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Short Note

Record of Blue tilapia *Oreochromis aureus* (Steindachner, 1864) in the Eerste River catchment, Western Cape province, South Africa

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***Oreochromis aureus* was imported from Israel into South Africa in 1959 but data on its current status in South Africa are lacking. Genomic DNA was extracted and the COI gene amplified at the South African Institute for Aquatic Biodiversity. The identity of the sequences and specimens was determined using the Barcode of Life Data Systems and GenBank. Morphological and genetic assessment demonstrated that 11 specimens collected from two farm dams in the Eerste River System, Western Cape province, were *Oreochromis aureus*. A MaxEnt model compiled using global distribution, rainfall and temperature data predicted that large areas of southern Africa were climatically suitable for this species, indicating considerable invasion debt in southern Africa. As a result, surveys to assess for the extent of the invasion in South Africa and eradication of existing populations, if feasible, are recommended management actions.**

Keywords: COI gene, DNA barcoding, invasion risk, Israeli tilapia, MaxEnt, mitochondrial DNA

Online Supplementary Material: Table S1: Summary of the importance of the variables for the model based on all occurrences, and Figure S1: ROC curve for the MaxEnt Model for Blue tilapia *Oreochromis aureus*, is available at DOI: 10.2989/16085914.2018.1455576

Introduction

Cichlids of the genus *Oreochromis* have been widely introduced for aquaculture and fisheries enhancements (Canonico et al. 2005). The most extensively utilised species are the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758), Blue (or Israeli) tilapia *Oreochromis aureus* (Steindachner, 1864) and their hybrids (Watanabe et al. 2002). *Oreochromis niloticus* and *O. aureus* are native to West Africa through to the Nile River and the Levant, with *O. niloticus* being the more widespread species with its distribution extending into East Africa (Trewavas 1983). Both species are geographically isolated from the East African *Oreochromis* species (e.g., *Oreochromis mossambicus* (Peters, 1852), *Oreochromis placidus* (Trewavas, 1941), *Oreochromis macrochir* (Boulenger, 1912), *Oreochromis andersonii* (Castelnau, 1841) and *Oreochromis mortimeri* (Trewavas, 1966) (Trewavas 1983). Both *O. niloticus* and *O. aureus* have been formally introduced into South Africa. Marr et al. (2017) evaluated the impact of *O. niloticus* in South Africa as massive *sensu* Blackburn et al. (2014), due to competition and/or hybridisation with populations of *O. mossambicus* in the Limpopo River basin (Moralee et al. 2000; Firmat et al. 2013; Zengeya et al. 2015). Similar impacts have been observed for *O. mortimeri* in Lake Kariba (Zengeya and Marshall 2008) and *O. andersonii* in the Kafue River (Deines et al. 2014).

Oreochromis aureus was imported (as "*Tilapia nilotica*") for experimental purposes from Israel to the Jonkershoek Hatchery near Stellenbosch in the Cape Fold Ecoregion in 1959 (van Schoor 1966). Offspring of these fish were released into farm dams in the Lourens and Eerste River catchments in 1961 and 1962 to evaluate their potential to survive the Western Cape winter (van Schoor 1966). Subsequently, van Schoor (1966) reported survival of *O. aureus* in 73% of the dams. As is the case with many introductions, subsequent introduction and distribution information on this species is largely anecdotal. Accounts in de Moor and Bruton (1988) include an established population in Rozendal Dam, Rozendal Farm, Jonkershoek valley, Western Cape in 1967; persistence in farm dams in the Lynedoch district, Eerste River catchment (unknown

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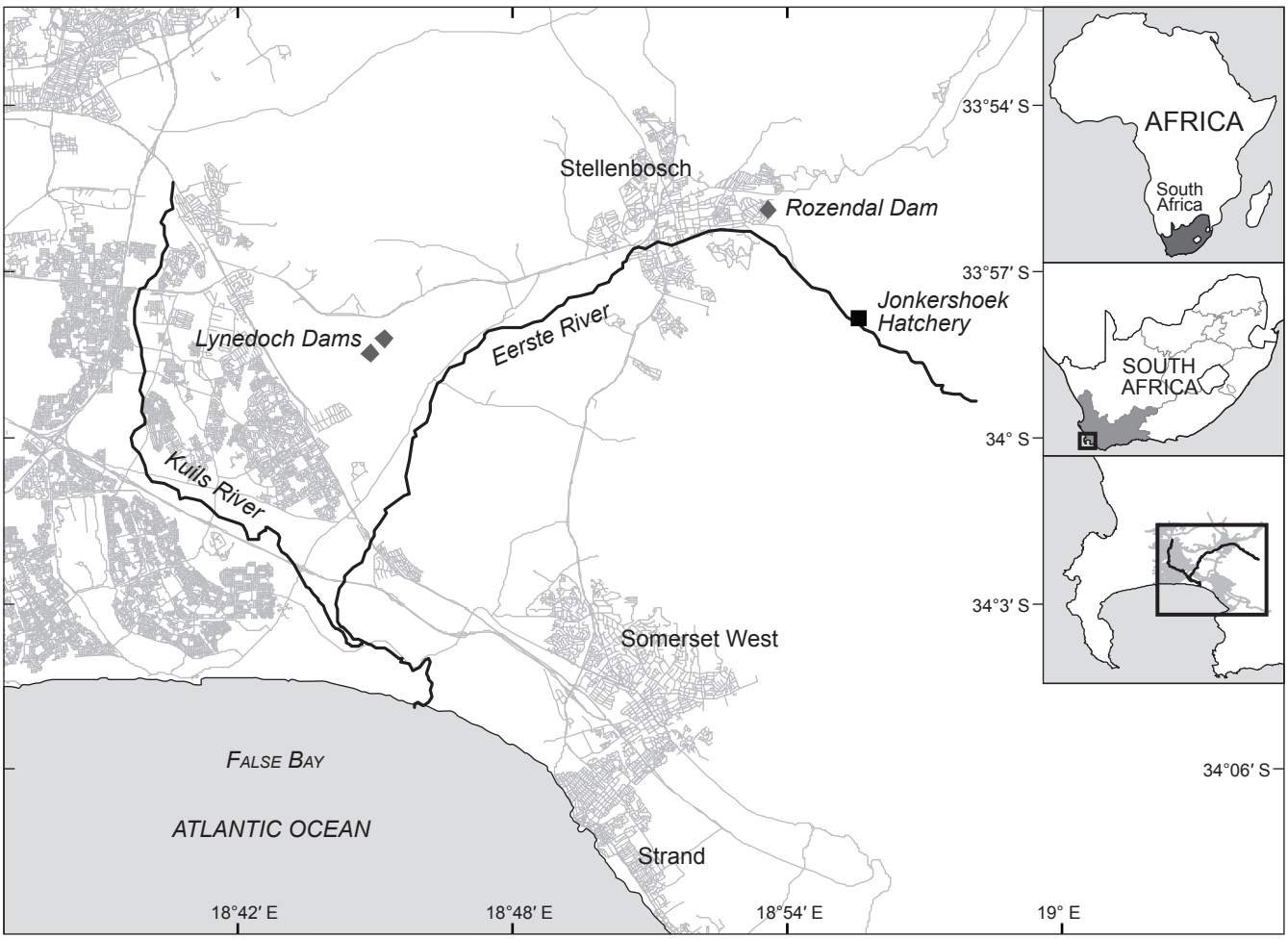

Figure 1: Map depicting the Eerste River catchment in relation to the Jonkershoek Hatchery (source of fish), the Rozendal dam where *Oreochromis aureus* was first recorded in the Eerste River catchment and the location of the Lynedoch dams visited during this study

origin and date of introduction; Figure 1); the introduction of *O. aureus* (and *O. noliticus*) into a small dam in northern KwaZulu-Natal in 1978 from the Amatikulu Hatchery in KwaZulu-Natal and a 1982 introduction of *O. aureus* (and *O. niloticus*) into the Dudley Pringle Dam, Wewe River catchment, KwaZulu-Natal. Because the persistence of these populations was never evaluated, and because the species had not been formally reported in almost 30 years, Ellender and Weyl (2014) evaluated its introduction as failed.

In response to anecdotal reports that *O. aureus* populations may have persisted in some dams near Stellenbosch in the Eerste River catchment, a survey was completed to determine the status of *O. aureus* in these dams using DNA barcoding data and morphological attributes; and to predict the potential distribution range of *O. aureus* in southern Africa using a species distribution model.

Materials and methods

Study area

Oreochromis sp. specimens were collected by CapeNature from two farm dams in the Lynedoch area, Eerste River catchment, Western Cape (Figure 1) on 7 December 2016 and 21 April 2017 using gill, seine and cast nets and angling (Dam 1: 18°45'12" E, 33°58'13" S and Dam 2: 18°44'54" E, 33°58'28" S). The samples were collected in accordance with CapeNature's ethical protocols as part of their invasive species monitoring programme. Because CapeNature collected the specimens and provided the fin clips to the South African Institute for Aquatic Biodiversity (SAIAB) for genetic analysis, no permit was required to conduct the study. A photograph was taken of each specimen captured and the fork length recorded. For genetic analysis, tissue samples (pectoral fin clips) were taken from each specimen and placed in 99% ethanol. The photographs, details of the capture localities and genetic samples were accessioned to the SAIAB collection (035059–035069).

Genetic analyses

Genomic DNA was extracted from each fin clip using the salting out procedure of Sunnucks and Hales (1996). The DNA barcoding fragment, *sensu* Hebert et al. (2003), of the cytochrome c oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR), using the primers VF2_t1 (Ivanova et al. 2007) and FishR1 (Ward et

al. 2005). The PCR conditions and thermocycling regime followed Ward et al. (2005). Successful amplification was determined by visualising products under ultraviolet light, following electrophoresis in a 1% agarose-TBE gel, stained with ethidium bromide. Amplicons were purified using an Exonuclease I-Shrimp Alkaline Phosphate (Exo/SAP, ThermoFisher Scientific) protocol (Werle et al. 1994). Sequencing reactions were performed using an ABI Big Dye v3.1 terminator chemistry kit (Applied Biosystems, Austin, Texas). Sequencing was conducted in both the forward (using M13F primer) and reverse directions. Resulting products were precipitated using an ethanol-EDTA procedure, suspended in formamide (HiDi) and analysed on an ABI-Hitachi 3500 Genetic Analyser (Applied Biosystems) at SAIAB. The resulting sequences were checked against their chromatograms for misreads and sequencing errors using ChromasLITE (Technylesium). Consensus DNA barcodes were compiled using Lasergene SeqMan Pro 9 (DNASTAR, Madison, Wisconsin).

The identity of the sequences and specimens was determined by submitting an identification query on the Barcode of Life Data Systems, BOLD: http://www.barcodinglife.org; Ratnasingham and Hebert (2007), for comparison against all barcoded specimens. Sequences were also subjected to a BLAST search (Altschul et al. 1990) against sequences available on GenBank. Positive matches with hybrids or data from unpublished sources where identifications could not be verified were excluded.

Distribution modelling

MaxEnt, a machine learning maximum entropy modelling programme (Phillips et al. 2006), was used to determine the potential invasive range of *O. aureus* in southern Africa. Global distribution data for *O. aureus* was downloaded from the Global Biodiversity Information Facility open access database (http://www.gbif.org) using the *gbif* function in the R package dismo (Hijmans et al. 2017). The MaxEnt model for *O. aureus* was developed using the *maxent* function in the R package dismo, using presence only data, default settings, *k*-fold cross validation (k = 5) and the current global distribution of the species. The environmental variables used to develop the model were the 30 arc second bioclimatic variables from the WorldClim database (http://www.worldclim.org). The bioclimatic variables were checked for collinearity following Merow et al. (2013) using $r = \pm 0.86$ as a threshold to identify collinear variables. A set of four temperature and five rainfall variables were selected from the 19 bioclimatic variables available. The data extraction and MaxEnt modelling was conducted using the R statistical software version 3.4.0 (R Development Core Team 2017). The Receiver Operator Curve (ROC) was used to determine the thresholds that represent greater than 50% and 90% habitat suitability for *O. aureus*, $p = 0.5$ and $p = 0.9$, respectively. Maps of habitat at the aforementioned levels of habitat suitability were prepared at Global and southern African levels.

Results

Six *Oreochromis* sp. specimens (190–404 mm TL) were sampled from Dam 1 on 7 December 2016 and five fish (380–460 mm TL) were sampled from Dam 2 on 21 April 2017. All fish captured at Dam 1 were adult males with the exception of one juvenile of indeterminate sex, whereas the gender of the fish captured at Dam 2 were not determined. The adult males had clear characteristics of *O. aureus* with a grey body, red fringed dorsal and caudal fins with light blue windows (Trewavas 1983); see Figure 2a, c and d. The juvenile fish superficially resembled *O. niloticus* with a blue patch on the cheek, bars on the body and a light golden body colour, but the caudal fin in all specimens lacked the characteristic "*regular and definite striping*" characteristic of *O. niloticus* (Trewavas 1983); see Figure 2b. It is not possible to positively distinguish between *O. aureus, O niloticus* and *O. mossambicus* based on lateral line scale, dorsal or anal fin rays and spine counts (Trewavas 1983) because of the broad overlap in these parameters between the species (Trewavas 1983). The fish captured in this study were found to have D XVI, 12, A III, 9 and lateral line scale counts of 32.

Genetic analysis

The 655 bp COI barcode sequences from each of the eleven individuals are lodged on GenBank under accession numbers MF817697-MF817707. A single COI barcode (i.e. haplotype) was shared among all specimens. The BOLD identification query with this haplotype returned a 100% match to published barcodes of *O. aureus* sampled from the species' native range, including several localities across Israel and the Lower Nile, Egypt (Shirak et al. 2009). In the nucleotide BLAST search, the highest scoring (1 210, with 100% query coverage and 100% sequence identity) matches were obtained with seven *O. aureus* sequences deposited on NCBI/GenBank (http://ncbi.nlm.nih.gov). Among these were the two complete mitochondrial genomes of *O. aureus*; GU477629: Jiang *et al.*, unpublished GenBank data; GU370125: He et al. (2011).

Potential distribution

The variables "Minimum temperature in the coldest month", "Precipitation in driest quarter", "Temperature annual range" and "Maximum temperature in the warmest month" contributed the most to the MaxEnt model (27%, 17%, 15% and 14%, respectively); see Supplementary Table S1. However, the permutational test revealed that "Minimum temperature in the coldest month", "Precipitation in driest quarter", "Temperature annual range" and "Precipitation in coldest quarter" were the most important variables (33%, 22%, 11% and 11%, respectively); see Supplementary Table S1. The ROC analysis found that the AUC was 0.964, which indicates that the model is a good fit. The ROC (Figure S1, Supplementary material) determined the habitat suitability thresholds for $p = 0.5$ and $p = 0.9$ to be 0.05 and 0.1 on the MaxEnt output, respectively. The MaxEnt model predicts a habitat suitability between 0.5 and 0.9 over the Senegal-Niger river native range of *O. aureus*; these are represented by grey areas in Figure 3. Globally, large parts of the southern United States, Mexico, Central America, Brazil, Argentina, Paraguay, Uruguay, Australia, India, south-east Asia and China are predicted to be suitable for *O. aureus*; these are represented by black areas in Figure 3.

Figure 2: Photographs of Blue tilapia *Oreochromis aureus* caught in summer from a dam in the Eerste River catchment, Stellenbosch, Western Cape. a) adult male (specimen A003); b) juvenile (specimen A006); c) detail of adult male head and d) detail of adult male tail

For southern Africa, MaxEnt predicts that the Olifants–Doring, Berg and Breede River catchments in the Western Cape, the Orange-Vaal catchment above Augrabies Falls excluding the high altitude areas, coastal catchments between Mossel Bay and Port Elizabeth, and East Coast catchments from the Gamtoos Catchment through the Eastern Cape and Kwa-Zulu Natal into Mozambique and the Lowveld areas of Mpumalanga and Limpopo provinces are suitable for the establishment of *O. aureus*; these are represented by grey and black areas in Figure 4.

Discussion

Morphological and genetic assessment confirmed the presence of *O. aureus* in South Africa in two farm dams in the Eerste River catchment. The knowledge that these populations have been present in the dams for more than 30 years demonstrates survival in the wild and the presence of a self-sustaining population, at least at the point of introduction. We therefore conclude that *O. aureus* is at least at a C3 invasion status using the Blackburn et al. (2011) unified framework for biological invasions definition; "*individuals are surviving in the wild in location where introduced, reproduction is occurring, and the population is self-sustaining"*. For C3 invasions, Blackburn et al. (2011) suggest containment or eradication as potential management options. Considering that *O. aureus* is known to hybridise with the indigenous *O. mossambicus* (Trewavas 1983), the impacts of an *O. aureus* invasion on indigenous *Oreochromis* species are likely to be similar to those described for *O. niloticus*; e.g. Firmat et al. (2013), Zengeya et al. (2013), Deines et al. (2014) and Zengeya et al. (2015).

A recent risk assessment conducted using the Fish Invasiveness Screening Kit (FISK) identified that *O. aureus* posed a high risk of becoming invasive in South Africa (Marr et al. 2017). This was supported by the MaxEnt model, which predicts that the species is likely to establish over large parts of southern Africa (Figure 4). It is therefore important to determine the contemporary distribution of this species in South Africa. Such a survey should first concentrate on the Eerste and Lourens systems in the Western Cape, including farm dams, and the Wewe River in KwaZulu-Natal, specifically the Dudley Pringle Dam where this species was introduced. This latter survey should include genetic screening of *Oreochromis* specimens to assess for potential hybrids.

Nile tilapia dominates the global tilapia market (Gupta and Acosta 2004), and there is considerable interest in farming *O. niloticus* in South Africa (OLFW pers. obs.). In addition,

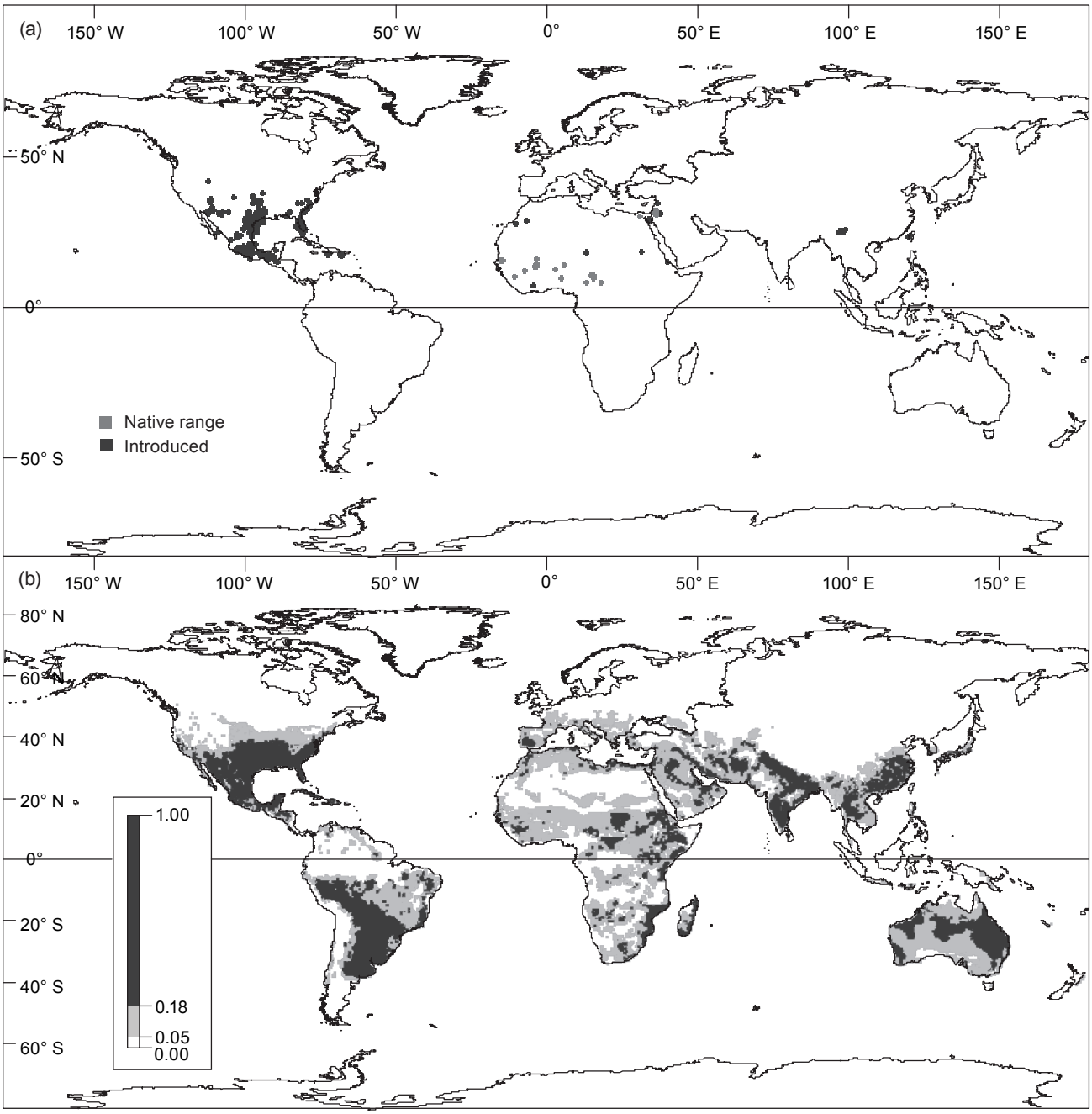

Figure 3: (a) The current global distribution of Blue tilapia *Oreochromis aureus*. Grey markers depict localities in the native range of the species, whereas black markers depict localities where the species has been introduced. (b) The MaxEnt predictions for the potential global distribution of *O. aureus* based on the current global distribution of the species. White represents regions with habitat suitability scores for *O. aureus* less than 0.5, grey regions depict 0.5 to 0.9 habitat suitability scores, whereas black regions depict greater than 0.9 habitat suitability scores.

introductions of *O. niloticus* for stock enhancements are well documented (see Ellender et al. 2014). As a result, the potential spread of the more cold tolerant *O. aureus* from the Lynedoch Dam populations is cause for concern and active measures for containment are recommended. Because *O. aureus* is not specifically listed in either the National List of Invasive Freshwater Fish Species (Republic of South Africa 2016a) or the list of Prohibited Freshwater Fishes. (Republic of South Africa 2016b), the legislative status of the species in South Africa urgently needs to be clarified. Considering the substantial risks posed to the indigenous *Oreochromis* species by this cold tolerant species, we recommend urgent inclusion of *O. aureus* in the NEMBA Alien Invasive Species list. As there are very limited socio-economic considerations constraining its removal, see Woodford et al. (2017), eradication of

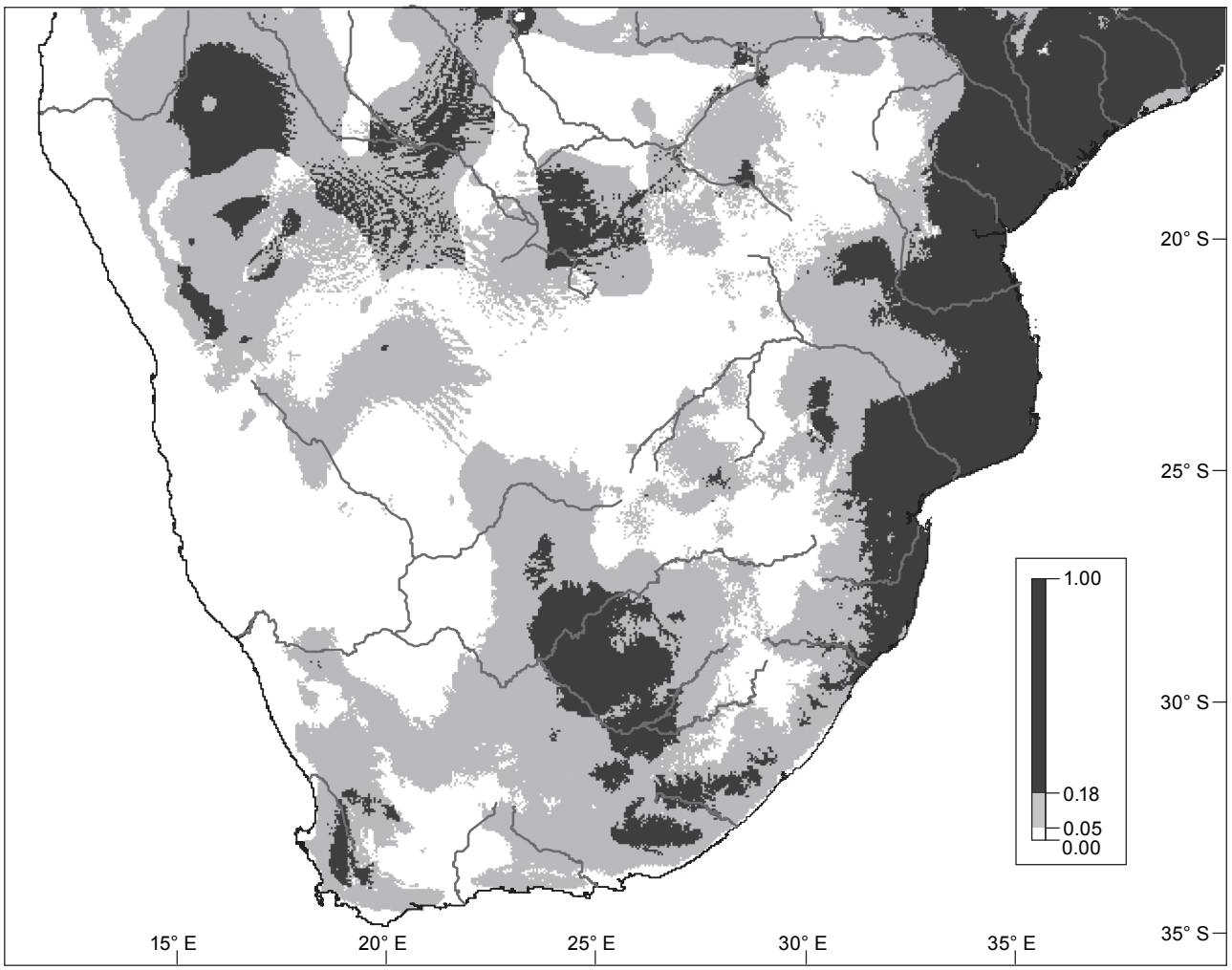

Figure 4: MaxEnt predictions for the potential southern Africa distribution of Blue tilapia *Oreochromis aureus* based on its current global distribution (Figure 3). White represents regions with habitat suitability scores for *O. aureus* less than 0.5, grey regions depict 0.5 to 0.9 habitat suitability scores, whereas black regions depict greater than 0.9 habitat suitability scores.

all potential source populations, if feasible, should be a minimum management objective.

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