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Genetic markers reveal a gradient of hybridization between cape hakes (*Merluccius capensis* and *Merluccius paradoxus*) in their sympatric geographic distribution.

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Abstract

The cape hakes *Merluccius capensis* and *Merluccius paradoxus* are important fishing resources for African countries such as Namibia and South Africa. In this study we have genetically analyzed adult samples from the overlapping distribution of these species. Eight microsatellite loci, the nuclear 5S rDNA locus and the Cytochrome Oxidase subunit I (COI) gene were employed as molecular markers. A North-South gradient of interspecific hybridization was found, with discordant mitochondrial and nuclear genotypes at the northernmost edge of *M. paradoxus* distribution. These results suggest intense introgression in North Benguela off the Namibian coast. Independent hake stock assessment is recommended in this region for sustainable management of this valuable resource.

Key words: Cape hakes, hybrid zones, Benguela; *Merluccius capensis; Merluccius paradoxus*.

1. Introduction

Hybrid zones may occur when two formerly separated species meet again (Avise and Wollenberg, 1997; Hewitt, 2001). They often arise at biogeographic borders and may occur for different taxa in what it is called a *suture zone* (Hewitt, 2000). In the marine realm hybrids between different animal species are relatively frequent (e.g. Palumbi, 1994; Gardner, 1997; Srinivasa Rao and Lakshmi, 1999; Miralles et al., 2013) because, amongst other reasons, many species have mass spawning and/or interspecific reproductive barriers may be weak. However, marine hybrid zones have been considered rare, perhaps because they have not been sufficiently studied (Arnold, 1997; Gardner, 1997). They have been reported for a few species, such as mussels of the genus *Mytilus* (e.g. Bierne et al., 2003, Riginos and Cunningham, 2005), redfish of the genus *Sebastes* (e.g. Roques et al., 2001), hakes of the genus *Merluccius* (Machado Schiaffino et al., 2010) and some coral reef fishes (Hobbs et al., 2009). A variety of genetic consequences can result from hybridization (Seehausen, 2004, 2006). In cases of hybridization but no introgression, no genetic consequences are expected (this would be an evolutionary dead end). When there is introgression through unidirectional gene flow, one species will lose its genetic identity. Introgression through bi-directional gene flow will potentially result in reverse speciation (Seehausen, 2006). Finally, another possible outcome is hybrids becoming a new lineage (see Seehausen, 2004).

Hybridization is not expected to occur with the same frequency in all the areas where two species are sympatric. Hybrids are more frequent in marginal populations, where mate choice may be relaxed (e.g. Ritchie, 2007), and in the colonization front when one of the species is displacing or expanding its distribution (e.g. Carson and Templeton, 1984; Horreo et al., 2011). It also happens where the two sympatric species are unequally abundant (e.g. Arnold, 1997; Hobbs et al., 2009). In these cases

asymmetric hybridization would be expected, the rarer species providing frequently the female in hybrid crosses (e.g. Wirtz, 1999).

Identification of hybrid zones is especially important for species subjected to exploitation because they may require a distinct management. Allendorf et al. (2001) have classified hybrid zones in six different types based on their origin (natural *versus* anthropogenic) and on the extent of introgression, with differential management and conservation priorities proposed for each of them. Cape hakes (*Merluccius capensis* and *Merluccius paradoxus*) are two of the most economically and ecologically important African fishing resources (Alheit and Pitcher, 1995; Boyer and Hampton, 2001), and have been subjected to sustainable management initiatives for the last decades (e.g. Butterworth and Rademeyer, 2005; Hutchings et al., 2009a). They overlap in large part of their distributions, along the coastlines of Namibia and South Africa (Figure 1), but they inhabit at different depths. *Merluccius capensis* is known as the shallow cape hake while *M. paradoxus* is called the deep cape hake (Alheit and Pitcher, 1995). Cape hakes' population structure has been described by von der Heyden et al. (2007b): there are no barriers to dispersal between Namibian and South African waters for *M. capensis* while for *M. paradoxus* there are significant spatial population genetic differences. Spawning of the two cape hakes overlaps temporally. In South African waters, spawning occurs from August to March with two apparent peaks, the first at the end of the year for both species and the second in the austral autumn mainly for *M. paradoxus* (Assorov and Berembeim, 1983; Botha, 1986). In Namibian waters, *M. capensis* spawns throughout the year, more intensely between July and October, while by now there is no evidence of *M. paradoxus* spawning there (Assorov and Berembeim, 1983; Alheit and Pitcher, 1995; Kainge et al., 2007). Although little is known about the spawning behavior of these two species, reproductive barriers between them seem to exist, at least

partially, due to depth. Von der Heyden et al. (2007a) and Stenevik et al. (2008) found eggs of *M. paradoxus* distributed in deeper waters than *M. capensis* eggs (with an average depth of 231 m. and 348 m. for *M. capensis* and *M. paradoxus* respectively). However, displacement of cape hakes has been reported in response to change in the oxygen content of bottom waters, *M. capensis* entering in contact with *M. paradoxus* (Hamukuaya et al., 1998). Since hybrid zones have been reported for other overlapping species of this genus (the North American hakes *Merluccius albidus* and *M. bilinearis*; Machado-Schiaffino et al., 2010), it is theoretically possible that the same phenomenon occurs also for Cape hakes.

The objective of this study was to examine the extent and direction of possible introgressive hybridization and to identify potential hybrid zones in cape hakes. For this purpose, adults of both species were sampled from different areas across the overlapping distribution and genotyped for eight microsatellite loci, the nuclear 5S rDNA locus and the Cytochrome Oxidase subunit I (COI) gene for genetic estimation of their hybrid status.

2. **Materials and Methods**

2.1. Sampling

A total of 296 cape hakes, *Merluccius capensis* and *M. paradoxus*, were collected during 2002-2003 from three different areas in the overlapping zone of both species in the south Atlantic Ocean (Figure 1): two within the Benguela current (North and South, 11-14ºE 22-26ºS and 15-18ºE 30-33ºS, respectively) and one within the Agulhas current (20-24ºE 34-36ºS). They were taxonomically identified *de visu* by local

experts. Tissue samples (muscle or fin biopsy of approx. 1 mm^3) were obtained from each individual and stored in absolute ethanol until analysis.

2.2. Genetic analysis

Eight microsatellite loci were assayed: Mmer-Hk3, Mmer-Hk9, Mmer-Hk20, Mmer-Hk29, Mmer-Hk34 (Morán et al., 1999), Mmer-UEAW01 (Rico et al., 1997), Maus7 and Maus32 (Machado-Schiaffino and Garcia-Vazquez, 2009). PCR conditions and protocols were slightly modified from Machado-Schiaffino et al. (2010) for optimizing amplification in *Merluccius capensis* and *Merluccius paradoxus* (Table 1). PCR products were separated using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems), with BigDye 3.1 Terminator system, in the Unit of Genetic Analysis of the University of Oviedo (Spain). Genotypes were determined employing the GeneMapper® Software Version 4.0.

The nuclear 5S rDNA coding gene was genotyped as described by Perez and Garcia-Vazquez (2004). *Merluccius capensis* yields one only fragment of 371 nucleotides and *M. paradoxus* provides two fragments of 371 and 494 nucleotides. Fragment sizes were determined in 2% agarose gels by comparison with a DNA mass ladder.

The mitochondrial COI gene was amplified employing the primers COIFish-F1 and COIFish-R1 (Ward et al., 2005). PCR reactions were carried out accordingly with the protocols described by Ward et al. (2005). PCR products were visualized, purified and sequenced as described in Machado-Schiaffino et al. (2009). PCR products were visualized in 50 ml 2% agarose gels 3µl of ethidium bromide (10mg/ml). Stained bands were excised from the gel and DNA was purified with a Wizard SV Gel and PCR Clean up system (Promega) prior to sequencing. Automated fluorescence sequencing was

performed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) in the Unit of Genetic Analysis of the University of Oviedo (Spain).

2.3. Data analysis

Microsatellite scoring errors, large allele dropout and null alleles were checked employing MICROCHECKER (van Oosterhout et al., 2004). GENEPOP (Raymond and Rousset, 1995) was employed to test for linkage disequilibrium and departure from Hardy-Weinberg equilibrium. Microsatellite variation parameters such as expected and observed heterozygosity were calculated with GENETIX Version 4.03 (Belkhir et al., 2004). FSTAT Version 2.9.3.2 (Goudet, 2001) was used to calculate microsatellite allelic richness. To identify individuals from each pure species, hybrids of first generation and backcrosses we employed NewHybrids (Anderson and Thompson, 2002), with settings of 300 000 Monte Carlo Markov Chain (MCMC) iterations after a burn-in period of 30 000 iterations. The Bayesian software STRUCTURE v.2.3.1 (Pritchard et al., 2000) was used to estimate the membership of each individual to each species with the "Admixture model" and K=2 (two expected genetic units, one corresponding to each species), which assumes that individuals may have mixed ancestry. Settings were a burn-in period of 100 000 steps followed by 1 000 000 MCMC iterations. Since there is no clear consensus about the proportion of membership considered as a signal of introgression (Allendorf et al., 2001), for conservative interpretation we have considered >25% the threshold for significant membership of a species as in Machado-Schiaffino et al. (2010). We have run STRUCTURE five times with K=2. We have also followed the methodology described by Schwartz and Beheregaray (2008) with two runs, using the species defined in the first run as a prior

for the second run. This is a test for each individual having an ancestor of the other species in the last two generations (Pritchard et al., 2000)

COI sequences were edited using the BioEdit Sequence Alignment Editor software (Hall, 1999). The edited sequences were compared with standard sequences of each species with the online software NCBI-BLAST (Altschul et al., 1990).

Species divergence time was estimated from COI sequences under a Bayesian MCMC framework using BEAST version 1.6.1 (Drummond and Rambaut, 2007). Bayesian intraspecific phylogenies are based on coalescent theory (Kingman, 1982) and allow the inference of past population dynamics and parameters from contemporary gene sequences. Following a burn-in of 10 million cycles, rates were sampled every 1 000 cycles from 60 million MCMC steps for an Extended Bayesian Skyline tree with a stepwise model for mitochondrial DNA and strict clock model. The substitution model of COI sequences and their priors (previously known information) were defined by jModeltest software version 0.11 (Posada, 2008) using the Akaike information criterion (AIC; Akaike, 1974). The COI gene mutation rate employed were 1.2% per MY (Bermingham et al., 1997). Three runs were performed to ensure that results do not reflect spurious probabilities. Tracer version 1.5 (Rambaut and Drummond, 2007) was used to check that chains converged to a stationary distribution and to visualize the results obtained.

3. Results

Microsatellite loci were employed to assign individuals to a species and to identify first-generation hybrids and brackcrosses with the programs NewHybrids and

STRUCTURE. Two microsatellites (Mmer-Hk29 and Mmer-Hk34; Morán et al., 1999) exhibited null alleles and were not used for the study of interspecific introgression. Null alleles and dropouts were not found for other microsatellites. The six loci retained for the study were highly variable (Table 2) and did not show significant differences between observed and expected heterozygosity, neither linkage disequilibrium for any sample ($p > 0.05$ in all cases). With NewHybrids software, hybrids of *M. capensis* and *M. paradoxus* were not identified in the Agulhas Bank sampling area (Figure 2 bottom). However, 4% hybrids were found in South Benguela (Figure 2 middle). Greater hybridization was found in North Benguela sample (Figure 2 top), with 5% hybrids issued from *M. capensis* females and 8.5% individuals backcrossed to *M. paradoxus*. This North-South gradient of interspecific hybridization was confirmed with STRUCTURE software. In the Agulhas Bank area, one *M. paradoxus* individual (1.3% of analyzed samples) exhibited 27% individual membership of *M. capensis* (Figure 3 top), indicating some degree of introgression. In South Benguela (Figure 3 bottom left), 13 *M. capensis* individuals (26.5% of analyzed samples) exhibited mixed membership (introgression), whereas *M. paradoxus* specimens had no introgression. Finally, numerous individuals of both *M. capensis* and *M. paradoxus* sampled from North Benguela had mixed membership (Figure 3 bottom right): 36% and 20% respectively. All the individuals identified as F1 and backcrosses by NewHybrids exhibited mixed membership with STRUCTURE. All the samples yielded 5S rDNA amplification patterns concordant with their assignation to a species (this marker cannot distinguish between *M. paradoxus* and hybrids).

The 622 nucleotide-long COI gene sequence obtained in this study was polymorphic and differed between species, as expected. The different haplotypes from the analyzed samples are available at GenBank (http://www.ncbi.nlm.nih.gov/genbank/)

with the accession numbers JF268612-JF268620. Comparing the sequences obtained with reference sequences from GenBank (JF493884.1 and JF493889.1 for *M. capensis* and *M. paradoxus* respectively), all the individuals identified *de visu* and by microsatellite genotypes as *M. capensis* exhibited typical *M. capensis* COI genes. It is an indicator that hybrids and introgressed *M. capensis* had been produced from crosses between *M. capensis* females and *M. paradoxus* males. On the other hand, all the 30 *M. paradoxus* individuals sampled from North Benguela and one from South Benguela exhibited typical *M. capensis* COI sequences. Therefore, these individuals were nuclear-mitochondrial discrepant. It can be explained by recurrent backcrosses of descendants of *M. capensis x M. paradoxus* hybrid females with *M. paradoxus* (Figure 4).

Concerning the level of mitochondrial variation in the studied areas, the Agulhas samples exhibited less haplotypes and lower diversities (Hd and π) than the Benguela samples (Table 2). According to the results explained above, F_{ST} values between pairs of samples were discordant for microsatellites and mitochondrial DNA mainly due to the North Benguela sample of *M. paradoxus* (Table 3). North Benguela *M. paradoxus* was not different from any *M. capensis* sample for mitochondrial DNA (Table 3, below diagonal) but was significantly different from the other *M. paradoxus*. For microsatellites (Table 3, above diagonal), North Benguela *M. paradoxus* were significantly different from two *M. capensis* samples (not from South Benguela *M. capensis*) and also from Agulhas *M. paradoxus* samples, but not from South Benguela *M. paradoxus*.

From the COI sequences found in this study, the estimated time for the most recent common ancestor (tMRCA) of South African cape hakes samples was 3.4 million years ago (MYA) with a standard deviation of 3.63×10^{-3} MYA and 95% HPD of 2.437 – 4.471 MYA.

4. Discussion

The results of this study revealed hybridization and introgression between the two cape hake species, with a clear North-South gradient. Higher proportion of introgressed individuals was found in the north (North Benguela exhibited the highest). It is geographically associated with the border of *M. paradoxus* distribution (Figure 1). Moreover, *M. paradoxus* seems to have captured the *M. capensis* mitochondrial genome in that region, as described in a few cases for other fish like Arctic char (Bernatchez et al., 1995) and also for North American hakes (Machado-Schiaffino et al., 2010). Although discordant mitochondrial and microsatellite population patterns can be explained based on different potential for natural selection, lack of mutation-drift equilibrium and/or sex-biased dispersal (DiBattista et al., 2012), the present case of species status discordance between markers could be due to repeated generations of backcrosses of hybrids *M. capensis* x *M. paradoxus* to *M. paradoxus*, leading to a molecular leakage classified as Type 2 hybridization or natural introgression by Allendorf et al. (2001). North Benguela (Namibian waters) could therefore be considered a hybrid zone for these species.

From the technical point of view, this study may encompass some ascertainment bias because the microsatellites employed were developed for other *Merluccius* species (*Merluccius merluccius* and *M. australis*). Ascertainment bias can complicate cross-species comparisons of genetic diversity (e.g. Annos et al., 2003) because the species from which DNA was used for microsatellite primer development often shows higher genetic diversity than other species for which the same primers are used. However in

this study genetic diversity was similar in the two species (Table 2), therefore ascertainment bias, if occurring, affected likely similarly the two species here compared.

Different factors can be invoked for explaining the hybridization found in North Benguela. On one hand, environmental alterations (natural and/or anthropogenic) promote the breakdown of interspecific barriers (e.g. Gilman and Behm, 2011; Crego-Prieto et al., 2012; and references therein). The North Benguela region is subjected to stressful processes such as overfishing and the Benguela regime shift (Hutchings et al., 2009b).Also it is intensely affected by the Benguela Niño and anoxic periods (Boyer and Hampton, 2001; Rouault et al., 2007; Monteiro et al., 2008). From the distribution of their eggs in the water column, it seems that *M. paradoxus* spawn in deeper waters than *M. capensis* (Von der Heyden et al., 2007a; Stenevik et al., 2008). It is possible that adverse environmental conditions (in the bottom and/or in the surface) force repeatedly spawning overlaps of these species in North Benguela, thus allowing interspecific mattings.

On the other hand, adverse environmental factors may not be necessary for explaining these results. Hybridization occurs naturally between other hake species (e.g. Machado-Schiaffino et al., 2010) and it could be an evolutionary mechanism in the genus *Merluccius* (Campo et al., 2009). Hybridization is most common and successful in recently diverged species (Mallet, 2005), as it is the case of these hakes (e.g. Roldan et al., 1999; Campo et al., 2009). Grant and Leslie (2001) suggested that most hake species diverged around 2-3 MYA and Quinteiro et al. (2000) estimated the divergence time of *M. capensis* and *M. paradoxus* between 3.8 and 4.5 MYA. Our estimation of species divergence sets the time for the most recent common ancestor in 3.4 MYA, very similar to previous estimates (Becker et al., 1988; Quinteiro et al., 2000). The gradient of introgression, more intense at the edge of *M. paradoxus* distribution, could be

explained as a consequence of relaxed sexual selection that could be expected in marginal populations (e.g. Ritchie, 2007), and/or as a strategy in the colonization front (e.g. Carson and Templeton, 1984; Horreo et al., 2011). If *M. paradoxus* expanded northwards from South Africa as it could be deduced from the phylogeny of the genus (Roldán et al., 1999; Campo et al., 2007; Campo et al., 2009), the phenomenon of hybridization would be essentially natural in this case (Type 2 from Allendorf et al., 2001). These interesting evolutionary hypotheses deserve further investigations. Comparing this pair of species with other sympatric *Merluccius* and combining genetic data with life history trait patterns could enlighten the mechanisms involved in speciation at sea, that are still largely unknown (Norris and Hull, 2012).

An alternative explanation could be scarcity of one of the two species (e.g. Arnold, 1997; Frisch and van Herwerden, 2006; Hobbs et al., 2009). Past *M. capensis* overfishing in Namibian waters (Isarev, 1983, 1988) combined with more general climate factors affecting this species (e.g. Rikter and Golubiatnikova, 1997), could have led to hybridization due to reduced abundance of this species. This hypothesis would also explain the asymmetric hybridization found in our results, issued from *M. capensis* females. Females of the rarer species would hybridize with more abundant species due to a lack of conspecific partners (e.g. Wirtz, 1999, Frisch and van Herwerden, 2006). Sneak mating like for example in Serranidae (Frisch and van Herwerden, 2006) and Salmonidae (e.g. Garcia-Vazquez et al., 2001), although less probable because hakes exhibit mass spawning (Alheit and Pitcher, 1995) cannot be discarded. On the other hand, bidirectional hybrid mating but reduced fitness of the offspring of one direction (*M. paradoxus* female x *M. capensis* male in our study) cannot be ruled out for explaining asymmetric hybridization. This last hypothesis would be compatible with

scarcer *M. paradoxus* breeders in Namibian waters (Assorov and Berembeim, 1983; Alheit and Pitcher, 1995; Kainge et al., 2007).

The lack of hybrids at the southernmost study site could be due to absence of hybridization and no immigration of hybrids from other sites. However, our study was carried out on adults only. Therefore we cannot know if hybridization is occurring at the southernmost study site, only that if it happens the hybrids are not reaching adulthood. The presence of hybrids at the northernmost study site may be due to a breakdown of pre-mating (for example relaxed mating choice as suggested above; Ritchie, 2007) or post-mating isolation mechanisms, and we do not know which.

Whatever factor is invoked for explaining the introgression between cape hakes found in this work, it seems that the North Benguela region is different from the other distribution areas. These hake populations should be managed carefully applying sustainable initiatives (Butterworth and Rademeyer, 2005; Hutchings et al., 2009a). Differences in interspecific introgression between this region and the rest of the distribution, clearly significant for *M. paradoxus*, suggest that it should be considered a separate population unit supporting Von der Heyden et al. (2007b), and managed accordingly.

5. Conclusions

In conclusion, introgressive hybridization between sympatric cape hakes, *Merluccius paradoxus* and *Merluccius capensis*, has been described employing microsatellite loci, the mitochondrial COI gene and the nuclear 5S rDNA locus. A hybrid zone was detected in the North Benguela area that could be either due to natural and/or anthropogenic factors. Management of cape hakes from that area as a separate population unit is recommended.

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FIGURE LEGENDS:

Figure 1. *Merluccius capensis* and *Merluccius paradoxus* distribution range (above). Sampling areas in Agulhas, South Benguela and North Benguela are marked in dark in the enlarged section (below).

Figure 2. Hybridization detected with NewHybrids software. Percentage of each species (*M*. *capensis* in dark blue and *M*. *paradoxus* in light blue), hybrids (intermediate blue) and backcrosses to *M*. *paradoxus* (white) per location.

Figure 3. Individual membership of Cape hake samples from the considered regions, estimated with STRUCTURE software. **(a)** Agulhas sampling area; **(b)** South and North Benguela sampling areas at left and right, respectively. Membership to each species is represented as *M*. *capensis* in dark blue and *M*. *paradoxus* in light blue. Each vertical bar represents one individual.

Figure 4. Example of a possible scenario to explain the nuclear-mitochondrial discordance found in this study. First-generation hybrids (F1) have 50% nuclear genes of each parental species. The nuclear genome of the non-recurrent parental species will be diluted in successive backcrosses. However, *Merluccius capensis* mitochondrial DNA (marked as ♀), of maternal origin, will remain the same across generations.

Table 1: Description of the eight microsatellite loci assayed for *M. capensis* (C) and *M. paradoxus* (P) and PCR conditions. T_a : annealing temperature.

Primer Name	Reference	Repeat Motif	GenBank Accession no.	T_a (ºC)	Mg^{2+} (mM)
Mmer-HK3	(Moran *et al*., 1999)	GT	AF136627	54	1.5
Mmer-HK9	(Moran *et al*., 1999)	GA	AF136628	54	1.5
Mmer-HK20	(Moran *et al*., 1999)	GT	AF137595	52	1.5
Maus7	(Machado-Schiaffino and García-Vázquez, 2009)	$(GT)_5AA(GT)_3AA$ $(GT)_7TA(GT)_{10}$	EU703880	C: 61 P: 60	2.5
Maus32	(Machado-Schiaffino and García-Vázquez, 2009)	$(CA)_9(TG)_2$	EU703883	C: 60 P: 63	C: 2.5 P: 2
UEAW01	(Rico *et al*., 1997)	$(CA)_2$ $(CA)_{11}$	X87461	60	1.5
Mmer-HK29	(Moran *et al*., 1999)	GT	AF137597	52	2
Mmer-HK34	(Moran *et al*., 1999)	GT	AF137596	52	C: 2.5 P: 2

Table 2: Diversity indices for microsatellite loci and mitochondrial DNA of the *Merluccius capensis* and *Merluccius paradoxus* samples analyzed.

	M. capensis			*M. paradoxus*		
	Agulhas (n=75)	S.B. (n=49)	N.B. (n=50)	Agulhas (n=75)	S.B. (n=17)	N. B. (n=30)
Microsatellite loci						
A. R.	11.653	11.097	10.592	11.682	13.537	11.407
N. A.	19.833	16.500	15.667	21.167	13.667	14.833
He	0.730	0.717	0.718	0.779	0.800	0.683
Ho	0.675	0.543	0.626	0.742	0.790	0.560
Mitochondrial DNA						
Hd	0.378	0.800	0.667	0.464	0.378	0.833
(π)	0.00096	0.00268	0.00161	0.00161	0.00151	0.00214
Nh	3	4	5	3	3	6

S.B., South Benguela; N.B., North Benguela; n, number of individuals; A.R, Allelic Richness; N.A., Average Number of Alleles per locus and population; He, Expected heterozygosity; Ho, Observed heterozygosity; Hd, Haplotype diversity; (π), Nucleotide diversity; Nh, Number of haplotypes

Table 3: Genetic differentiation between populations. Pairwise F_{ST} estimates between hake samples based on microsatellite loci (above diagonal) and mtDNA (below diagonal). Significant values are in bold. S.B., South Benguela; N.B., North Benguela. :

		M. capensis			*M. paradoxus*		
		Agulhas	S.B.	N.B.	Agulhas	S.B.	N.B.
M. capensis	Agulhas	-	0.0034	0.0030	**0.0612**	**0.0422**	**0.0176**
	S.B.	0.0372	-	0.0016	**0.0524**	**0.0329**	- 0.0002
	N.B.	-0.0256	-0.0505	-	**0.0505**	**0.0295**	**0.0162**
M. paradoxus	Agulhas	**0.9834**	**0.9728**	**0.9788**	-	0.0131	**0.0241**
	S.B.	**0.8789**	**0.8432**	**0.87411**	-0.0199	-	0.0000
	N.B.	0.0045	0.0068	0.0017	**0.9748**	**0.8652**	-

Figure 1

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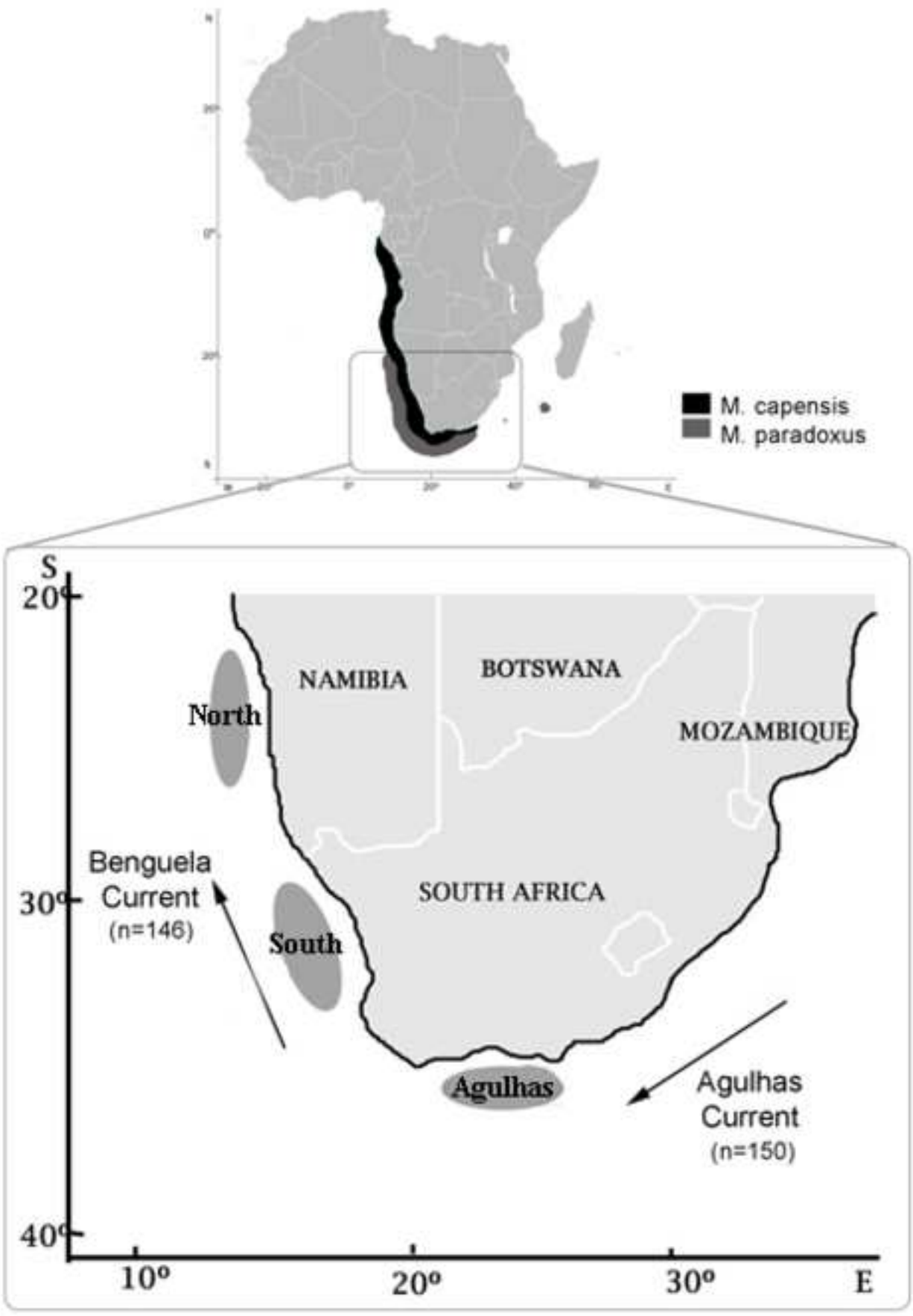

Figure 2

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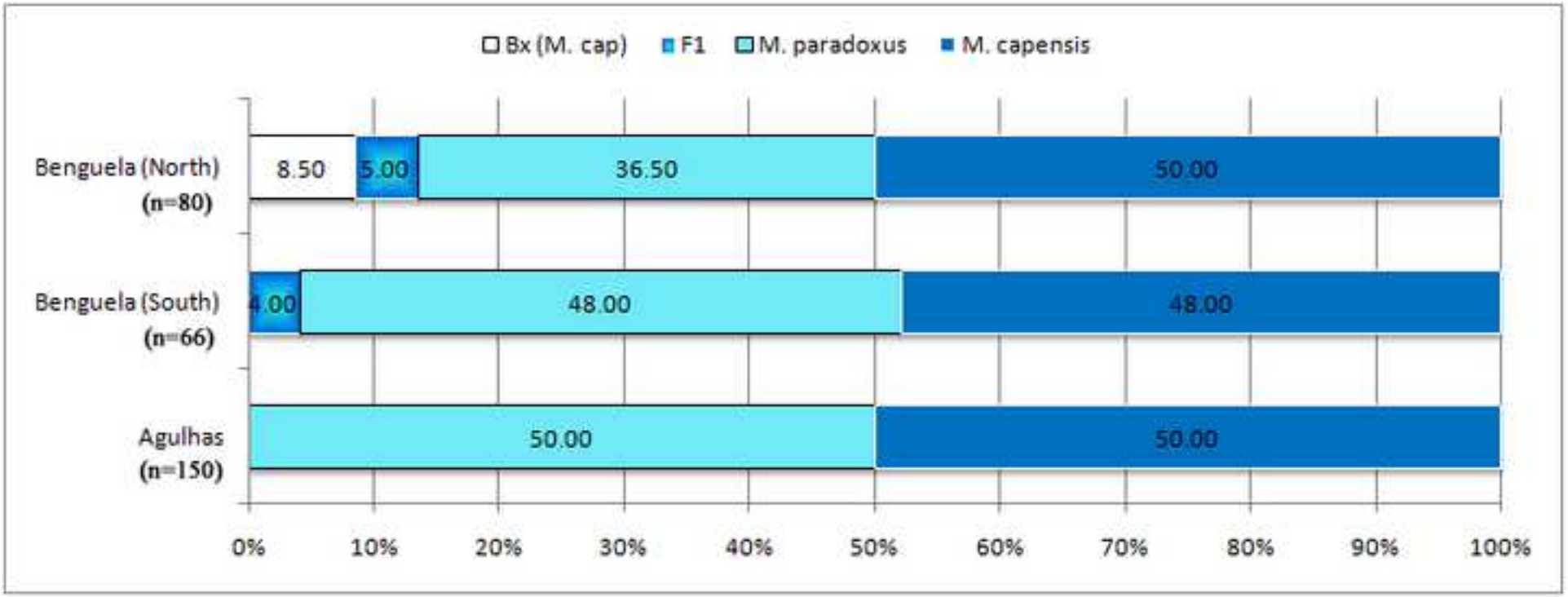

Figure 3

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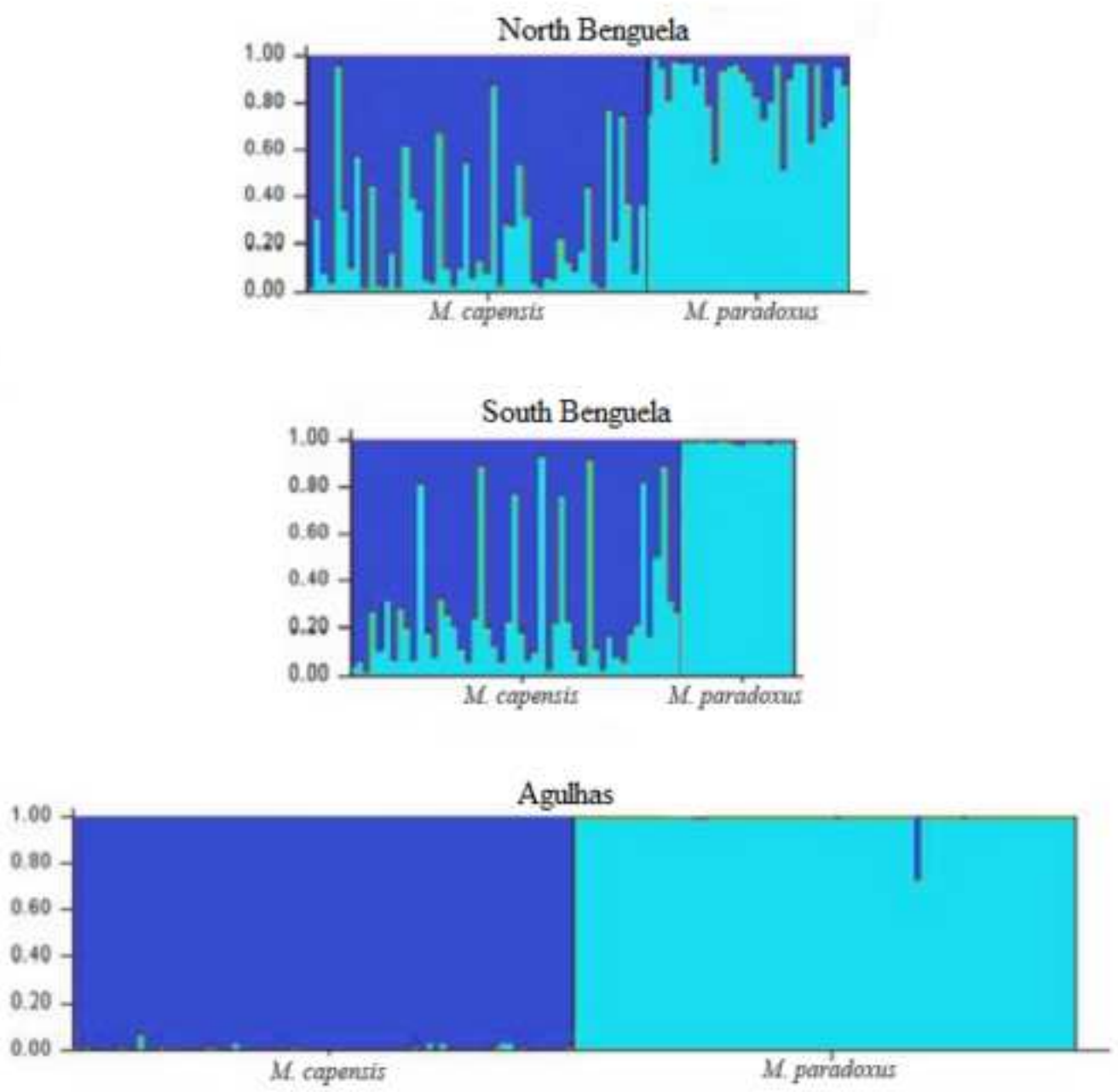

Figure 4

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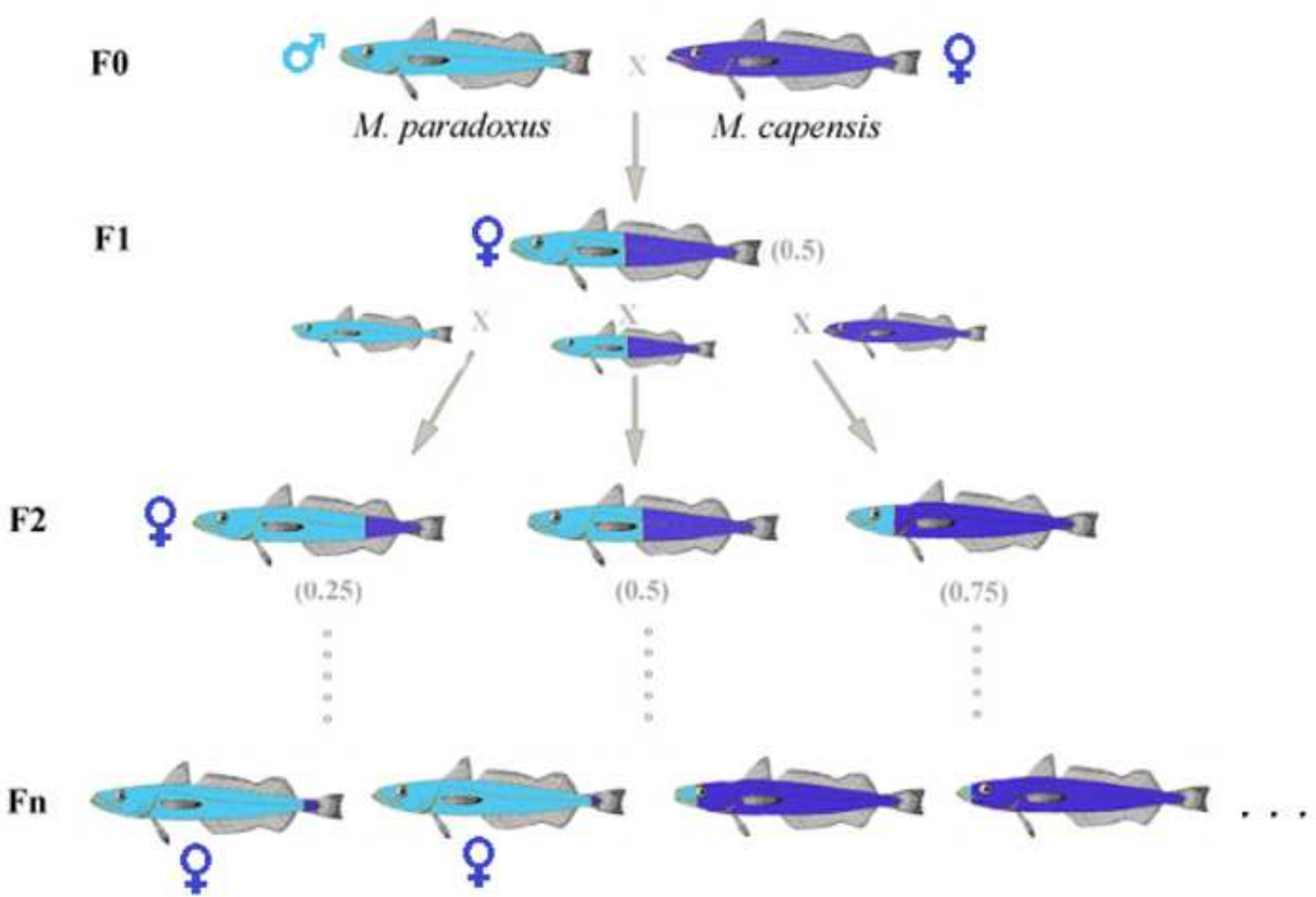

Editor's comments: The authors are thanked for making the revisions requested. The Reviewer is largely satisfied with the revisions but makes two additional recommendations for minor revisions. These recommendations are supported.

The authors are asked to please read the m/s through for typos and grammatical errors. In addition, please consider the second recommendation on estimating divergence times of the COIs of the two species mtDNA. This would add to the value of the m/s.

Response: OK. We have done the changes recommended.

Reviewers' comments:

Reviewer: After having read through the revised version of this manuscript, I am satisfied that they have addressed the concerns of all the reviewers. Most notably by adding two separate sections in the introduction on the biology of the species and genetic consequences of hybridization, methodological caveats are now discussed, in addition to raising the possibility that hybrids are formed but simply do not survive to adulthood in the south. I only have the following minor suggestions prior to publication:

1) I suggest one final pass through the paper to remove a number of apparent grammatical errors and typos.

Response: OK, done. We have revised carefully the language. We have also checked that the format of the citations and references follows the *Journal of Sea Research*'s style.

2) Pg. 11, line 58: These authors have the information necessary to estimate divergence time between the mtDNA genomes of the two species (COI), so I suggest that they do so.

Response: OK, done. We have estimated the divergence time between the mtDNA genomes of the cape hakes, from our COI sequences. We have obtained a value similar to previous estimates from other authors. This has been added in the new version of the manuscript (Material & Methods, Results, Discussion sections)