivity and total dissolved solids (TDS) were measured at each locality using portable electronic meters (Hanna Inc.). Water depth, stream width and the length of the stream segment were measured where fish population densities were measured (see below) using a graduated pole and tape measure. At each site surveyed, habitat types and flow conditions were visually assessed and classified as either pool or riffle depending on the depth and flow velocity. Deep areas with slow flow and smooth surface appearance were classified as pools while shallow areas with fast flow and uneven surface appearance were classified as riffles. Flow velocity was visually assessed and scored as static (0), slow (1), moderate (2) or fast (3).

At each locality, dominant substratum was visually assessed and recorded according to particle sizes as mud/silt (< 0.063 mm); sand (0.063–2 mm); gravel (>2–64 mm); cobbles (>64–256 mm); boulders (>256–330 mm) or bedrock following Quinn and Hickey (1990). Aquatic vegetation was scored as absent (0), scarce (< 30%), moderate (30–60%) or abundant (>60%). The extent of stream shading was visually assessed depending on the presence or absence of marginal vegetation and was scored as absent (0), scarce (< 30%), moderate (30–60%) or abundant (>60%). Riparian vegetation was assessed and classified as woody vegetation, mixed vegetation or grassland.

Classification trees (Breiman, Friedman, Olshen, & Stone, 1984) were used to investigate habitat preferences of *Galaxias* sp. 'Joubertina'. The analysis was carried out in R version 2.15 (R Development Core Team, 2012) using R Libraries Tree (for classification trees) and Random Forests (Breiman, 2001, 2002). Classification tree analysis explains variation of response variables based on a set of independent explanatory predictors, either numerical or categorical (Breiman et al., 1984; De'ath, 2002; Ripley, 1996). The analysis differentiates between pre-identified variables in the data as present or absent (De'ath & Fabricius, 2000). Classification trees were constructed using *Galaxias* sp. 'Joubertina' presence or absence data for each site sampled. Physico-chemical variables (temperature, pH, TDS and conductivity), extent of shading, altitude, abundance of aquatic vegetation, type of riparian vegetation and bottom substrate were used as predictors in the analysis. The best predictive tree with the smallest predicted mean square error was selected using cross-validation (De'ath & Fabricius, 2000). The accuracy of the predicted tree models was improved using Random Forests (a non-parametric bootstrapping procedure that constructs a set of trees by resampling the data and averaging the predictions from the bootstrap iterations) (Breiman, 2001). Heterogeneity between data sets within Random Forest trees was determined by the Gini index of variable importance

(Breiman, 2002). A higher Gini index value indicates greater importance of the variable.

2.2 | Assessment of population densities

A single assessment of the abundance and population densities of *Galaxias* sp. 'Joubertina" was made in April 2012 at four localities, two in the upper Wabooms and two in the upper Granaat (Figure 1). These sites were chosen for this assessment because their catchments are least affected by humans (the riparian zones are still intact, the stream reaches have not been bulldozed, there are no non-native fishes in the reaches and water flow has not been modified as there are no weirs, dams or water abstraction points above the localities) indicating that these are the remaining near-natural populations of this lineage. Population densities of the fish were estimated as number per square metre using the three pass depletion method (Lockwood & Schneider, 2000; Zippin, 1956) using an electrofisher.

2.3 | Assessment of threats

Threats were assessed following the guidelines provided by both the IUCN and the Global International Waters Assessment (GIWA, 2001). Major threats to the lineage were classified using the IUCN threat classification scheme version 3.0 (IUCN, 2001; Kostoski, Albrecht, Trajanovski, & Wilke, 2010; Salafsky et al., 2008). According to the IUCN, the threats could be in the past, ongoing, or in the future, using a time frame of three generations or 10 years, whichever is longer (but not exceeding 100 years in the future), as is required by the Red List Categories and Criteria (IUCN, 2001). The presence of non-native fishes, invasive plants, in-stream or off-stream dams, physical in-stream impacts resulting from river channelization, inter-basin transfers (IBTs), canalized rivers and weirs and groundwater abstraction were noted in the assessment criteria. To obtain an overview of the threats to *Galaxias* sp. 'Joubertina', these were identified and the levels of impacts were scored according to the degree of severity on the tributary and the lineage, following the scoring scheme provided by GIWA (2001). If the estimated average impact score was <0.5, it was considered that there was no impact on the tributary; the level of impact was regarded to be slight if the average impact score was between 0.5 and 1; moderate if the average impact score was between 1 and 1.5; high if the average impact score was between 1.5 and 2, and severe if the average impact score was >2 and/or if the maximum impact score of at least one threat was 3.

2.4 | Assessment of the threatened status of the lineage

The risk of extinction for *Galaxias* sp. 'Joubertina' was assessed according to the IUCN Red List categories and criteria (IUCN, 2001, 2003) in which all taxa listed as Critically Endangered, Endangered or Vulnerable are described as threatened.

For each of these threat categories there is a set of five main criteria, A–E, with a number of sub-criteria within A, B and C and an additional sub-criterion in D for the Vulnerable category (IUCN, 2001). The qualifying thresholds within the criteria A–E differ between the threat categories, but classification in any one of these criteria qualifies a taxon for listing (IUCN, 2001). The number of mature individuals was estimated using the equation $d \times A \times p$, where d is an estimate of population density, A is an estimate of area, and p is an estimate of the proportion of individuals that are mature (IUCN, 2011).

The estimation of the area of occupancy (AOO) and the extent of occurrence (EOO) for *Galaxias* sp. 'Joubertina' was performed using the GeoCAT tool (Bachman, Moat, Will, de la Torre, & Scott, 2011) based on the occurrence data that were generated from surveys conducted in 2011 and 2012. These metrics were calculated without the Krom population, which is thought to have been introduced from the Twee River (Gamtoos River system) through inter-basin water transfer canals (Chakona et al., 2015). This population is considered, therefore, to be extralimital, so does not meet the criteria (IUCN, 2003) regarding introduced taxa and subpopulations. The AOO was estimated using the IUCN recommended tetrad (2 × 2 km) spatial scale (IUCN, 2011).

3 | RESULTS

3.1 | Description and distribution

Galaxias sp. 'Joubertina' was found in only six mountain tributaries of the Krom and Gamtoos river systems (Figure 1). The Krom River system has only one population that is restricted to the upper Krom, while five populations occur in tributaries of the Gamtoos River system – the Wabooms, Diepkloof, Granaat, Krakeel and Twee streams. All known populations of *Galaxias* sp. 'Joubertina' do not occur with other native fishes, except at one locality in the Krakeel River where its distribution overlaps with the Cape kurper, *Sandelia capensis* (Cuvier, 1831).

Based on genetic patterns from Chakona et al. (2015), the reconstructed historical range of *Galaxias* sp. 'Joubertina' is inferred to have encompassed both upland tributaries and mainstem sections of the Gamtoos tributaries in the Kouga subcatchment (Figure 2a). The remnant populations of this lineage therefore represent a small proportion of its inferred historical distribution range (Figure 2b). These populations are highly fragmented and now occur in short headwater sections of the streams, many of them only 0.5–1.2 km in length (Figure 2b).

3.2 | Habitat

The elevation of the surveyed sites ranges from 209 to 817 m above sea level, but *Galaxias* sp. 'Joubertina' occurred in a narrow altitudinal range (568 to 648 m above sea level). The surveyed streams range from <1 to 3.5 m in average width. The banks of the Gamtoos tributaries were sheltered, except for the Twee stream that had previously been bulldozed. The dominant vegetation along these streams was riverine fynbos (mainly shrubs of the family Ericaceae and grasses) although submerged macrophytes (aquatic vegetation) were also common in some reaches (Figure 3). The upper Krom tributary was very narrow (±1 m wide) and the banks were heavily vegetated with fynbos (mainly reeds and shrubs), and in some places the stream was completely shaded. Habitat descriptions of localities where *Galaxias* sp. 'Joubertina' was collected are summarized in Table 1. Conductivity

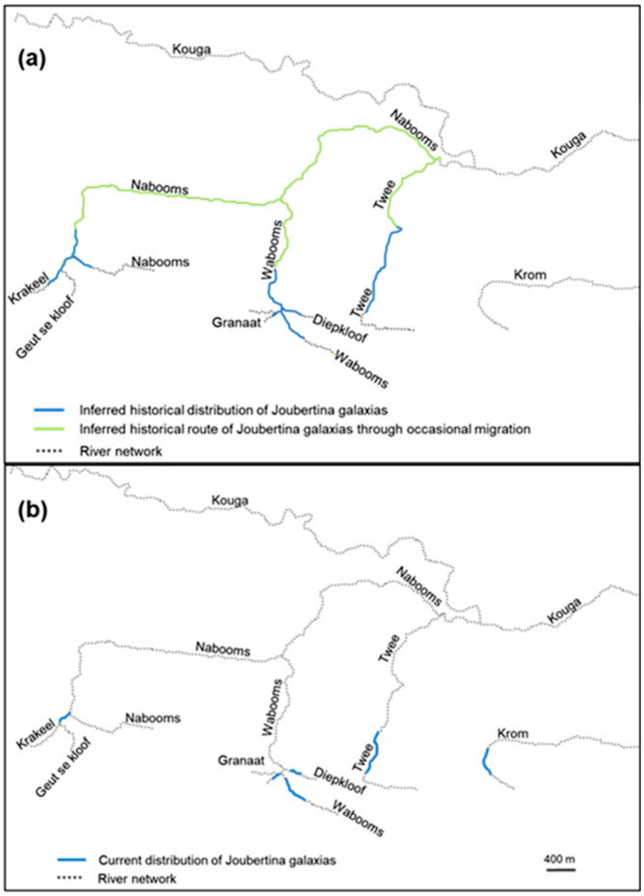

FIGURE 2 **(a)** Inferred historical, and **(b)** current distribution of *Galaxias* sp. 'Joubertina'

(range 38–80 µS cm^{-1}) and total dissolved solids (range 19–40 mg L^{-1}) were generally low, which is typical of mountain tributaries in the CFE. The pH values (range 5.0–6.3) suggested that the water was generally in the acidic range.

Galaxias sp. 'Joubertina' was largely associated with pool and riffle habitats with gentle currents and vegetated banks, but was also collected in static pools. Classification tree analysis indicated that altitude, conductivity and the presence of aquatic vegetation were the habitat variables that strongly influence the distribution of *Galaxias* sp. 'Joubertina' (Appendix S1, Supplementary information). Individuals of this lineage were collected from stream reaches within a narrow altitudinal range (554–634 masl), with water conductivity >37 µS cm^{-1} and where aquatic and marginal vegetation were abundant.

3.3 | Abundance and density

The upper Granaat and upper Waboms were the only sections where *Galaxias* sp. 'Joubertina' still occurs in near-natural conditions. The Krakeel and Twee populations were severely affected by agricultural activities (bull-dozing and channelization), while the Diepkloof has been affected by sedimentation and invasion by non-native fishes. The Krom population was excluded from further analysis as it is possible that this may be a newly established population. The densities for *Galaxias* sp. 'Joubertina' were calculated only for the Granaat and Waboms as the other four populations from the Gamtoos system were considered to be severely affected and may not be viable in the long term owing to severe impacts. The estimated population sizes ranged from 1148 to 4420 individuals in the Waboms and 120–1337 individuals in the Granaat, and densities ranged from 0.54–1.3 fish m^{-2} and 0.16–0.99 fish m^{-2} for the two streams, respectively (Table 2). *Galaxias* sp. 'Joubertina' was uncommon in the other streams, particularly the Twee, Krakeel and Diepkloof.

Very few mature individuals (> 35 mm SL) were caught per population during sampling surveys, with sub-adults (20–35 mm SL) being the most common size class. The estimated number of mature individuals for the Granaat ranged from 36 to 401, while for the Waboms, the estimates ranged from 344 to 1326 individuals (Table 3). Estimates from the present study suggest that *Galaxias* sp. 'Joubertina' may be represented by fewer than 2500 mature individuals.

3.4 | Decline of historical range

The present distribution range of *Galaxias* sp. 'Joubertina' is only a small proportion of the inferred historical range (Figure 2). All remaining populations are now extremely fragmented and isolated, due to the presence of dams, degradation of intervening habitats among populations, and the presence of non-native predators in the mainstem habitats.

3.5 | Key threats and impacts

Key threats to the upper Gamtoos and upper Krom River systems were assessed as defined by the IUCN threats classification scheme version 3.0 (Table 4). Four classes of threats were assessed for *Galaxias* sp. 'Joubertina' (Table 5). Of the six streams in which *Galaxias* sp. 'Joubertina' occurs at present, only the catchments of the upper Granaat and Waboms have not been directly affected by human activities (Table 5).

The most serious threat to *Galaxias* sp. 'Joubertina' is the presence of non-native predators in the Gamtoos and Krom River catchments, as well as agricultural activities and their associated impacts on instream and riparian habitats (Table 5). *Galaxias* sp. 'Joubertina' has been eliminated from sections of the Waboms, Diepkloof and Krom streams that have been invaded by *Micropterus salmoides*. The Granaat population is protected from invasion by a waterfall which prevents upstream movement of non-native fishes.

Mountain tributaries of the Gamtoos and Krom River systems naturally have perennial flow, but excessive water extraction has transformed downstream sections of many streams into seasonal dry-beds during the dry season. Water is extracted from the surface through weirs (Krakeel) and canals (Krom) and from aquifers with pumps (Twee and Krakeel) and used for agricultural and domestic purposes (Figure 4). The Diepkloof, Krakeel, Twee and Krom populations have been severely affected by habitat loss caused by over-abstraction of water for agriculture. Major portions of the streams run dry, especially the Twee and Krakeel streams where most of the flow has been diverted for agriculture. Population sizes in these

FIGURE 3 Typical habitats where *Galaxias* sp. 'Joubertina' were collected

TABLE 1 Habitat description of six tributaries in the Krom and Gamtoos River systems where *Galaxias* sp. 'Joubertina' was collected

	Habitat description				
Tributary	Channel flow conditions	Substratum	Riparian vegetation type	Aquatic vegetation	Stream shade
Twee	Single short pool up to 3 m deep upstream with static and no flow downstream	Cobble with abundant boulders with occasional bedrock	Non-native hardwood dominated	Moderate	Scarce
Krakeel	Small pools less than 1 m deep with very slow flow upstream and downstream	Diverse, including boulders, cobbles, mud and silt	Mixed vegetation (grass & non-native conifer)	Abundant	Scarce
Wabooms	Single short pool up to 1.5 m deep bounded with riffles upstream and downstream with occasional static flow	Diverse, including bedrock, boulders, cobbles, gravel and silt	Shrub (*Erica* Family)	Scarce	Moderate
Diepkloof	Mostly riffles, no pools observed	Mud and silt with occasional cobble, boulders and bedrock	Non-native conifer dominated	Scarce	Abundant
Granaat	Riffles upstream and small pools downstream	Bedrock with occasional boulders, cobbles and sand	Mixed vegetation (grass & non-native conifer)	Scarce	Moderate
Krom*	Small pools less than 1 m deep bounded with riffles upstream and downstream	Bedrock with some boulders and large cobbles and mostly small sandy pools	Grassy fynbos and Ericaceae species	Moderate	Abundant

*= potential extralimital population.

streams were found to be low. Stream structure and critical habitats for fish have also been destroyed directly through bulldozing, especially in the Twee and Krakeel streams. Physical damage to habitats, destruction of riparian vegetation, excessive stream-bank erosion and in-stream sedimentation were observed in all tributaries (Figure 4).

There are several dams that have been built in catchments of the Gamtoos and Krom river systems to store water for agricultural and domestic use. Non-native fishes were subsequently introduced for angling. These dams, in particular Joubertina Dam, are therefore acting as reservoirs for the spread of invasive non-native fishes, which potentially repopulate the lower sections of the mountain tributaries and

TABLE 2 Population densities of *Galaxias* sp. 'Joubertina' for the two sites sampled in each of the Granaat and Wabooms tributaries. N is the number of fish captured per pass and T/N is the total number of fish captured in each pool; se = standard error

Site	N pass 1	N pass 2	N pass 3	T/N	Pool length (m)	Pool width (m)	Pool area (m^2)	N.se	Density (N m^{-2})
Wabooms 1	26	11	9	46	12.9	3.18	41.07	6.22	1.30
Wabooms 2	10	6	2	18	17.5	1.98	34.71	1.90	0.54
Granaat 1	5	3	2	10	15	4.47	67.00	2.15	0.16
Granaat 2	25	17	10	52	24.6	2.73	67.24	11.49	0.99

TABLE 3 Estimated ranges of population sizes and the number of mature individuals of *Galaxias* sp. 'Joubertina' in the Granaat and Wabooms tributaries calculated from the densities obtained from the four sampled sites in Table 2

Tributary	Length (m)	Width (m)	Total area of distribution (m^2)	Density (N m^{-2})	Population size (N)	Mature individuals (N)
Wabooms	850	2.5–4	2125–3400	0.54–1.3	1148–4420	344–1326
Granaat	600	1.25–2.25	750–1350	0.16–0.99	120–1337	36–401

TABLE 4 Scoring table for the Krom and Gamtoos river systems showing key threats as defined by IUCN threats classification scheme version 3.0, current impact and expected future impact changes (shown using arrows). Four scores which are: 0-no known impact; 1-slight impact; 2-moderate impact and 3-severe impact, were used for the assessment of the current impacts affecting the two river systems following the scoring scheme provided by GIWA (2001). For each threat class, the average scores were calculated and maximum values were also given. The level of knowledge regarding these threats was subjectively estimated. The arrow indicates the likely direction of future changes: ↑- impact increases; →- no change; ↓- impact decreases

Threat class	Key threat	Knowledge of threat	Impact	Average impact	Maximum impact
1 Residential and commercial development	1.1 Housing and urban areas	-	0	0	0
	1.2 Commercial and industrial areas	-	0		
	1.3 Tourism and recreation areas	-	0		
2 Agriculture and aquaculture	2.1 Annual and perennial non-timber crops	Well known	2↑	1	2
	2.2 Wood and pulp plantations	Less known	1↑		
	2.3 Livestock farming and ranching	Less known	1↑		
	2.4 Marine and freshwater aquaculture	-	0		
3 Energy production and mining	3.1 Oil and gas drilling	-	0	0	0
	3.2 Mining and quarrying	-	0		
	3.3 Renewable energy	-	0		
4 Transportation and service corridors	4.1 Roads and railroads	-	0	0	0
	4.2 Utility and service lines	-	0		
	4.3 Shipping lanes	-	0		
	4.4 Flight paths	-	0		
5 Biological resource use	5.1 Hunting and trapping terrestrial animals	-	0	0	0
	5.2 Gathering terrestrial plants	-	0		
	5.3 Logging and wood harvesting	-	0		
	5.4 Fishing and harvesting aquatic resources	-	0		
6 Human intrusions and disturbance	6.1 Recreational activities	-	0	0	0
	6.2 War, civil unrest and military exercises	-	0		
	6.3 Work and other activities	-	0		
7 Natural system modifications	7.1 Fire and fire suppression	Moderately known	1↑	2.3	3
	7.2 Dams and water management/use	Well known	3↑		
	7.3 Other ecosystem modifications	Less known	3↑		
8 Invasive and other problematic species and genes	8.1 Invasive non-native/non-native species	Well known	3↑	1	3
	8.2 Problematic native species	-	0		
	8.3 Introduced genetic material		0		
9 Pollution	9.1 Domestic and urban waste water	-	0	0.5	2
	9.2 Industrial and military effluents	-	0		
	9.3 Agricultural and forestry effluents	Moderately known	2↑		
	9.4 Garbage and solid waste	-	0		
	9.5 Air-borne pollutants	Less known	1↑		
	9.6 Excess energy	-	0		
10 Geological events	10.1 Volcanoes	-	0	0	0
	10.2 Earthquakes/tsunamis	-	0		
	10.3 Avalanches/landslides	-	0		
11 Climate change and severe weather	11.1 Habitat shifting and alteration	-	0	0	0
	11.2 Droughts	-	0		
	11.3 Temperature extremes	-	0		
	11.4 Storms and flooding	-	0		
	11.5 Other impacts	-	0		

TABLE 5 Criteria used for the assessment of threats to *Galaxias* sp. 'Joubertina' using four scores which are: 0-no known impact; 1-slight impact; 2-moderate impact and 3-severe impact

	Impact of threats						
Tributary	Agriculture & aquaculture	Natural system modifications	Invasive & other problematic species & genes	Pollution	Average impact	Maximum impact	Level of impact
Waboom s	1	1	1	0	0.75	1	Slight
Krakeel	3	3	1	1	2	3	Severe
Granaat	0	0	0	0	0	0	None
Twee	3	3	2	1	2.25	3	Severe
Diepkloof	3	3	2	1	2.25	3	Severe
Krom*	2	2	3	1	2	3	Severe

*Potentially extralimital.

FIGURE 4 Some of the major threats to *Galaxias* sp. 'Joubertina': **(a)** over-abstraction of water and bulldozing in the Twee River; **(b)** sedimentation, erosion and siltation of the Wabooms and Granaat streams due to the Joubertina dam; **(c)** invasion of the riparian zone by *Pinus* trees along the Wabooms River; **(d)** increased water turbidity in the Diepkloof River resulting from irrigation back-flow; **(e)** habitat homogenization caused by bulldozing in the Krakeel River, and **(f)** canal (in which bass was collected) diverting water from the Krom River into a farm dam

therefore prevent recolonization by native fishes. Canals that connect the Krom and Twee streams could have facilitated, and may still be facilitating, the spread of non-native fishes between the Gamtoos and Krom river systems.

3.6 | IUCN conservation status assessment

Galaxias sp. 'Joubertina' has an EOO of 27.175 km^2 and remnant populations are now completely isolated (Figure 2b). The extent of occurrence has declined and this is likely to be a continuing process for three of the populations (Krakeel, Twee and Diepkloof) that are not protected from potential invasion by alien piscivores owing to a lack of instream physical barriers. The Granaat and Wabooms are the only secure populations. The estimated densities ranged from 0.16–1.30 fish m^{-2} and the number of mature individuals was estimated to be fewer than 2500. Furthermore, this lineage has an AOO of 20 km^2 (based on the IUCN approach of applying a 2 km^2 grid over distributional point data), but its actual AOO is less than 10 km^2. *Galaxias* sp. 'Joubertina' could potentially be classified as Critically Endangered because of its restricted geographical range, severely fragmented populations and the continuous decline that is likely in the extent of occurrence, area of occupancy, habitat quality and number of mature individuals. However, the occurrence in five natural locations (Granaat, Waboms, Krakeel, Diepkloof and Twee), the presence of more than 250 mature individuals and the presence of relatively secure populations in the Wabooms and Granaat populations, make the Endangered classification more realistic (Table 6).

4 | DISCUSSION

4.1 | Threats to *Galaxias* sp. 'Joubertina'

The Cape Fold Ecoregion contains the highest concentration of threatened freshwater fishes in southern Africa (Ellender, Wasserman, Chakona, Skelton, & Weyl, 2017; Tweddle et al., 2009). The discovery of unique lineages and novel species that were previously included under these threatened 'species' (Chakona & Skelton, 2017; Chakona & Swartz, 2013; Chakona, Swartz, & Gouws, 2013; Chakona et al., 2014; Swartz et al., 2007; 2009; Wishart et al., 2006) makes it imperative to re-evaluate the conservation status of freshwater fishes in the CFE to inform conservation and management decisions. *Galaxias* sp. 'Joubertina' is the first lineage within the Cape galaxias species complex to be assessed formally to determine its conservation status. This lineage was classified as Endangered, indicating that it faces 'a very high risk of extinction in the wild in 10 years or three generations' (IUCN, 2001). There is therefore a need for urgent conservation actions and management plans that will be effective within this time frame. *Galaxias* sp. 'Joubertina' is threatened by multiple impacts which include restricted distribution range, habitat degradation, hydrological modification and the presence of non-native species. These factors have been identified as the main threats to other freshwater fishes in the CFE and elsewhere (Cambray, 2003; Cowx, 2002; Ellender et al., 2017; Hardie, Jackson, Barmuta, & White, 2006; Skelton, 2002; Tweddle et al., 2009; Van Rensburg et al., 2011; Woodford & Impson, 2004).

4.2 | Consequences of restricted distribution range and small population size

Remnant populations of *Galaxias* sp. 'Joubertina' are highly fragmented and confined to six mountain tributaries (including the extralimital Krom population). There are several potential risks associated with the isolated nature of remnant populations of this lineage in fragmented habitats. Small, isolated populations are vulnerable to

TABLE 6 Summary of the thresholds for the IUCN criteria (IUCN, 2001) and results for the assessment of *Galaxias* sp. 'Joubertina' in South Africa

	Thresholds			Criteria for qualifying	
Criterion	*Critically Endangered*	*Endangered*	*Vulnerable*		Result
B. Small geographic range + fragmented, declining or fluctuating	Extent of occurrence <100 km^2 OR area of occupancy <10 km^2 + two of the following: (i) severely fragmented or only a single location (ii) continuing decline (iii) extreme fluctuations	Extent of occurrence <5000 km^2 OR area of occupancy <500 km^2 + two of the following: (i) severely fragmented or no more than 5 locations (ii) continuing decline (iii) extreme fluctuations	Extent of occurrence 20000 km^2 OR area of occupancy <2000 km^2 + two of the following: (i) severely fragmented or no more than 10 locations (ii) continuing decline (iii) extreme fluctuations	CR: B1(a, b) CR:B2(a, b)	EOO has been reduced to 27.175 km^2 and is continuing to decline. Populations are severely fragmented and at least four of the five natural populations have become isolated. There are instream barriers that protect two of the remnant populations from invasion by non-native species which are present in the lower sections of the rivers. AOO has been reduced to 8 km^2 and is continuing to decline. Populations are severely fragmented and at least four of the five natural populations have become isolated. There are instream physical barriers that protect two of the remnant populations from invasion by non-native species which are present in the lower sections of the rivers.
C. Small population and declining	<250 mature individuals, population declining	<2500 mature individuals, population declining	<10000 mature individuals, population declining	EN: C1	Population size is estimated to be 380–1727 mature individuals and only the Waboom and Granaat are likely to have more than 250 mature individuals each. The other four subpopulations probably have fewer than 250 mature individuals and ongoing decline is likely. These four subpopulations face a high risk of being extirpated because they are susceptible to invasion by non-native fishes because there are no barriers.

long-term stochastic processes and potential loss of genetic heterogeneity (Frankham, 2005; Frankham, Ballou, & Briscoe, 2002). These threats are particularly severe for populations in small headwater streams as these habitats are characterized by extreme variability in environmental conditions and are highly susceptible to stochastic events such as dewatering of stream channels and forest fires that could result in loss of aquatic biota (Rieman & Clayton, 1997; Schlosser & Angermeier, 1995).

Adequate levels of gene flow are necessary to prevent the deleterious effects of inbreeding (Frankham, 2005; Frankham et al., 2002). While gene flow levels among all *Galaxias* sp. 'Joubertina' populations could have been naturally low (Chakona et al., 2015), the current situation where all known populations of this lineage are highly fragmented, and the presence of non-native predators in river reaches among them, makes it unlikely that migration could occur among remnant populations. This could affect the lineage's long-term viability and evolutionary potential owing to higher chances of inbreeding, which in turn could cause a loss of genetic variability and increase the risk of extinction (Frankham, 2005; Frankham et al., 2002). Although baseline data on densities are unavailable, population sizes are likely to have been larger in the past, so although migration levels might have been low, higher abundance would have increased the effective population size and reduced the general effects of inbreeding (Newman & Pilson, 1997). Future studies should determine the population sizes required to maintain long-term viability of remnant populations of *Galaxias* sp. 'Joubertina', while at the same time estimating the size of habitat required to support those populations.

4.3 | Potential impacts of habitat degradation

Freshwater fishes in the CFE evolved in perennial rivers with clear (or peat stained) water with low suspended solids and heterogeneous substrates dominated by cobbles, pebbles and boulders. However, catchment disturbance through unsustainable agricultural practices, bulldozing and channelization, construction of weirs and excessive or complete abstraction of water have severely transformed many rivers and streams in the region (Nel et al., 2007). Destruction of instream habitat in the Gamtoos catchment represents a significant threat to *Galaxias* sp. 'Joubertina', because increased input of suspended sediments and sedimentation have been found to influence the spawning and abundance of galaxiids in Australia and New Zealand (Hardie & Chilcott, 2017; Rowe, Hicks, & Richardson, 2000). Although the breeding biology of *Galaxias* sp. 'Joubertina' is unknown, studies from Australia and New Zealand have shown that other members of this genus are lithophilic species which spawn in sediment-free rocky habitats (Hardie & Chilcott, 2017). Loss of habitat heterogeneity and increased water turbidity may therefore affect spawning and recruitment success for the South African galaxiids that evolved in mountain habitats with rocky substrates and clear, flowing water. The near-natural stream reaches where *Galaxias* sp. 'Joubertina' occurred were dominated by heterogeneous substrates (a mix of cobbles, pebbles, some boulders, gravel and sand) with the banks covered by marginal and bank-trailing vegetation. This habitat complexity is important as it may provide cover for the fishes, and also provide important habitat for aquatic invertebrates which are the main food source for the Cape galaxias (Skelton, 2001).

4.4 | Threats from non-native species

Non-native species have been identified as the leading threat to freshwater fishes in many regions of the world (Cowx, 2002; Cucherousset & Olden, 2011; Hardie et al., 2006; Tweddle et al., 2009). In southern Africa, non-native species have caused localized extinctions and range reductions of several fish species, with some of the key examples being the extirpation of the Breede-Berg white fish '*Pseudobarbus*' *capensis* (previously *Barbus andrewi*) from the Berg River system (Clark et al., 2009), the extirpation of the Berg river redfin *Pseudobarbus burgi* from the Eerste River system (Skelton, 1988) and the extirpation of the Maloti minnow *Pseudobarbus quathlambae* from the Mkomazana River (Jubb, 1966). During surveys for the present study, native fishes were seldom collected in areas invaded by non-native piscivorous fishes. *Micropterus salmoides*, *Micropterus dolomieu* and *Clarias gariepinus* were collected in the lower reaches and mainstem areas during surveys for the present study. Although the impact of non-native fishes in the Gamtoos River system has not been empirically studied, they have the potential to be detrimental to a small species such as *Galaxias* sp. 'Joubertina'. A number of studies have reported that *Galaxias* and other native fishes in the CFR are particularly susceptible to predation by non-native piscivores (Ellender et al., 2017; Shelton et al., 2015; Shelton, Day, & Griffiths, 2008; Van Der Walt, Weyl, Woodford, & Radloff, 2016; Woodford & Impson, 2004). In the present study, no specimens of *Galaxias* sp. 'Joubertina' were collected in river sections and canals that were invaded by *M. salmoides*. Restriction of *Galaxias* sp. 'Joubertina' to stream habitats that have not been invaded by non-native fishes is an indication of the susceptibility of this lineage to predation. In addition to impacts that could have resulted in population decline, presence of non-native fishes in mainstem habitats and lower reaches of tributary streams is likely to prevent recolonization and recovery of *Galaxias* sp. 'Joubertina'. Because non-native fishes currently occur in the lower reaches of the tributaries that harbour remnant populations of *Galaxias* sp. 'Joubertina' populations, there are concerns that, if not managed, they may invade the upper reaches where the lineage still persists.

4.5 | Potential threats from projected global climate change

Simulations under different climate change scenarios predict that southern Africa will experience severe decline in river discharge through reduced precipitation and increased evapotranspiration (Palmer et al., 2008; Xenopoulos et al., 2005). Dallas and Rivers-Moore (2014) reported that the CFE is one of the 'hotspots of concern' owing to projected increases in the frequency and intensity of extreme events (droughts) and reduction in mean annual precipitation. Similar patterns have been projected for other Mediterranean-type ecosystems where climate change is predicted to have significant impacts on stream flows and habitat diversity in mountain rivers and streams that contain highly threatened species such as *Salmo farioides* (Papadaki et al., 2016). The impacts of climate change will be exacerbated by the likely increase in human demand for water, mainly for agricultural purposes in the CFE, which may in turn alter flow regimes of the rivers through excessive or complete abstraction of surface water and utilization of aquifers that sustain baseflow during the dry season. A recent assessment by Shelton et al. (unpublished data) has highlighted that almost all native fishes that were considered in the study were highly vulnerable to global climate change and the associated human impacts in the CFE. However, lack of information on the biology and ecology of stream fishes in the CFE is a crucial knowledge gap that limits an understanding of the impacts of climate change in the region. Future research efforts should address this problem by focusing on the reproductive biology, life histories, habitat preferences, feeding ecology and responses of native fishes to manipulations of their habitat (e.g. alteration of flow and temperature regimes, as well as to changes in habitat quality).

4.6 | Recovery planning and management issues

As an immediate measure, there is need for conservation authorities to find ways of protecting critical *Galaxias* sp. 'Joubertina' habitat to secure the remaining populations. The sub-catchments of the Kouga River that contain remnant populations of *Galaxias* sp. 'Joubertina' and the recently described cyprinid, *Pseudobarbus swartzi* (Chakona & Skelton, 2017), have been demarcated as fish sanctuaries in the national Freshwater Ecosystem Priority Areas (FEPAs) of South Africa (Nel et al., 2011). These critical biodiversity areas (or FEPAs) need to be maintained in natural or near-natural condition to maintain key ecological processes, and introduction of non-native species is prohibited (Nel et al., 2011). However, long-term conservation strategies should not focus only on conserving biological patterns, but should also seek to maintain evolutionary processes (Moritz, 1994, 1999). Rehabilitation of habitats and eradication of alien species (Weyl, Finlayson, Impson, Woodford, & Steinkjer, 2014) will result in re-establishing connectivity among remaining populations of *Galaxias* sp. 'Joubertina' and facilitate expansion and migration of individuals of this lineage. This will allow larger and more stable populations (e.g. Waboomsand Granaat) to 'rescue' smaller populations (e.g. Diepkloof), thereby reducing potential deleterious effects of inbreeding. There is also a need to assess the minimum stream flow that is required to sustain all life-history stages of *Galaxias* sp. 'Joubertina'. Care should be taken in designing rehabilitation projects, because restoration may allow the spread and establishment of unwanted non-native species.

Given that a large proportion of *Galaxias* sp. 'Joubertina' habitat occurs within privately owned land, there is a need to liaise with landowners and stakeholders regarding management options. Although involvement of people in local communities has long been identified as one of the important factors in successful conservation projects (Cambray & Pister, 2002), the greatest challenge currently facing conservation authorities in the study area is poor awareness of the issues of water and freshwater biodiversity conservation. There is therefore a need for environmental education, explaining the ecological impacts of excessive water abstraction and the spread of non-native fishes and plants. The lower reaches of almost all of the mountain tributaries where *Galaxias* sp. 'Joubertina' occur have been severely degraded by a range of activities, particularly bulldozing and channelization, which caused severe degradation of fish habitat. A key action is to provide local farmers and landowners with appropriate knowledge and expertise to develop low-impact flood management practices that minimize the destruction of instream habitats.

The primary measure of success for conservation and recovery measures will be the down-listing of the current 'Endangered' status of *Galaxias* sp. 'Joubertina'. A more effective way to achieve the down-listing would be to secure the remaining populations and putting measures in place that would enable more populations to recover to more than 2500 mature individuals. Recovery after rehabilitation should be monitored to assess any changes in the status of the remaining populations.

ACKNOWLEDGEMENTS

This work was part of GC's MSc research which was funded by the South African Institute for Aquatic Biodiversity (SAIAB), Rhodes University Margaret Smith bursary and the DST/NRF Centre of Excellence for Invasion Biology through OLF Weyl's core team member grant. Nkosinathi Mazungula, Bruce Ellender, Christine Coppinger, Tuuli Makinen and Roger Smith are thanked for assistance with fieldwork, and Wilbert Kadye for assistance with data collection and analysis. The authors acknowledge that opinions, findings and conclusions or recommendations expressed here are those of the authors and that the NRF accepts no liability whatsoever in this regard.

ORCID

Albert Chakona http://orcid.org/0000-0001-6844-7501

REFERENCES

Abell, R., Thieme, M. L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., … Petry, P. (2008). Freshwater ecoregions of the world: A new map of biogeographic units of freshwater biodiversity conservation. *Bioscience*, *58*, 403–414.

Bachman, S., Moat, J., Will, A. W., de la Torre, J., & Scott, B. (2011). Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. *ZooKeys*, *150*, 117–126.

Bloomer, P., & Impson, N. D. (2000). Mitochondrial DNA differentiation in the Critically Endangered Berg River Redfin (*Pseudobarbus burgi*). *The Journal of Heredity*, *91*, 122–127.

Breiman, L. (2001). Random forests. *Machine Learning*, *45*, 5–32.

Breiman, L. (2002). *Manual on setting up, using, and understanding random forests*, v3.1. //oz.berkeley.edu/users/breiman/Using_random_forests_V3.1.pdf

Breiman, L., Friedman, J. H., Olshen, R. A., & Stone, C. J. (1984). *Classification and regression trees*. Washington DC: Chapman & Hall/CRC.

Cambray, J. A. (2003). The global impact of alien trout species: A review, with reference to their impact in South Africa. *African Journal of Aquatic Science*, *28*, 61–67.

Cambray, J. A., Bok, A., & Smith, R. (1995). Range extensions for *Galaxias zebratus* Castelnau, 1861 (Galaxiidae), Krom and Gamtoos River systems, Eastern Cape. *Southern African Journal of Aquatic Science*, *21*, 99–102.

Cambray, J. A., & Pister, E. P. (2002). The role of scientists in creating public awareness for the conservation of fish species: African and American case studies. In M. J. Collares-Pereira, I. G. Cowx, & M. M. Coelho (Eds.), *Conservation of freshwater fishes: Options for the future* (pp. 414–423). Oxford UK: Fishing News Books, Blackwell Science.

Chakona, A., & Skelton, P. H. (2017). A review of the *Pseudobarbus afer* (Peters, 1864) species complex (Teleostei, Cyprinidae) in the eastern Cape Fold Ecoregion of South Africa. *ZooKeys*, *657*, 109–140.

Chakona, A., & Swartz, E. R. (2013). A new redfin species, *Pseudobarbus skeltoni* (Cyprinidae, Teleostei) from the Cape Floristic Region, South Africa. *Zootaxa*, *3686*, 565–577.

Chakona, A., Swartz, E. R., & Gouws, G. (2013). Evolutionary drivers of diversification and distribution of a southern temperate stream fish assemblage: Testing the role of historical isolation and spatial range expansion. *PLoS ONE*, *8*. e70953

Chakona, A., Swartz, E. R., Gouws, G., & Bloomer, P. (2013). A freshwater fish defies ancient mountain ranges and drainage divides: Extrinsic and intrinsic influences on the evolutionary history of a recently identified galaxiid. *Journal of Biogeography*, *40*, 1399–1412.

Chakona, A., Swartz, E. R., & Skelton, P. H. (2014). A new species of redfin (Teleostei, Cyprinidae, *Pseudobarbus*) from the Verlorenvlei River system, South Africa. *ZooKeys*, *453*, 121–137.

Chakona, G., Swartz, E. R., & Chakona, A. (2015). Historical abiotic events or human-aided dispersal: Inferring the evolutionary history of a newly discovered galaxiid fish. *Ecology and Evolution*, *5*, 1369–1380.

Clark, B. M., Impson, D., & Rall, J. (2009). Present status and historical changes in the fish fauna of the Berg River, South Africa. *Transactions of the Royal Society of South Africa*, *64*, 142–163.

Cowx, I. G. (2002). Analysis of threats to freshwater fish conservation: Past and present challenges. In M. J. Collares-Pereira, I. G. Cowx, & M. M. Coelho (Eds.), *Conservation of freshwater fishes: Options for the future* (pp. 201–220). Oxford, UK: Blackwell Scientific.

Cucherousset, J., & Olden, J. D. (2011). Ecological impacts of non-native freshwater fishes. *Fisheries*, *36*, 215–230.

Dallas, H. F., & Rivers-Moore, N. A. (2014). Ecological consequences of global climate change for freshwater ecosystems in South Africa. *South African Journal of Science*, *110*, 48–58.

De'ath, G. (2002). Multivariate regression trees: A new technique for modeling species–environment relationships. *Ecology*, *83*, 1105–1117.

De'ath, G., & Fabricius, K. E. (2000). Classification and regression trees: A powerful yet simple technique for the analysis of complex ecological data. *Ecology*, *81*, 3178–3192.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., … Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews*, *81*, 163–182.

Ellender, B. R., Wasserman, R. J., Chakona, A., Skelton, P. H., & Weyl, O. L. F. (2017). A review of the biology and status of Cape Fold Ecoregion freshwater fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *27*, 867–879.

Ellender, B. R., & Weyl, O. L. F. (2014). A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. *Aquatic Invasions*, *9*, 117–132.

Frankham, R. (2005). Genetics and extinction. *Biological Conservation*, *126*, 131–140.

Frankham, R., Ballou, J. D., & Briscoe, D. A. (2002). *Introduction to conservation genetics*. Cambridge: Cambridge University Press.

GIWA. (2001). Methodology handbook. Scaling and scoping, //www.unep.org/dewa/giwa/methodology/RevScalScop_Meth_10July2001.PDF, accessed in March 2012.

Goldblatt, P., & Manning, J. C. (2002). Plant diversity of the Cape region of Southern Africa. *Annals of the Missouri Botanical Garden*, *89*, 281–302.

Hardie, S. A., & Chilcott, M. A. (2017). Water levels in a highland lake control the quantity and quality of spawning habitat for a littoral-spawning galaxiid fish. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *27*, 24–38.

Hardie, S. A., Jackson, J. E., Barmuta, L. A., & White, R. W. G. (2006). Status of galaxiid fishes in Tasmania, Australia: Conservation listings, threats and management issues. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *16*, 235–250.

Harrison, I. J., & Stiassny, M. L. J. (1999). The quiet crisis: A preliminary listing of freshwater fishes of the world that are extinct or 'missing in action'. In R. D. E. MacPhee (Ed.), *Extinctions in near time* (pp. 271–331). New York, NY: Kluwer Academic/Plenum.

Helfman, G. S. (2007). *Fish conservation: A guide to understanding and restoring global aquatic biodiversity and fishery resources* (pp. 3–16 & 97–249). Washington DC: Island Press.

Hinton-Taylor, C., Pollock, C. M., Chanson, J. S., Butchart, S. H. M., Oldfield, T. E. E., & Katariya, V. (2009). State of the world's species. In J.-C. Vié, C. Hilton-Taylor, & S. N. Stuart (Eds.), *SWildlife in a changing world: An analysis of the 2008 IUCN red list of threatened species* (pp. 15–41). Gland, Switzerland: IUCN.

IUCN. (2001). *IUCN red list categories and criteria: Version 3.1. IUCN Species*. Gland, Switzerland: Survival Commission, IUCN.

IUCN. (2003). *Guidelines for Application of IUCN Red List Criteria at Regional Levels: Version 3.0*. Gland, Switzerland and Cambridge, UK: IUCN Species Survival Commission.

IUCN. (2011). *IUCN Red List of Threatened Species*. Version 2011.2. www.iucnredlist.org. Downloaded on 07 March 2012.

Jubb, R. A. (1966). *Labeo (?) quathlambae*, a rare freshwater fish now feared extinct. *Piscator*, *67*, 78–80.

Kadye, W. T., Chakona, A., & Jordaan, M. S. (2016). Swimming with the giant: Coexistence patterns of a new redfin minnow *Pseudobarbus skeltoni* from a global biodiversity hot spot. *Ecology and Evolution*, *6*, 7141–7155.

Kostoski, G., Albrecht, C., Trajanovski, S., & Wilke, T. (2010). A freshwater biodiversity hotspot under pressure - assessing threats and identifying conservation needs for ancient Lake Ohrid. *Biogeosciences*, *7*, 5347–5382.

Linder, H. P., Johnson, S. D., Kuhlmann, M., Matthee, C. A., Nyffeler, R., & Swartz, E. R. (2010). Biotic diversity in the Southern African winter-rainfall region. *Current Opinion in Environmental Sustainability*, *2*, 109–116.

Lockwood, R. N., & Schneider, J. C. (2000). Stream fish population estimates by mark and-recapture and depletion methods. In J. C. Schneider (Ed.), *Manual of fisheries survey methods II: With periodic updates*. Ann Arbor, MI: Michigan Department of Natural Resources, Fisheries Special Report 25.

Magurran, A. E. (2009). Threats to freshwater fish. *Science*, *325*, 1215–1216.

Moritz, C. (1994). Defining 'Evolutionarily Significant Units' for conservation. *Trends in Ecology and Evolution*, *9*, 373–375.

Moritz, C. (1999). Conservation units and translocations: Strategies for conserving evolutionary processes. *Hereditas*, *130*, 217–228.

Nel, J. L., Driver, A., Strydom, W. F., Maherry, A., Peterson, C., Hill, L., … Smith-Adao, L. B. (2011). *Atlas of freshwater ecosystem priority areas in South Africa: Maps to support sustainable development of water resources*. Pretoria, South Africa: Water Research Commission Report No. TT 500/11.

Nel, J. L., Roux, D. J., Maree, G., Kleynhans, C. J., Moolman, J., Reyers, B., … Cowling, R. M. (2007). Rivers in peril inside and outside protected areas: A systematic approach to conservation assessment of river ecosystems. *Diversity and Distributions*, *13*, 341–352.

Newman, D., & Pilson, D. (1997). Increased probability of extinction due to decreased genetic effective population size: Experimental populations of *Clarkia pulchella*. *Evolution*, *51*, 354–362.

Palmer, M. A., Liermann, C. A. R., Nilsson, C., Flörke, M., Alcamo, J., Lake, P. S., & Bond, N. (2008). Climate change and the world's river basins: Anticipating management options. *Frontiers in Ecology and the Environment*, *6*, 81–89.

Papadaki, C., Soulis, K., Muñoz-Mas, R., Martinez-Capel, F., Zogaris, S., Ntoanidis, L., & Dimitriou, E. (2016). Potential impacts of climate change on flow regime and fish habitat in mountain rivers of the south-western Balkans. *Science of the Total Environment*, *540*, 418–428.

Quinn, J. M., & Hickey, C. W. (1990). Magnitude of effects of substrate particle size, recent flooding, and catchment development on benthic invertebrates in 88 New Zealand rivers. *New Zealand Journal of Marine and Freshwater Research*, *24*, 411–427.

R Development Core Team. (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3–900051–07-0, URL //www.R-project.org/.

Rieman, B., & Clayton, J. (1997). Wildfire and native fish: Issues of forest health and conservation of sensitive species. *Fisheries*, *22*, 6–15.

Ripley, B. D. (1996). *Pattern recognition and neural networks*. Cambridge, UK: Cambridge University Press.

Rouget, M., Richardson, D. M., Cowling, R. M., Lloyd, W., & Lombard, A. T. (2003). Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the Cape Floristic Region. *Biological Conservation*, *112*, 63–85.

Rowe, D., Hicks, M., & Richardson, J. (2000). Reduced abundance of banded kokopu (*Galaxias fasciatus*) and other native fish in turbid rivers of the North Island of New Zealand. *New Zealand Journal of Marine and Freshwater Research*, *34*, 547–558.

Salafsky, N., Salzer, D., Stattersfield, A. J., Hilton-Taylor, C., Neugarten, R., Butchart, S. H. M., … Wilkie, D. (2008). A standard lexicon for biodiversity conservation: Unified classifications of threats and actions. *Conservation Biology*, *22*, 897–911.

Schlosser, I. J., & Angermeier, P. L. (1995). Spatial variation in demographic processes of lotic fishes: Conceptual models, empirical evidence, and implications for conservation. In J. L. Nielsen (Ed.), *Evolution and the aquatic ecosystem: Defining unique units in population conservation* (pp. 392–401). Symposium 17, American Fisheries Society: Bethesda, MD.

Shelton, J. M., Day, J. A., & Griffiths, C. L. (2008). Influence of largemouth bass, *Micropterus salmoides*, on abundance and habitat selection of Cape galaxias, *Galaxias zebratus*, in a mountain stream in the Cape Floristic Region, South Africa. *African Journal of Aquatic Science*, *33*, 201–210.

Shelton, J. M., Samways, M. J., & Day, J. A. (2015). Predatory impact of non-native rainbow trout on endemic fish populations in headwater streams in the Cape Floristic Region of South Africa. *Biological Invasions*, *17*, 365.

Skelton, P. H. (1988). A taxonomic revision of the redfin minnows (Pisces, Cyprinidae) from southern Africa. *Annals of the Cape Provincial Museum (Natural History)*, *16*, 201–307.

Skelton, P. H. (2001). *A complete guide to the freshwater fishes of Southern Africa*. Cape Town, South Africa: Struik.

Skelton, P. H. (2002). An overview of the challenges of conserving freshwater fishes in South Africa. In M. J. Collares-Pereira, I. G. Cowx, & M. M. Coelho (Eds.), *Conservation of freshwater fishes: Options for the future* (pp. 221–236). London, UK: Fishing News Books.

Skelton, P. H., Cambray, J. A., & Lombard, A. (1995). Patterns of distribution and conservation status of freshwater fishes in South Africa. *South African Journal of Zoology*, *30*, 71–81.

Swartz, E. R., Chakona, A., Skelton, P. H., & Bloomer, P. (2014). The genetic legacy of lower sea-levels: Does the confluence of rivers during the last glacial maximum explain the contemporary distribution of a primary freshwater fish (*Pseudobarbus burchelli*, Cyprinidae) across isolated river systems? *Hydrobiologia*, *726*, 109–121.

Swartz, E. R., Skelton, P. H., & Bloomer, P. (2007). Sea-level changes, river capture and the evolution of populations of the Eastern Cape and fiery redfins (*Pseudobarbus afer* and *Pseudobarbus phlegethon*, Cyprinidae) across multiple river systems in South Africa. *Journal of Biogeography*, *34*, 2086–2099.

Swartz, E. R., Skelton, P. H., & Bloomer, P. (2009). Phylogeny and biogeography of the genus *Pseudobarbus* (Cyprinidae): Shedding light on the drainage history of rivers associated with the Cape Floristic Region. *Molecular Phylogenetics and Evolution*, *51*, 75–84.

Tweddle, D., Bills, R., Swartz, E., Coetzer, W., Da Costa, L., Engelbrecht, J., … Smith, K. G. (2009). The status and distribution of freshwater fishes. In W. R. T. Darwall, K. G. Smith, D. Tweddle, & P. Skelton (Eds.), *The status and distribution of freshwater biodiversity in Southern Africa* (pp. 21–37). Gland, Switzerland and SAIAB, Grahamstown, South Africa: IUCN.

Van Der Walt, J. A., Weyl, O. L. F., Woodford, D. J., & Radloff, F. G. T. (2016). Spatial extent and consequences of black bass (*Micropterus* spp.) invasion in a Cape Floristic Region river basin. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *26*, 736–748.

Van Rensburg, B. J., Weyl, O. L. F., Davies, S. J., van Wilgen, N. J., Spear, D., Chimimba, C. T., & Peacock, F. (2011). Invasive vertebrates of South Africa. In D. Pimentel (Ed.), *Biological invasions: Economic and environmental costs of alien plant, animal and microbe species* (2nd ed.) (pp. 325–380). USA: CRC Press.

Waters, J. M., & Cambray, J. A. (1997). Intraspecific phylogeography of the Cape *Galaxias* from South Africa: Evidence from mitochondrial DNA sequences. *Journal of Fish Biology*, *50*, 1329–1338.

Weyl, O. L. F., Finlayson, B., Impson, N. D., Woodford, D. J., & Steinkjer, J. (2014). Threatened endemic fishes in South Africa's Cape Floristic Region: A new beginning for the Rondegat River. *Fisheries*, *39*, 270–279.

Wishart, M., Hughes, J., Stewart, B., & Impson, D. (2006). Extreme levels of intra-specific divergence among Cape Peninsula populations of the Cape *Galaxias*, *Galaxias zebratus* Castelnau 1861, reveal a possible species complex. *African Journal of Aquatic Science*, *31*, 99–106.

Wishart, M. J., & Day, J. A. (2002). Endemism in the freshwater fauna of the south-western Cape, South Africa. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie*, *28*, 1752–1756.

Woodford, D. J., & Impson, D. N. (2004). A preliminary assessment of the impact of alien rainbow trout (*Oncorhynchus mykiss*) on indigenous fishes of the upper Berg River, Western Cape Province, South Africa. *African Journal of Aquatic Science*, *29*, 107–111.

Xenopoulos, M. A., Lodge, D. M., Alcamo, J., Märker, M., Schulze, K., & Van Vuurens, D. P. (2005). Scenarios of freshwater fish extinctions from climate change and water withdrawal. *Global Change Biology*, *11*, 1557–1564.

Zippin, C. (1956). An evaluation of the removal method of estimating animal populations. *Biometrics*, *12*, 163–189.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Chakona G, Swartz ER, Chakona A. The status and distribution of a newly identified endemic galaxiid in the eastern cape fold ecoregion of South Africa. *Aquatic Conserv: Mar Freshw Ecosyst*. 2018;28:55–67. https://doi.org/10.1002/aqc.2850