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# Invasion status of Florida bass *Micropterus floridanus* (Lesueur, 1822) in South Africa

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Largemouth bass *Micropterus salmoides* are a popular North American angling species that was introduced into South Africa in 1928. To enhance the largemouth bass fisheries, Florida bass *Micropterus floridanus* were introduced into KwaZulu Natal, South Africa, in 1980. Knowledge on the status of *M. floridanus* in South Africa is required, because it lives longer and reaches larger sizes than *M. salmoides*, which may result in heightened impacts on native biota. Because *M. floridanus* are morphologically similar, but genetically distinct from *M. salmoides*, the distribution of this species was assessed by genetically screening 185 *Micropterus* sp. individuals sampled from 20 localities across South Africa using the mitochondrial ND2 gene. Individuals with mitochondrial DNA matching *M. salmoides* were recovered from 16 localities, whereas *M. floridanus* mitochondrial DNA was recovered from 13 localities. At nine localities (45%), the mitochondrial DNA of both species was detected. These results demonstrate *M. floridanus* dispersal to multiple sites across South Africa.

**Keywords:** black bass, largemouth bass, introduced species, mitochondrial DNA, species spread

**Online Supplementary Material A:** List of ND2 gene sequences downloaded from GenBank used to generate a minimum spanning network of *Micropterus salmoides* and *Micropterus floridanus* collected from South Africa and North America and **Online Supplementary Material B:** List of all ND2 gene sequences generated for the current study, based on sampling of 20 South African water bodies, are available online at <http://dx.doi.org/10.2989/16085914.2017.1398131>

## Introduction

The purposeful introduction of non-native fishes for the development of fisheries is one of the main pathways driving freshwater fish invasions globally (Cucherousset and Olden 2011; Ellender et al. 2014). One of the most widely introduced freshwater sport fishes is the largemouth bass *Micropterus salmoides* (Lacepède, 1802), a species native to North America, which has been introduced outside of its native range on all continents, except Australia and Antarctica (Jackson 2002; Hargrove et al. 2015; Taylor et al. 2015). Although such introductions have resulted in the desired fisheries objectives, the often superior performance of *M. salmoides* over native predators (Alexander et al. 2014) has often resulted in negative impacts on native biota (e.g. Ribeiro and Leunda 2012; Ellender and Weyl 2014).

The initial introduction of alien fishes into South Africa is well documented (see de Moor and Bruton 1988). *Micropterus salmoides* were imported in 1928 to provide angling opportunities in areas that were too warm for trout *Oncorhynchus mykiss* (Walbaum, 1792) and *Salmo trutta* (Linnaeus, 1758), which were introduced for sport fishing in the 1890s (de Moor and Bruton 1988; Ellender and Weyl 2014). In a detailed study using microsatellite DNA, Hargrove

et al. (2017) reconstructed the introduction history of *M. salmoides* in South Africa, and found that these populations harboured extremely low neutral genetic diversity, consistent with the records of small numbers of fish being introduced into the country. This low genetic diversity does not appear to have limited the successful establishment of this species in South Africa (Hargrove et al. 2017). By 1950, the offspring of these fish had been spread, with the help of government stocking programmes, throughout South Africa (Ellender et al. 2014) and there is currently a large sport fishery based on this and other *Micropterus* species (Hargrove et al. 2015; Weyl and Cowley 2016).

To enhance *M. salmoides* fisheries, Florida bass *Micropterus floridanus* (Lesueur, 1822), were imported to the Umgeni Hatchery, Kwa Zulu Natal, South Africa in 1980 (de Moor and Bruton 1988). Until relatively recently, *M. floridanus* was considered a subspecies of *M. salmoides*, because the two species are difficult to separate morphologically and because they hybridise when they occur in sympatry (Warren 2009). Kassler et al. (2002), however, demonstrated that *M. salmoides floridanus* differed both morphologically and genetically, in both mitochondrial DNA and allozyme

variation, from *M. salmoides* and recommended that *M. floridanus* be recognised as a valid species. This recommendation has been accepted in several subsequent assessments (e.g. Near et al. 2003, 2004) and *M. floridanus* is now listed as a valid name in the California Academy of Sciences Catalog of Fishes (Eschmeyer and Fong 2017).

*Micropterus floridanus* is often introduced to enhance *M. salmoides* fisheries (Kassler et al. 2002), because it is considered to be better adapted to warmer climates (Philipp and Whitt 1991), where it has a longer spawning season (Iseley 1987; Rogers et al. 2006), grows faster, and lives longer (Philipp and Whitt 1991; Neal and Noble 2002). As a result of more rapid growth and increased longevity relative to *M. salmoides*, larger sizes are frequently attained by both *M. floridanus* and the *M. floridanus* × *M. salmoides* intergrade populations (Neal and Noble 2002). Indeed, the angling record for *M. salmoides* in southern Africa remained relatively stable at ca. 4 kg for more than 50 years, until the introduction of *M. floridanus* into the region in 1980 resulted in a rapid increase to 8.3 kg in 2004 (Figure 1).

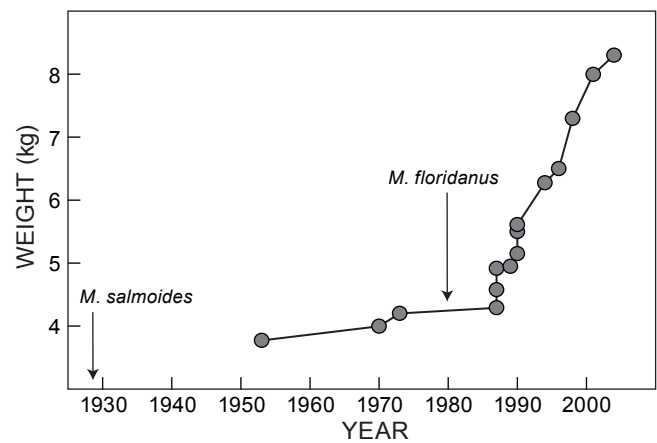
Knowledge of the extent of the current distribution of *M. floridanus* in South Africa is increasingly important, because the current alien species legislation in the country aims to limit the spread of invasive fishes by prohibiting their movement to catchments where they do not already occur (Woodford et al. 2017). The distribution of *M. floridanus* in South Africa is, however, currently unknown, largely because of the private and generally unreported nature of introductions following the cessation of government support to stocking programmes in the early 1990s (Ellender et al. 2014), but also because this species and its hybrids are morphologically difficult to distinguish from *M. salmoides*.

The current study sought to test for the presence of *M. floridanus* in a series of South African water bodies in order to provide a better understanding of the current status of this species in the country. To do this, mitochondrial ND2 gene region data were generated from bass sampled from 20 South African reservoirs and compared to haplotypes of known provenance to test the hypothesis that *M. floridanus* have expanded their distribution despite the existence of legislation that explicitly prohibits their translocation.

## Materials and methods

In total, 185 *Micropterus* sp. specimens measuring between 175 and 580 mm fork length (FL) were sampled from 20 locations across 15 river systems in six of South Africa's nine provinces between January 2011 and April 2012 (Figure 2). These included samples from the populations in five localities that, on the basis of the rapid assessment presented here, were targeted for a more comprehensive microsatellite-DNA based analysis by Hargrove et al. (2017).

Samples were collected by angling, either by a survey team or by tournament anglers who donated their catches. Fish identified a priori as *M. salmoides* were measured for FL to the nearest mm, and a ca. 1 cm<sup>2</sup> finclip was taken from one pelvic fin and stored in 99% ethanol. All fish that were alive at the time of sampling were released following the collection of the fin clip. Sample sizes and locality details are provided in Table 1.

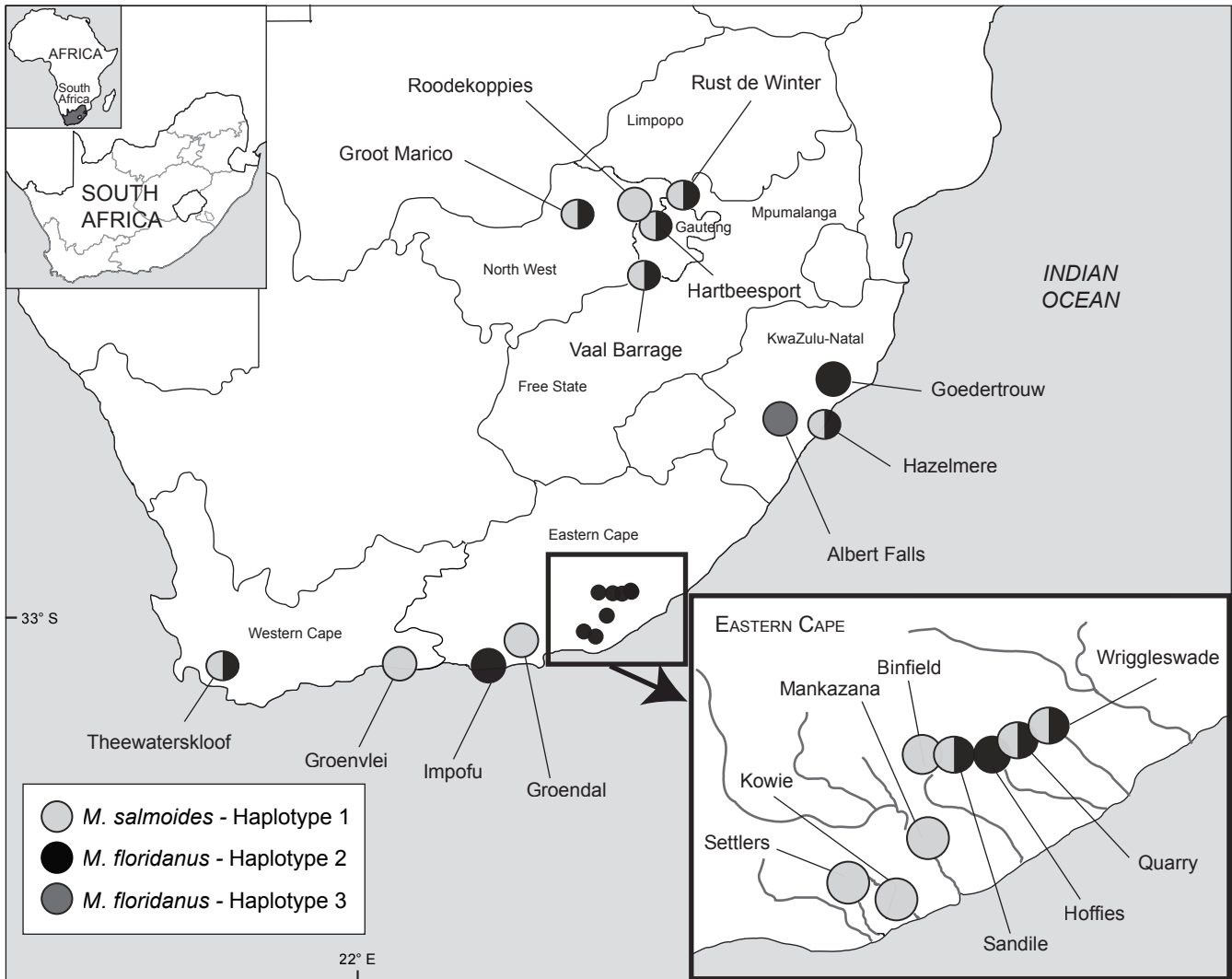


**Figure 1:** The southern African (South Africa and Zimbabwe) largemouth bass angling record (kg) in relation to the dates of introduction of *Micropterus salmoides* and *Micropterus floridanus* into the region (source: A Williams and the South African Bass Anglers Association, unpublished data)

Genomic DNA was extracted using the Wizard® Genomic DNA purification kit (Promega, Madison USA) following manufacturer's instructions. Extracted DNA was quantified using a Nanodrop 2000 spectrophotometer (Nanodrop, Wilmington, Delaware) and all DNA concentrations were standardised to a final concentration of 20 ng µl<sup>-1</sup> prior to polymerase chain reaction (PCR) amplification.

The mitochondrial NADH subunit 2 (ND2) gene was used to identify *M. salmoides* and *M. floridanus* lineages based on earlier phylogenetic analyses that identified the presence of diagnostic, fixed differences between the two species (Near et al. 2003, 2004; 2005; Bangs et al. 2008). A 1 047 base pair fragment was amplified using the primers GLN\_F (5' CTA CCT GAA GAG ATC AAA AC 3') and ASN\_R (5' CGC GTT TAG CTG TTA ACT AA 3') (Kocher et al. 1995). PCR volumes of 25 µl contained 10X PCR buffer, 2.5 mM MgCl<sub>2</sub>, 0.8 mM dNTPs, 0.2 pmol of each primer, 1U Super-Therm Taq DNA polymerase, 50–100 µl of DNA template and adjusted to the final volume with molecular-grade water. Thermocycling parameters were as follows: an initial denaturation of 3 min at 94 °C, followed by 35 cycles of 30 sec at 94 °C, 30 sec at 51 °C, and 1 min 30 sec at 72 °C. Each reaction finished with a final extension for 5 min at 72 °C. The presence of DNA fragments of proper length were confirmed via electrophoresis on 2% ethidium bromide stained gel. All successful PCR products (i.e., product amplified and of appropriate length) were further processed (i.e. purified and sequenced) at Macrogen Inc., South Korea, using their standard protocols.

All sequences were edited and checked manually in SeqMan (DNASTAR) and aligned with publicly available ND2 gene sequence data for *M. salmoides* and *M. floridanus* were retrieved from GenBank (Supplementary Material A) using the ClustalX algorithm with default parameters (Thompson et al. 1997). The combined sequence set was collapsed into unique haplotypes using FaBox (v 1.41; Villesen 2007). Because DNA sequences varied in length, final alignment was trimmed to the shortest sequence using FaBox (v



**Figure 2:** Largemouth bass sampling localities in South Africa, indicating the presence of *Micropterus salmoides* (light grey), *Micropterus floridanus* (black or dark grey) haplotypes in the sampled population

1.41; Villesen 2007). Haplotypes were collapsed such that each novel haplotype was represented only once in our final alignment. A minimum spanning haplotype network was generated in POPART (Leigh and Bryant 2015) with the explicit purpose of being to place haplotype variants recovered from South Africa in the context of published sequences. Because mitochondrial DNA is only maternally inherited (Ballard and Whitlock 2004), no attempt was made to identify hybrids. Population level haplotype diversity (HD) (Nei 1987) and nucleotide diversity ( $\pi$ ) (Nei and Tajima 1981; Nei 1987) were estimated using DnaSP (Rozas et al. 2003) for South African samples only. All generated sequences were deposited in GenBank under accession numbers MS177522-MS177706 (Supplementary Material B).

**Results**

A total of three haplotypes were recovered from the 185 individuals screened using mitochondrial DNA. On

comparison with published sequences, 125 were identified as *M. salmoides* and the other 60 were identified as *M. floridanus* based on comparison with published sequences (Table 2). The final ND2 alignment used in our minimum spanning network was 609 base pairs in length and included 11 largemouth bass and 19 Florida bass haplotypes, respectively (Figure 2). A clear separation of haplotypes by species was evident and the majority of Florida bass gene sequences were separated by few mutational differences.

Geographic data were unavailable for many of the sequences retrieved from GenBank, which prevented us from placing South African haplotypes into potential source regions from within the native range. In addition, North American ND2 sequences were shared across wide geographic regions (e.g. accession no. KJ 669255 was recovered from water bodies in South Carolina, Minnesota, and Wisconsin (Max Bangs, Auburn University, pers. comm.)). Nonetheless, the minimum spanning network

**Table 1:** Sampling localities ordered by province and river system, showing number of sampled specimens ( $n$ ) identified as ND2 mitochondrial DNA sequences of either *Micropterus floridanus* or *Micropterus salmoides* origin. Diversity indices are given as number of haplotypes ( $N_H$ ), haplotype diversity ( $H_D$ ) (Nei 1987) and nucleotide diversity ( $\pi$ ) (Nei and Tajima 1981; Nei 1987). nMF = individuals with *M. floridanus* mitochondrial DNA; nMS = individuals with *M. salmoides* mitochondrial DNA; EC = Eastern Cape; GP = Gauteng; KZN = KwaZulu-Natal; LP = Limpopo; NW = North-West; WP = Western Cape

Province	River system	Locality	°S	°E	$n$	$N_H$	$H_D$	$\pi$	nMF	nMS
EC	Great Fish	Mankazana Dam	33°10'0.48"	26°57'15.48"	10	1	0.00	0.00	0	10
EC	Kariega	Settlers Dam	33°24'46.44"	26°30'30.96"	8	1	0.00	0.00	0	11
EC	Kei	Hoffies Dam	32°40'3.36"	27°18'40.32"	3	1	0.00	0.00	3	0
EC	Kei	Quarry Dam	32°39'49.68"	27°21'21.96"	5	2	0.40	0.01	1	5
EC	Kei	Wriggleswade Dam	32°35'50.64"	27°34'3"	12	2	0.48	0.02	4	8
EC	Keiskamma	Binfield Dam	32°41'17.88"	26°54'16.2"	10	2	0.47	0.02	0	10
EC	Keiskamma	Sandile Dam	32°43'2.28"	27°6'16.2"	11	2	0.55	0.02	4	6
EC	Kowie	Kowie Weir	33°32'31.56"	26°45'39.96"	10	1	0.00	0.00	0	10
EC	Krom	Impofu Dam	34°5'32.64"	24°41'21.12"	10	1	0.00	0.00	10	0
EC	Swartkops	Groendal Dam	33°41'27.96"	25°15'56.16"	11	2	0.18	0.01	0	12
GP	Orange/Vaal	Vaal Barrage Dam	26°45'47.16"	27°41'43.8"	10	2	0.47	0.02	3	7
KZN	Mdloti	Hazelmere Dam	29°35'54.96"	31°2'32.64"	11	2	0.33	0.01	9	2
KZN	Mthlathuze	Goedertrouw Dam	28°46'22.08"	31°28'3.36"	9	1	0.00	0.00	9	0
KZN	Umgeni	Albert Falls Dam	29°25'49.44"	30°25'26.4"	1	1	0.00	0.00	1	0
LP	Olifants	Rust de Winter Dam	25°13'23.88"	28°28'59.88"	10	2	0.36	0.01	2	8
NW	Limpopo	Groot Marico River	25°38'24.72"	26°25'10.56"	10	2	0.20	0.01	1	9
NW	Limpopo	Haartbeespoort Dam	25°44'51"	27°52'0.84"	10	2	0.36	0.01	2	8
NW	Limpopo	Roodekoppies Dam	25°53'38.04"	28°43'8.76"	10	1	0.00	0.00	0	10
WP	Breede	Theewaterskloof Dam	34°4'26.4"	19°17'13.2"	14	2	0.44	0.02	10	4
WP	Coastal lake	Groenvlei	34°1'45.48"	22°50'43.08"	10	1	0.00	0.00	0	10

**Table 2:** Exact matches of haplotypes found in South Africa to published sequences on GenBank (same haplotype). GenBank haplotype matches cover the entire gene region sequenced in the current study.  $n$  = number of individuals

Haplotype	$n$	Species	GenBank number and references
1	125	<i>Micropterus salmoides</i>	AY225735 (Near et al. 2003), HQ391896 (Li et al. 2012), JX502856 (Illinois River system, Illinois, Baker et al. 2013) and KJ669250 (Bangs et al. 2008)
2	59	<i>Micropterus floridanus</i>	KJ669260 and KJ669279 (Bangs et al. 2008)
3	1	<i>Micropterus floridanus</i>	KJ669266 (Bangs et al. 2008)

identified that few haplotypes from the native range of *M. floridanus* are represented in contemporary South Africa samples.

*Micropterus salmoides* mitochondrial DNA was recorded at 16 of the 20 localities (80%), *M. floridanus* mitochondrial DNA was found at 13 localities (65%), and 9 of the populations (45%) has mitochondrial DNA of both species (Table 1, Figure 3). Samples from four water bodies contained solely *M. floridanus* haplotypes and seven water bodies contained only *M. salmoides* haplotypes. The number of haplotypes recovered from individual populations was consistently low (either one or two haplotypes). Haplotype diversity ranged from 0.00–0.55 and nucleotide diversity ranged from a low of 0.00–0.02 (Table 1). Of the two *M. floridanus* haplotypes recovered, one was found only in the Albert Falls population.

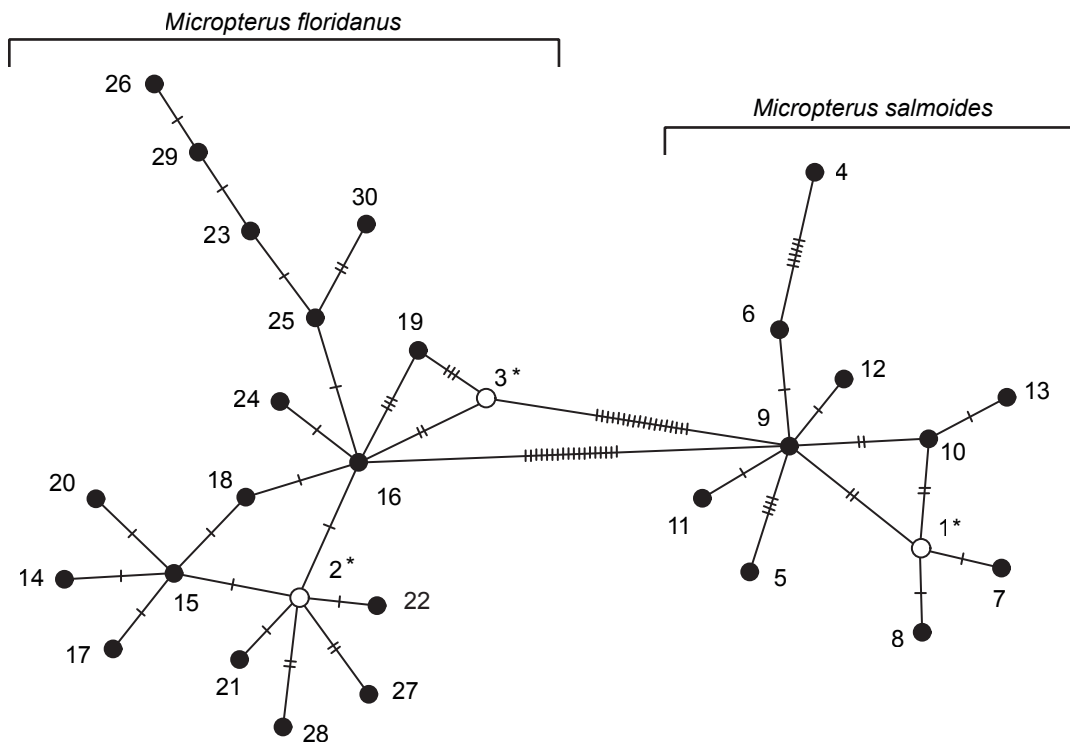
## Discussion

The results of this study demonstrate that *M. floridanus* genes have spread beyond the initial area of introduction.

Although the use of maternally inherited mitochondrial markers in the current study may have resulted in failure to detect hybrid individuals within populations, the available data demonstrate that *M. floridanus* genes now occur in the largest river basins of the country, the Limpopo and Orange (Table 1). Because the interprovincial transfer of largemouth bass (and, by association, of Florida bass) has been prohibited in South Africa since the 1970s (McCafferty et al. 2012) the presence of *M. floridanus* haplotypes outside the KwaZulu-Natal Province suggests that South Africa is no exception to the global problem of illegal introductions (e.g. Johnson et al. 2009; Cowx et al. 2010).

Spread from the point of introduction is a fundamental criterion for invasiveness (*sensu* Blackburn et al. 2011), and the results presented in this paper therefore demonstrate that, in South Africa, *M. floridanus* is a 'fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence'. What is interesting is that, as is the case with *M. salmoides* (Hargrove et al. 2017), low genetic diversity in *M. floridanus*





**Figure 3:** Minimum spanning network based on ND2 gene sequences generated from the native (North America) and non-native range (South Africa) of largemouth bass (*Micropterus salmoides*) and Florida bass (*Micropterus floridanus*). Individual tick marks denote one base pair difference between haplotypes. White nodes (○) with asterisks (\*) correspond to ND2 sequences collected from South Africa, black nodes (●) represent native range populations. A list containing all sequences utilised can be found in the Supplementary material A and B

does not appear to have hampered their establishment success. Only a very small number of *M. floridanus* haplotypes, which represented a subset of those present in their native distribution, were identified across a large number of South Africa's bass populations. Five populations examined in the current study were also included Hargrove et al. (2017), and the estimated species composition in both studies was congruent. The combined genetic and distribution data suggest that *M. salmoides* and *M. floridanus* are capable of colonising diverse environments, based on founding populations consisting of very few individuals (e.g. <20 individuals; Hargrove et al. 2017).

From a conservation perspective, the spread of *M. floridanus* throughout South Africa is relevant, because of a series of biological characteristics specific to this species and that might lead to its enhanced performance over *M. salmoides*. For example, *M. floridanus* become sexually mature at a younger age than *M. salmoides* (Clugston 1964) and have a more protracted spawning season (Isely et al. 1987; Rogers et al. 2006), during which they can spawn multiple times (Hargrove and Austin 2017). The longer spawning seasons reduce the risk of spawning failure, particularly in summer rainfall regions, where reservoir drawdowns coincide with *M. salmoides* spawning (e.g. Weyl and Hecht 1999). Faster growth rates may also provide a foraging advantage over *M. salmoides*, which have been hypothesised to result in higher rates of survival of *M. floridanus* and of their hybrids with *M. salmoides* (Maceina et al. 1988).

In parts of their introduced range (e.g. California, USA) *M. floridanus* have been shown to live longer and reach larger sizes than *M. salmoides*, in part due to their lower vulnerability to capture by anglers, resulting in populations that had larger mean size (Bottroff and Lembeck 1978). Because predator pressure is considered a major factor threatening South African freshwater fishes (Ellender and Weyl 2014), the increased fitness of *M. floridanus* may result in heightened impacts on native biota.

Because fish introductions are a continuous process, it is important to determine the full extent of invasion in South Africa, especially if the current legislation prohibiting introductions of *M. floridanus* into 'catchments where they do not already occur' (Woodford et al. 2017) is to be correctly interpreted. Because *M. floridanus* and *M. salmoides* are not only morphologically almost indistinguishable, but also readily hybridise, future work should consider employing large numbers of genome-wide markers (e.g. single nucleotide polymorphisms SNPs) that can accurately detect the extent of introgression between closely related species (Li et al. 2015). Such detailed understanding of the distribution of *M. floridanus* and its potential hybrids in South Africa would represent an important source of information, as spatially explicit models could be used to manage and/or prevent the spread of this alien species.

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