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# ITS1-rDNA-Based Methodology To Identify World-Wide Hake Species of the Genus *Merluccius*

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Species-specific DNA-based tags are valuable tools for the management of both fisheries and commercial fish products. In this study, we have developed a two-step molecular tool to detect the presence of hake DNA (*Merluccius* spp.) and to identify the exact hake species present in an blind sample. The first test involves PCR amplification of an ITS1-rDNA fragment of 193 bp using nested primers that are interspecifically conserved in *Merluccius* spp. and Atlantic cod, *Gadus morhua*. The second test consists of the PCR amplification of a 602–659 bp DNA fragment spanning part of the ribosomal cluster 18S–ITS1–5.8S and digesting it with four restriction enzymes whose targets map at interspecifically nonconserved sites of the ITS1. Alternatively, the identification of hake species can be achieved by FINS or BLAST, using the nucleotide sequence of either the whole ITS1 sequence or its nested fragment of 193 bp. Because of their high reproducibility and ease of execution, these procedures allow for routine analysis and constitute high reliable tools for the rapid identification of 12 species of hake.

KEYWORDS: Hakes; *Merluccius* spp.; species identification; preliminary exclusion test; RFLPs; ITS1rDNA marker; food science; traceability

# INTRODUCTION

The genus *Merluccius* is composed of at least 14 species of hakes that occupy most temperate and tropical continental shelves except the Asian shores of the Pacific Ocean (1, 2). Most hakes have been heavily fished along the last century (3), and their fisheries have attracted considerable interest from marine ecologists, fishery scientists, and commercial managers. In particular, there are key unresolved issues related to their conservation, exploitation, and traceability. However, the lack of a conspicuous identification key for each species seriously limits the assessment of both their commercial importance and the impact of fisheries on their sustainability. For instance, the existence of broad areas of bathymetric overlap between hakes (1) results in the simultaneous catching of two species in the same area, thus hampering independent management of each species' fishery. The industrial and legal sectors also face difficulties in regulating the commerce of products from mixedspecies fisheries. The marked differences in price and marketability between hakes, together with declining fishing catches, increase the opportunities for fraudulent substitution with cheaper species of hakes or similar taxa (4).

The development of diagnostic tools for the unambiguous identification of hakes is a technology that would benefit both

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basic research studies on species biogeography and hybridization as well as applied fields such as fishery management, conservation genetics, fishery forensics, and commercial traceability. Even though experienced researchers can identify hakes using morphology (1), many samples such as those from museum collections, historical fishery surveys, or processed commercial fish products are devoid of morphological traits. Allozyme electrophoresis has been the most popular method used to describe genetic variation in hakes (5, 6). However, the frequency dependence of the interspecific genetic distinctiveness, together with the low allelic variation of allozymes, do not allow us to unambiguously assign individuals to species for many practical purposes. Biochemical analyses based on speciesspecific sarcoplasmic proteins, using techniques such as isoelectrofocusing (IEF), two-dimensional electrophoresis (2DE), and SDS-PAGE, have been adapted to assist in the industrial identification of some hake species (7-9). However, most of those identification methods are based on tissue-dependent protein analyses, which depend heavily on the heat lability of proteins or on the maintenance of high levels of biological activity. Although species-specific heat-resistant muscle proteins could partially overcome such problems, closely related species usually share identical or apparently identical protein sequences, i.e., replacements between basic amino acids that do not bring about modifications in the net charge of the protein can appear as isoforms by electrophoresis.

Most of the disadvantages of protein-based identification methods can be circumvented using DNA-based techniques (10).

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Table 1. Names, Natural Ranges, and Sampling Locations of Hake Species from the Genus Merluccius

hake species	codes	common names	ranges	country <sup>a</sup>	sampling coordinates
Merluccius merluccius	ME	European hake	21–62° N	Spain Italy*	37° 35′ N/08° 50′ W 38° 03′ N/12° 56′ E 55° 30′ N/04° 36′ E
Merluccius senegalensis	SE	Senegalese hake	10–33° N	Senegal* Namibia	55 50 N/04 58 E 15° 01′ N/18° 00′ W 18° 10′ N/16° 20′ W
Merluccius polli	PO	Benguela hake	20° N–19° S	Mauritania Senegal* Mauritania	21° 40' N/17° 55' W 15° 01' N/18° 00' W 19° 37' N/17° 06' W
Merluccius capensis	CA	shallow-water cape hake	0–34° S	Spain–Morocco* Angola* Namibia	27° 15′ N/14° 10′ W 17° 10′ S/ 11° 21′ W 24° 10′ S/14° 23′ E
Merluccius paradoxus	PA	deep-water cape hake	south 22° S	South Africa* South Africa* South Africa*	25° 33′ S/15° 13′ E 25° 33′ S/15° 13′ E 34° 10′ S/17° 10′ E
Merluccius productus	PR	Pacific hake	25–51° N	Canada* Canada	48° 08' N/122° 20' W 49° 10' N/123° 10' W
Merluccius gayi	GA	Peruvian hake Chilean hake	3–10° S 23–47° S	Perú-Chile* Chile* Perú-Chile	08° 50' S/80° 00' W 24° 40' S/70° 50' W 30° 00' S/71° 55' W
Merluccius australis	AU	Antarctic queen hake New Zealand hake	40–57° S south 40° S	Chile* Australia	41° 20′ S/74° 35′ W 43° 40′ S/169° 25′ E 52° 40′ S/63° 35′ W
Merluccius hubbsi	HU	Patagonian hake	25–54° S	Argentina* Argentina	46° 30′ S/60° 45′ W 48° 30′ S/60° 45′ W 48° 30′ S/61° 30′ W
Merluccius albidus	AL	offshore hake	20–35° N	United States* United States*	35° 21′ N/70° 50′ W 37° 21′ N/73° 33′ W
Merluccius hernandezi Merluccius bilinearis	HE BI	California hake silver hake	5–23° N 36–47° N	Mexico* United States* United States* United States	29° 50' N/113° 20' W 39° 00' N/73° 10' W 40° 40' N/72° 00' W 42° 30' N/68° 33' W

<sup>a</sup> The asterisks indicate the samples containing the individuals whose ITS1 were sequenced to calibrate the identification method. About 20 individuals were collected at each sampling location.

Ancient DNA samples or commercial products often contain DNA that can be amplified and compared to known "sample types", provided they had been standardized with reconstruction methods developed for phylogenetic analyses (11). Two previous DNA-based methods developed to identify hakes involved the use of four and seven restriction enzymes to digest a part of the mtDNA control region (12) and a cytochrome *b* fragment (13), respectively. However, the extremely large variation characterizing the left domain of the mtDNA control region (14) and the lack of species-specific restriction patterns of cytochrome *b* for several pairs of closely related hakes (13) seriously limit the utility of those methods for the full diagnostic identification within genus *Merluccius*.

The ITS1 spacer of the rDNA gene family accounts for hundreds of copies in fish genomes (15) and has the property to evolve concertedly within species and independently between species (16). Because concerted evolution results in sequence homogenization across most members of the rDNA family within reproductive units (17), the ITS1-rDNA spacer has been successfully applied in fish phylogeography (18) as well as in phylogenetic inference (19). This spacer is therefore one of the most promising DNA regions for species authentication and identification of closely related taxa (20). The goal of this work is to document the development of two ITS1-based diagnostic tools that allow detecting DNA from *Merluccius* and identifying 12 species of hakes from this genus, respectively.

# MATERIALS AND METHODS

Sampling and Morphological Identification of *Merluccius* spp. To assess the impact of intraspecific genetic variation that could weaken the diagnostic signal, we sampled each of 12 hake species at distant sites of their oceanographic distributions (**Table 1**). This worldwide sampling was performed in cooperation with local fishermen, commercial factory ships, and research vessels across Euro-African (East-Atlantic) and American (West-Atlantic and Pacific) fisheries. Approximately 20 specimens per sample were frozen upon collection, and their GPS codes were recorded on board. Whole specimens were boiled to facilitate bone cleaning and were identified using species-specific morphological traits. Shape and length of clean structures such as otoliths, abdominal vertebrae (parapophysis), crane, and pectoral fins were inspected with optical microscopy to classify them according to criteria previously established for this genus (1). The ITS1-rDNA of two identified individuals per species was sequenced to calibrate the identification method. A total of 15 specimens per sample and 1-3samples per species were used to assess the reproducibility of the identification method.

**DNA Extraction and Purification.** Genomic DNA was extracted by homogenization of 100 mg of gill tissue following an optimized DNA extraction method that combines the salting-out method (21) with the standard phenol/chloroform method (22) and is suitable for removing the mucopolysaccharides present in fish tissues.

Amplification and Sequencing of ITS1-rDNA. The ITS1-rDNA spacer was PCR-amplified from total DNA using a set of primers selected from coding regions of the 18S (*XelaITS1.1*: 5'-AAG-TAAAAGTCGTAACAAGGTTTCCGTAGG-3') and the 5.8S (*Onmy-ITS1.2*: 5'-CAAGCCGAGTGATCCACCGC-3') genes of *Xenopus laevis* (23) and *Salmo gairdneri* (24), respectively. PCR amplifications of 50  $\mu$ L containing 3 mM MgCl<sub>2</sub> were performed at 95 °C for 10 min and 35 cycles of 95 °C for 50 s, 55 °C for 40 s, and 72 °C for 2 min and 30 s, followed by a final step at 72 °C for 30 min. Amplicons of two individuals per species (**Table 1**) were purified from preparative gels (Marligen Biosciences, Valencia, Spain). Double-stranded DNA sequences were prepared in double with both the BigDye Terminator Cycle Sequencing Standard and the dGTP BigDye Terminator Ready Reaction Kit, using the primers *XelaITS1.1* and *OnmyITS1.2*. Sequenc-

ing reactions consisted of a denaturing cycle of 98 °C for 5 min, followed by 30 cycles of 96 °C for 30 s, 50 °C for 20 s, and 65 °C for 3 min and 30 s. Sequences were electrophoresed in an ABI Prism 310 DNA sequencer (Applied Biosystems, Madrid, Spain). All polymorphic sites were verified by re-amplifying all templates with two different Taq polymerases (Promega, Barcelona, and Eppendorf, Madrid, Spain) and resequencing them as described above. The consensus ITS1 sequence of each species was derived from single-strand chromatograms per template DNA using the CHROMAS software available online (http://www.technelysium.com.au/chromas.html).

Selection of Specific Primers for the Genus Merluccius. The 3' end of the 18S gene and the 5' end of the 5.8S gene were used to align the ITS1 sequences of 12 species of hakes using the SeqLab program from the GCG software package (25). This alignment allowed the identification of highly conserved regions between species, which were used to select several pairs of nested primers within the interspecifically conserved regions of the ITS1, using Oligo 4.05 (26). To check for the specificity of the primers to PCR-amplify exclusively in the genus Merluccius, all of them were also tested in closely related taxa (tailed hakes, Macruronus novaezelandiae and Macruronus magellanicus; Atlantic cod, Gadus morhua), as well as in distantly related taxa (salmonids, Salmo salar, Salmo trutta, and Oncorhynchus mykiss; flatfishes, Scophthalmus maximus, Scophthalmus rhombus, and Platichthys flexus; and mollusks, Octopus vulgaris and Mytilus galloprovincialis). All PCR products were visualized in 3% agarose gels to verify the length of the amplification products.

Selection of Diagnostic ITS1 Targets and Establishment of Species-Specific Restriction Patterns. The restriction maps of the 602-659 bp fragments spanning the ITS1 spacer were developed with WEBCUTTER 2.0 (27) and the enzymatic database REBASE (28) (New England Biolabs, Barcelona, Spain). The comparison of ITS1 restriction maps from each species allowed the selection of a minimum number of restriction enzymes necessary to achieve a complete discrimination of species. The consistency of the restriction patterns predicted was assessed by digesting 15 individuals per sampling site (45 individuals per species, including Gadus morhua) with four restriction enzymes. Independent digestions of 1  $\mu$ g of the amplicon per each enzyme were allowed to proceed for 5 h, and the products were electrophoresed in 3% agarose gels at 70 V for 1 h. The restriction patterns of each species were established from the gels by (i) their comparison with a molecular weight marker, (ii) side by side comparisons of the patterns obtained for each pair of species, and (iii) verification of the exact size of fragments as determined from the ITS1 sequence of each species.

**Phylogenetic Assessment of the Diagnostic Power.** Because all conspecific samples showed the same restriction pattern for all of the enzymes, a rooted tree was constructed with one individual per species to verify the correct phylogenetic discrimination of species. The polymorphism parsimony method of DOLLOP from PHYLIP 3.6 (29) was used to find the most parsimonious trees built from a data matrix of presence–absence of restriction fragments (*30*). To search for the best tree, the analyses were performed by randomizing the input order of species through 100 iterations. The parsimonious trees recovered were summarized in a consensus tree using CONSENSE from PHYLIP 3.6 (29). The consensus nodal values were considered as a measure of the resolution power achieved at identifying species.

**Species Identification using FINS and BLAST.** The use of FINS (*31*) to identify hake species consisted of three steps. First, the DNA of test samples were extracted and purified following the procedures described above. Second, the nested fragment of 193 bp from the ITS1-rDNA spacer of 10–45 individuals per species were PCR-amplified and sequenced using capilar electrophoresis. Third, a phylogenetic reconstruction of the ITS1 (either from the whole ITS1 sequence or from the 193 bp nested fragment) was performed using sample types of the 12 species and the ITS1 sequences from all samples. This analysis allowed the confirmation of the assignment accuracy of samples to species upon their ascribing into each species' cluster.

A second alternative to the PCR-RFLPs identification method developed was the calculation of the expectation value of random sequence identity using the BLAST package (*32*). For this calculation, the ITS1 nucleotide sequences (from either the whole ITS1 sequence

or the 193 bp sequence) of tested samples were compared to ITS1-rDNA sequences of all hakes made available in GenBank (accession numbers in **Figure 1**).

#### RESULTS

The double sequencing of each template as well as the use of two additional *Taq* DNA polymerases to amplify the ITS1 region, followed by its further resequencing, served at verifying the accuracy of the ITS1 sequences recovered. Identical ITS1 sequences were obtained for all individuals of the same species across independent amplifications and sequencing methods (**Figure 1**). The alignment yielded a nucleotide matrix of 692 bp that comprised the ITS1 sequence plus 53 bp from the 3' end of the 18S gene and 20 bp from the 5' end of the 5.8S gene. Most gaps introduced in the alignment to properly pair the ITS1 sequences from the 12 hake species were due to repetitive regions of simple nucleotide stretches (**Figure 1**).

The low number of ambiguities in the ITS1 alignment provided large conserved regions between species that were used to design three pairs of conserved primers. Two primer pairs were not specific of *Merluccius* and amplified the same length product in *Gadus morhua*, *Macruronus novaezelandiae*, *Macruronus magellanicus*, *Salmo salar*, *Salmo trutta*, and *Oncorhynchus mykiss*. Amplification was not observed in flatfish species or in mollusks; therefore, the latter were used as negative controls (data not shown). The third primer pair termed *MerITS1Nes1* and *MerITS1Nes2* (Figure 1) rendered a 193 bp PCR product of satisfactory quantity and quality in *Merluccius* spp. and in *G. morhua* (data not shown).

The nucleotide differences between the ITS1 sequences of the species provided the basis for selecting diagnostic enzymes from the ITS1 restriction maps of each species. The application of four restriction enzymes to aliquots of ITS1 amplicons allowed the full discrimination of all hake species and the Atlantic cod, with all composite haplotypes being speciesspecific (Table 2). The four enzymes recognized and cut the targets GT!AC/CA!TG (Afa I), GGCGC!C/C!CGCGG (Nar I), TGG!CCA/ACC!GGT (Mlu NI), and CCTC(N)7!/GGAG(N)6! (Mnl I). The restriction patterns generated after the digestion with Afa I allowed the identification of M. polli, M. hubbsi, M. bilinearis (Table 2 and Figure 2), and G. morhua (Table 2) and Figure 3). The remaining species were grouped in two clusters by the similarity of their restriction patterns, i.e., M. merluccius, M. senegalensis, M. capensis, M. paradoxus, and M. albidus, in one group, and M. productus, M. gayi, M. australis, and M. hernandezi, in a second group. After the identification of the first four species with Afa I, the enzyme Nar I allowed the direct discrimination of M. merluccius and M. senegalensis and also of M. capensis, M. paradoxus, M. albidus, and M. australis when combined with the pattern of the enzyme Afa I (Table 2 and Figure 2). The enzyme Mlu NI allowed the distinction of *M. productus* from *M. gayi* and *M.* hernandezi (Table 2 and Figure 2). The enzyme Mnl I was used to distinguish M. gavi from M. hernandezi by a 164 bp band specific to the latter species (Table 2 and Figure 4). The composite pattern of the four enzymes gave a full diagnosis between the 12 species. It should be noted that the agarose gels used did not allow the clear resolution of the smallest restriction fragments (<30 bp), otherwise unnecessary for species identification, and that digestions of the ITS1 sequence in some species did not go to completion, leaving some of the original ITS1 fragment uncut (e.g., Figure 2).

The presence–absence matrix of restriction fragments from the digestion of the ITS1 amplicon of 10–45 individuals per

Species	GenBank #	5'-ITS1-rDNA —	$\rightarrow$				
M. senegalensis	AY323936	ACGTTTGGAGCAGC	CCGTGCGGACG	GTCTTGCCGA	CG	-AAAGAAA-	GCCC
M. polli	AY323937	ACGTTTGGAGCAGC	CCGTGCGGACG	GTCTTGCCGA	CG	-AAA-A	GCAG-CCC
M. paradoxus	AY323938	ACGTTTGGAGCAGC	CCGTGCGGACG	GTCTTGCCGAAA	CG	- AAA - AAA -	GCCC
M. hernandezi	AY323939	ACGTTTTGAGCAG-	A-CCGTGCGGACG	GTCTCGCCGA	CACAA	 a	CCC
M. albidus M. apponaía	AY323940	ACGTTTTGAGCAGC	CCGTGCGGACG	GTCTCGCCGA	CACACACA		GCAA-CCC
M. morluggiug	AI323941 AV222042	ACGITIGGAGCAGC	CCGIGCGGACG	CTCTIGCCGA	CG=	-AAAGAAA-	
M. meridecius	AI323942	ACGITTGGAGCAGC	A - CCGTGCGGACG	GICIIGCCGA	CARACRC	- AAAGAAAA	GCCCACCC
M hilinearig	AU323943	ACGITTTGAGCAGC	AFCCGTGCGAACG	GICICGCCGA	CARACAC		GCAA-CCC
M hubbai	AT222244	ACGITTTGAGGAG-	-TCCGTGCGIACG	GICICGCCGAA-	CACAC-IA		
M cavi	AV323945	ACGTTTTGAGCOG-	A - CCGTGCGGACG	GTCTCGCCGA	CACAA		
M productus	AY323947	ACGTTTTGAGCAG-	A - CCGTGCGGACG	GTCTCGCCGA	CACAA		
in productus							
		1 10	20	30 4	0 5	0	60
Species							
M. senegalensis	AACACAACCCGÃ	GGGTCGAGCTCTGGGGG	G		TCCGCCCC	cccccG	AGTTCCCCGA
M. polli	AG-AGCCCGA	GGGTCGAGCTCCGGGGG	GTGTGGGGTGGGAC	CGGTTTAGGCCG	GCTCCCCCC	CCCTCTGCG	AGTTCCCCGA
M. paradoxus	AG-GACCCGA	GGGTCGAGCTCCGGGG-	TGGCAC	-GGTTTAG-CCG	-TTCCCCCCG	CCCACG	AGT CCCGA
M. hernandezi	ACAACC-GA	GGGTCGAGC			TCCCC	G	AGTTCCCCGA
M. albidus	AACC-GA	GGGTCGAGC			TCCC	G	AGTTCCCCGA
M. capensis	AACACAACCCGA	GGGTCGAGCT-TGGGGG	G		TCCGCCCC	CCG	AGTTCCCCGA
M. merluccius	A-CACAACC-GA	GGGTCGAGCT - TGGGGG	A CGGGCA		TCCGCCCC	ACCCCG	AGTTCCCCGA
M. australis	AACC-GA	GGGTCGAGC			TCCCCC	G	AGTTCCCCGA
M. bilinearis	ACC-GA	GGGTCGAGC			TCCCCCCC	G	AGTTCCCCGA
M. hubbsi	AGACC-GA	GGGTCGAGC			TCCCCCCC	G	AGTTCCCCGA
M. gayi	ACAACC-GA	GGGTCGAGC			TCCCC	G	AGTTCCCCGA
M. productus	ACAACC-GA	GGGTCGAGC			TCCCC	G	AGTTCCCCGA
	70 90		100 110	120	120	140	150
	/0 00	90	100 110	120	150	140	130
Species		"MorTTS	INegl" 5/ too	aaaaataaaat	tag 3/		$\longrightarrow$
M generalensis	CC-DADADA-C-	CTCARCC-ACCCCCA	A CCT CTTCC	GAGGATAGCGGC		CAGACA_CC	C-TCAAA
M polli	GG-DDDCM	GTCAACC-ACCCCCA	ACCICIIGG	GAGGATAGCGGC	TACCCGTCTG	CAGACA-CC	CC-GACAAC-
M. perri	CC-DDACDD	GTCAACC ACCCCCCCC	CTTGG	- ACCATACCCCT	T_CCCGTCTC	CAGACA CC	C-TGACAAC-
M hernandezi	GG-AAAAC-	GTCLARCE ACCCCCCC	CTTGG	GAGGATAGCGGT	TACCCGTCTG	CAGACA-CC	C-TGAACG
M albidus	GG-AAAAGC-	GTCARCC ACCCGCCCA		CAGGATAGCGGT	TACCCGTCTG	CAGACA-CC	C-TGAACG
M. capensis	GG-AAAAAA-C-	GTCAACC-ACCCCCA	ACCTCTTGC	GAGGATAGCGGC	TACCCGTCTG	CAGACA-CC	C-TGAAA
M merluccius	GG-AAAAAAAC-	GTCAACCAACCCA	CCTCTTGG	GAGGATAGCGGT	CACCCGTCTG	CAGACA-CC	C-TGAAA
M australis	GG-AAAAC-	GTCAACCAACCCCCCCCA	CTTGC	GAGGATAGCGGT	TACCCGTCTG	CAGACA-CC	C-TGACA-CG
M hilinearig	GGTAAAACT	GTCABCC-ACCCCCCCA	CTCGG	CACCATACCCCT	AACCCGTCTG	CAGACA-CC	C-TGACA-CG
M. hubbsi	GG-AAAAT	GTCAACCCA	CTTGG	GAGGATAGCGGT	TACCOUTTG	CAGACACCC	CC-GAACG
M gavi	GG-AAAAC-(	GTCAACC-ACCCCCCCA	CTTGG	GAGGATAGCGGT	TACCCGTCTG	CAGACA-CC	C-TGAACG
M. productus	GG-AAAAC-	GTCAACC-ACCCCCCCA	CTTGG	GAGGATAGCGGT	TACCCGTCTG	CAGACA-CC	C-TGAACG
	160	170 180	190	200	210	220	230
Species							
							00000 0 <b>0</b> 000
M. senegalensis	TA-GTCCCCCCG	TTTCGCTGACTCT-GTT	GGCGCCCTCGCT-	CC-GCACAC	-AT-TCTCCG	AGGTCCGAC	GGGGAGTGCG
M. senegalensis M. polli	TA-GTCCCCCCG GTCCCCC-G	ITTCGCTGACTCT-GTT ITTCGCTGACCCT-GTT	GGCGCCCTCGCT- GGCGCT-GCTC	CC-GCACAC GCTCCCGCACAC	-AT-TCTCCG	AGGTCCGAC AGGTCAGAG	GGGGAGTGCG GGAGAGTGCG
M. senegalensis M. polli M. paradoxus	TA-GTCCCCCCG GTCCCCC-G GTCCC-ACG	TTTCGCTGACTCT-GTT TTTCGCTGACCCT-GTT TTTCGCTGACCCC-GTT	GGCGCCCTCGCT- GGCGCT-GCTC GGC-TC-T-ACTC	CC-GCACAC GCTCCCGCACAC GCTCC-GCACAC	-AT-TCTCCG. -AT-TCTCCG. -AT-TCTCCG.	AGGTCCGAC AGGTCAGAG AGGTAAGAG	GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG
M. senegalensis M. polli M. paradoxus M. hernandezi	TA-GTCCCCCCG GTCCCCC-G GTCCC-ACG TATGACCCCC-G	ITTCGCTGACTCT-GTT ITTCGCTGACCCT-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT	GGCGCCCTCGCT- GGCGCT-GCTC GGC-TC-T-ACTC GGCGCCCTT-CT-	CC-GCACAC GCTCCCGCACAC GCTCC-GCACAC GCC-GCACAC	-AT-TCTCCG. -AT-TCTCCG. -AT-TCTCCG. -ATATCTCCG.	AGGTCCGAC AGGTCAGAG AGGTAAGAG AGGTACGAG	GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG
M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus	TA-GTCCCCCCG GTCCCCC-G GTCCC-ACG TATGACCCCC-G TATGACCCCC-G	ITTCGCTGACTCT-GTT ITTCGCTGACCCT-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCCGACCCT-GTT	GGCGCCCTCGCT- GGCGC-TC-GCTC GGC-TC-T-ACTC GGCGCCCTT-CT- GGCGCCCTCT-	CC-GCACAC GCTCCCGCACAC GCTCC-GCACAC GCC-GCACAC GCC-GCACAC	-AT-TCTCCG. -AT-TCTCCG. -AT-TCTCCG. -ATATCTCCG. -ATATCTCCC.	AGGTCCGAC AGGTCAGAG AGGTAAGAG AGGTACGAG AGGTATGAG	GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG
M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis	TA-GTCCCCCCG GTCCCCC-G GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG	TTTCGCTGACCCT-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCCGACCCC-GTT TTTCGCCGACCCT-GTT TTTCGCCGACCCT-GTT	GGCGCCCTCGCT GGCGC-T-GCTC GGC-TC-T-ACTC GGCGCCCTT-CT- GGCGCCCT-CT- GGCGCCCT-CT-	CC-GCACAC GCTCCCGCACAC GCTCC-GCACAC GCC-GCACAC GCC-GCACAC GCTCC-GCACAC	-AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -ATATCTCCG -AT-TCTCCT -AT-TCTCCC	AGGTCCGAC AGGTCAGAG AGGTAAGAG AGGTACGAG AGGTATGAG AGGTCCGAC	GEGGAGIGCG GGAGAGIGCG GGAGAGIGCG GGAGAGIGCG GGGGAGIGCG
M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius	TA-GTCCCCCCG GTCCCC-AG TATGACCCCC-G TATGACCCCC-G TATGACCCCCCG TA-GTCCCCCCG	ITTCGCTGACTCT-GTT TTTCGCTGACCCT-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCCGACCCT-GTT ITTCGCTGACTCT-GTT ITTTGCTGACTCTTGTT	GGCGCCCTCGCT- GGCGC-T-T-GCTC GGC-TC-T-ACTC GGCGCCCTT-CT- GGCGCCCT-CT- GGCGCC-T-ACTC GGCGCC-TCGCT-	CC-GCACAC GCTCCCGCACAC GCTCC-GCACAC GCC-GCACAC GCC-GCACAC GCTCC-GCACAC CCG-CACAC	-AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -ATATCTCCG -ATATCTCCG -AT-TCTCCC -AT-TCTCCG -AT-TCTCCG	AGGTCCGAC AGGTCAGAG AGGTAAGAG AGGTACGAG AGGTATGAG AGGTCCGAC AGGTCTGAC	GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG
M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis	TA-GTCCCCCCCG GTCCCC-G TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCCG TA-GTCCCCCCCG TA-GTCCCCCCCG TATGACCCCCC-G	TTTCGCTGACTCT-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCCGACCCC-GTT TTTCGCCGACCCT-GTT TTTCGCTGACTCT-GTT TTTTGCTGACTCT-GTT TTTTGCCGACTCC-GTT	GGCGCCCTCGGT- GGCGCT-GCTC GGC-TC-T-ACTC GGCGCCCTT-CT- GGCGCCCTT-CT- GGCGCC-T-ACTC GGCGCC-TCGCT- GGCGCC-TCGCT-	CC - GCACAC GCTCCC-GCACAC G CC - GCACAC G CC - GCACAC GCTCC - GCACAC GCTCC - GCACAC CCG - CACAC CC - GCACAC	-AT - TCTCCG -AT - TCTCCG -AT - TCTCCG -ATATCTCCG -AT - TCTCCC -AT - TCTCCG -AT - TCTCCG - TATCTCCG	AGGTCCGAC AGGTCAGAG AGGTAAGAG AGGTACGAG AGGTCCGAC AGGTCCGAC AGGTCCGAC	GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG
M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCC-G TATGACCCCC-G TACGACCCCC-G	TTTCGCTGACTCT-GTT TTTCGCTGACCCT-GTT TTTCGCTGACCCC-GTT TTTCGCCGACCCC-GTT TTTCGCCGACCCT-GTT TTTCGCTGACTCT-GTT TTTTGCTGACTCCTGTT TTTCGCCGACCCC-GTT	GGCGCCTCGGT GGCGC-T-GCTC GGCGCCTT-CT- GGCGCCCTT-CT- GGCGCCT-CT- GGCGCC-T-ACTC GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCC-TCGCT-	CC - GCACAC GCTCCC - GCACAC G CC - GCACAC G CC - GCACAC GCTCC - GCACAC CC - GCACAC CC - GCACAC CC - GCACAC	-AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -ATATCTCCG -AT-TCTCCCT -AT-TCTCCCG -AT-TCTCCCG -TATCTCCCG CA-ATCTCCCG	AGGTCCGAC AGGTCAGAG AGGTACGAG AGGTACGAG AGGTCCGAC AGGTCCGAC AGGTCCGAG AGGTACGAG AGGTATGAG	GGGGAGTIGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG
M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. hubbsi	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGTCCCCCC-G	TTTCGCTGACTCT-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACTCT-GTT TTTTGGCTGACTCTTGTT TTTCGCCGACTCC-GTT TTTCGCCGACTCC-GTT TTTCGCCGACTCC-GTT	GGCGCCTCGCT GGCGC - T - GCTC GGCGCCT - CT - GGCGCCCT - CT - GGCGCCCT - CT - GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT	CC-GCACAC GCTCCCGCACAC GCTCC-GCACAC GCC-GCACAC GCC-GCACAC GCTCC-GCACAC CCG-CACAC CCGCACAC CC-GCACAC CC-GCACAC	-AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -ATATCTCCG -AT-TCTCCC -AT-TCTCCCG -TATCTCCCG CA-ATCTCCCC -AT-TCTCCCG	AGGTCCGAG AGGTAAGAG AGGTACGAG AGGTACGAG AGGTCCGAC AGGTCCGAC AGGTCTGAC AGGTACGAG AGGTACGAG AGGTACGAG	GGGGAGTIGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> <li>M. gayi</li> <li>M. acaductus</li> </ul>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCC-G TATGACCCCC-G TATGACCCCCC-G TATGACCCCCC-G TATGACCCCCC-G	TTTCGCTGACCT-GTT TTTCGCTGACCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCT-GTT TTTTGCTGACTCTTGTT TTTTGCCGACCCC-GTT TTTCGCCGACCCC-GTT TTTCGCCGACCCC-GTT TTTCGCCGACCCC-GTT	GGCGCCTCGCT GGC-TC-T-ACTC GGCGCCTT-CT- GGCGCCCT-CT GGCGCC-T-CT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT-	CC-GCACAC GCTCCCGCACAC GCTCC-GCACAC GCC-GCACAC GCC-GCACAC GCC-GCACAC CCG-CACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC	-AT - TCTCCG -AT - TCTCCG -AT - TCTCCG -AT - TCTCCG -AT - TCTCCG -AT - TCTCCG -TT - TCTCCG CA - ATCTCCCG -AT - TCTCCG -AT - TCTCCG -AT - TCTCCG	AGGTCCGAC AGGTAAGAG AGGTACGAG AGGTACGAG AGGTCCGAC AGGTCCGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG	GGGGAATTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> <li>M. gayi</li> <li>M. productus</li> </ul>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G	TTTCGCTGACTCT-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACTCT-GTT TTTCGCTGACTCT-GTT TTTCGCCGACTCC-GTT TTTCGCCGACTCC-GTT TTTCGCCGACCCC-GTT TTTCGCTGACCCC-GTT	GGGGCCTCGCT GGGGC - T - GCT GGCGCCT - CT GGCGCCTT - CT GGCGCCT - CT GGCGCC - TCGCT GGCGCC - TCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT	CC-GCACAC GCTCC-GCACAC GCC-GCACAC GCC-GCACAC GCC-GCACAC GCC-GCACAC CCG-CACAC CCG-CACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC	-AT - TCTCCG -AT - TCTCCG -AT - TCTCCG -ATATCTCCG -AT - TCTCCG -AT - TCTCCG -AT - TCTCCG CA - ATCTCCCG -AT - TCTCCG -AT - TCTCCG -ATATCTCCG -ATATCTCCG	AGGTCCGAC AGGTCAGAG AGGTAAGAG AGGTACGAG AGGTCCGAC AGGTCCGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG	GGGAATTGCG GGAGATTGCG GGAGACTGCG GGAGACTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGCGCG GGAGAGTGCG GGAGAGTGCG
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> <li>M. gayi</li> <li>M. productus</li> </ul>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G	TTTCGCTGACTCT-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCCGACCCT-GTT TTTCGCTGACTCT-GTT TTTCGCTGACTCCT-GTT TTTCGCCGACTCC-GTT TTTCGCCGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT	Gegecerteger Geget-T-ACT GegecerT-CT- Gegecert-CT- Gegecert-ACT Gegecerteger Gegecerteger Gegecerteger Gegecerteger Gegecerteger Gegecerteger Gegecerteger	CC-GCACAC GGTCCCGCACAC GGTCC-GCACAC GCC-GCACAC GGTCC-GCACAC GGTCC-GCACAC CCG-CACAC CCG-CACAC CCG-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC	-AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCCG -AT-TCTCCCG -AT-TCTCCCG -AT-TCTCCCG -AT-TCTCCCG -AT-TCTCCCG -AT-TCTCCCG -ATATCTCCC	AGGTCCGAC AGGTAAGAG AGGTAAGAG AGGTACGAG AGGTCCGAC AGGTCCGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG	GGGGAGTIGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> <li>M. gayi</li> <li>M. productus</li> </ul>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G	ITTCGCTGACTCT-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCTGACTCT-GTT ITTTGGCTGACTCT-GTT ITTCGCCGACTCC-GTT ITTCGCCGACTCC-GTT ITTCGCCGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT	GGGGCCTCGCT GGCC-TC-T-ACTC GGCGCCTT-CT- GGCGCCCT-CT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- 270	CC-GCACAC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC GCC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC	-AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -TAT-TCTCCG -TATCTCCG -AT-TCTCCCG -AT-TCTCCCG -AT	AGGTCCGAC AGGTAAGAG AGGTAAGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG	GGGAGATTGCG GGAGATGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG J10
M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. hubbsi M. gayi M. productus Species	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G	ITTCGCTGACCT-GTT ITTCGCTGACCC-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCT-GTT ITTCGCTGACCCT-GTT ITTCGCCGACCCC-GTT ITTCGCCGACCCC-GTT ITTCGCCGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT ITTCGCTGACCCC-GTT	GGGGCCTCGCT GGC-TC-T-ACTC GGCGCCTT-CT- GGCGCCCT-CT GGCGCC-T-ACTC GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- 270	CC-GCACAC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC GCC-GCACAC CCG-CACAC CCG-CACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC 280 2	-AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCCG -ATATCTCCCG -ATATCTCCCG -ATATCTCCCG -3TATCTCCCCG -3TATCTCCCCG -3TATCTCCCCG -3TATCTCCCCG -3TATCTCCCCG -3TATCTCCCCG -3TATCTCCCCG -3TATCTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	AGGTCCGAC AGGTCAGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG 00 3' atacq	GGGAAGTGCG GGAAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG 310 ataggggctt
M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. hubbsi M. productus Species M. senegalensis	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G 240 GC-GCAGTGCGT	TTTCGCTGACTCT-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCT-GTT TTTTGCTGACTCTTGTT TTTCGCCGACTCC-GTT TTTCGCCGACCCC-GTT TTTCGCCGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT 250 260 CCGG-AGAGGCCCT-CC	GGGCCCTCGCT- GGCGC - T - GCT GGCGCCT - CT- GGCGCCCT - CT- GGCGCC - TCGCT- GGCGCC - TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- 270 CGTCAGGA TG2	CC-GCACAC GGTCC-GCACAC GCC-GCACAC GCC-GCACAC GCC-GCACAC CCG-CACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC 280 2	-AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -ATATCTCCG	AGGTCCGAC AGGTAGAGA AGGTACAGA AGGTACCAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG 00 3' atacg GC ATCC	GGGAAFTGCG GGAAGTGCG GGAGATGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG 310 attaggggctt TATCCCCGAA
M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. hubbsi M. productus Species M. senegalensis M. polli	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCC-G CACCCCC-G CACCCCC-G CACCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACCCCCC-G CACGACCCCCC-G CACGACCCCC-G CACGACCCCC-G CACGACCCCCC-G CACGACCCCCC-G CACGACCCCCC-G CACGACCCCCC-G CACGACCCCCC-G CACCCCCCC-G CACCCCCCC-G CACCCCCCC-G CACCCCCCC-G CACCCCCCCC-G CACCCCCCCC-G CACCCCCCC-G CACCCCCCCC-G CACCCCCCCC-G CACCCCCCC-G CACCCCCCC-G CACCCCCCC-G CACCCCCCC-G CACCCCCCCC-G CACCCCCCCC-G CACCCCCCC-G CACCCCCCCC-G CACCCCCCCC-G CACCCCCCCC-G CACCCCCCCC-G CACCCCCCCCCCC-G CACCCCCCCC-G CACCCCCCCC-G CACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	TTTCGCTGACTCT-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCCGACCCC-GTT TTTCGCTGACTCT-GTT TTTCGCTGACTCT-GTT TTTCGCCGACTCC-GTT TTTCGCCGACTCC-GTT TTTCGCCGACTCC-GTT TTTCGCTGACCCC-GTT CCGCGACGCCC-GTT CCGG-AGAGGCCCT-CC CAAG-GGAGGCCCTACC	GGGCCCTCGCT- GGCGC-T-GCTC GGCGCCT-CT- GGCGCCCT-CT- GGCGCC-T-GT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- 270 CGTCAGGA-TGA CGTCAGGA-TGA	CC-GCACACC GCTCC-GCACAC GCC-GCACAC GCTCC-GCACAC GCTCC-GCACAC GCTCC-GCACAC CCG-CACAC CCG-CACAC CC-GCACAC CC-GCACAC CC-GCACAC 280 2 	-AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -ATATCTCCG	AGGTCCGAC AGGTAGAGA AGGTACGAG AGGTACGAG AGGTCCGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG 3' atacg GGCATCC GGCGTATGC	GGGAAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG ataggggctt TATCCCCGA
<pre>M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bilinearis M. hubbsi M. gayi M. productus Species M. senegalensis M. polli M. paradoxus</pre>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGGAGCCCC-G CAGGAGCGCG GC-GCAGTGCGT GC-GCAGTGCGC	TTTCGCTGACTCT-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACCCC-GTT TTTCGCTGACTCT-GTT TTTCGCCGACTCC-GTT TTTCGCCGACTCC-GTT TTTCGCCGACTCC-GTT TTTCGCCGACCCC-GTT TTTCGCTGACCCC-GTT 250 260 CCGG-AGAGGCCCT-CC CCGG-GGAGGCCCTACC CCGG-GGAGGCCCTACC	GGGCCCTCGCT GGCC-T-ACTC GGCGCCTT-CT- GGCGCCTT-CT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- 270 CGTCAGGA-TCTG GCTCAGG-TCTGG CGTCAGG-TCTGG	CC-GCACAC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC GCTCC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC 280 2 TTGTCTC TTGTCTC	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - ATATCTCCG - AT-TCTCCG - AT-TCTCCG - TATCTCCG TATCTCCG TATCTCCG - ATATCTCCG - ATATCTCCG - ATATCTCCG - ATATCTCCG - ATATCTCCG - ATATCTCCG - ATACTCCCG - ATACTCCCCG - ATACTCCCCCG - ATACTCCCCG - ATACTCCCCG - ATACTCCCCG - ATACTCCCCCG - ATACTCCCCG - ATACTCCCCG - ATACTCCCCG - ATACTCCCCCG - ATACTCCCCG - ATACTCCCCCCCCG - ATACTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	AGGTCCGAC AGGTACAGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG 3' atacg GGC - ATCC GGCGTATGC GGCCTATGC	GGGAATTGCG GGAGATGCG GGAGATGCG GGAGAGTGCG GGGGAATTGCG GGGGAATTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG J10 TATCCCCGA- TATCCCCGA-
M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. hubbsi M. productus Species M. senegalensis M. polli M. paradoxus M. hernandezi	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CATGACCCCC-G CATGACCCCC-G CATGACCCCC-G CC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGC	$\label{eq:constraint} \begin{split} & \text{ITTCGCTGACCT-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTTGGCTGACTCTTGTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITCGCCGACCCC-CCC} \\ & \text{CCGG-AGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCTACC} \\ & \text{CCGG-GGAGGCCCTACC} \\ & \text{CCGG-GGAGGCCCTACC} \\ & \text{CCGG-GGAGGCCCTACC} \\ & \text{CCGGTGGAGGCCCTACC} \\ & \text{CCGGTGGAGGCCCCCACC} \\ & \text{CCGGTGGAGGCCCCCC} \\ & \text{CCGGTGGAGGCCCCCC} \\ & \text{CCGGTGGAGGCCCCCCC} \\ & \text{CCGGTGGAGGCCCCCCC} \\ & \text{CCGGTGGAGGCCCCCCCC} \\ & \text{CCGGTGGAGGCCCCCCCCCCCCCCCCCCCC} \\ & CCGGTGGAGGCCCCCCCCCCCCCCCCCCCCCCCCCCCCC$	GGGGCCTCGCT GGC-TC-T-ACTC GGCGCCTT-CT- GGCGCCCT-CT GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- 270 CGTCAGGA-TCTG CGTCAGGA-TCTGG CGTCAGG-TCTGG C-TCAG-TCTAG	CC-GCACACC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC CCG-CACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC 280 2 	-AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -ATATCTCCCG -ATATCTCCG -ATATCTCCG -ATATCTCCG -ATATCTCCG -ATATCTCCG -ATATCTCCG -ATATCTCCG -ATATCTCCG -ATATCTCCG -ATATCTCCG -ATATCTCCG -ATATCTCCG -ATATCTCCG -ATAT	AGGTCCGAC AGGTCAGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG 00 3' atacg GGCATCC GGCGTATGC GGCGTATGC	GGGAAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG 310 ataggggctt TATCCCCGAA TATCCCCGAA
<pre>M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bubbsi M. pubbsi M. gayi M. productus Species M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus</pre>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCCG TA-GTCCCCCCCG TA-GTCCCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CACGCCCCC-G CACGCCCCC-G CACGCCCCC-G CC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT	$\label{eq:constraint} \begin{split} & \text{ITTCGCTGACCCT-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTTGGCTGACTCTTGTT} \\ & \text{ITTTGGCCGACTCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITCGCCGACCCC-GTT} \\ & \text{ITCGCCGACCCC-CT} \\ & \text{ITCGCCGG-GAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCTAC} \\ & \text{CCGGTGGAGGCCCT-CC} \\ & \text{CCGGTGGAGGCCCT-CC} \\ & \text{CCGGTGGAGGCCCT-CC} \\ & \text{CCGGG-GGAGGCCCT-CC} \\ & \text{CCGGTGGAGGCCCT-CC} \\ & \text{CCGGTGGAGGCCCCT-CC} \\ & \text{CCGGTGGAGGCCCT-CC} \\ & \text{CCGGTGGAGGCCCT-CC} \\ & \text{CCGGTGGAGGCCCT-CC} \\ & \text{CCGGTGGAGGCCCT-CC} \\ & \text{CCGGTGGAGGCCCCT-CC} \\ & \text{CCGGTGGAGGCCCT-CC} \\ & \text{CCCGTGGGACGCCCT-CC} \\ & \text{CCCGTGGGCCCCT-CC} \\ & \text{CCCGTGGGACGCCCT-CC} \\ & \text{CCCGTGGACGCCCT-CC} \\ & \text{CCCGTGGACGCCCT-CC} \\ & \text{CCCGTGCCCCCC-CC} \\ & \text{CCCCC} \\ & \text{CCCCCC} \\ & \text{CCCC} \\ & \text{CCCCC} \\ & \text{CCCC} \\ & \text{CCCCC} \\ & \text{CCCC} \\ & \text{CCC} \\ & \text{CCCC} \\ & \text{CCC} \\ & \text{CCCC} \\ & \text{CCC} \\ & \text{CCCC} \\ & \text{CCC} \\ & \text{CCCC} \\ & \text{CCC} \\ & \text{CCCC} \\ & \text{CCC} \\ & \text{CCCC} \\ & CCCC$	GGGCCCTCGCT GGGC-T-GCTG GGGCCCT-CT- GGCGCCCT-CT- GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- CTCAGA-TCGG CTCAGG-TCTGG C-TCAG-TCCAGG C-TCAG-TCCAGG	CC-GCACACC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC CCG-CACAC CCG-CACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC 	- AT - TCTCCG - AT - TCTCCG - AT - TCTCCG - AT AT - TCTCCG - AT ATCTCCG - AT AT ATCTCCG - AT ATCTCCG - AT ATCTCCG - AT ATCTCCG - AT ATCTCCG - AT ATCTCCG - AT AT ATCTCCG - AT AT ATCTCCG - AT AT ATCTCCG - AT AT AT ATCTCCG - AT ATCTCCG - AT ATCTCC	AGGTCCGAC AGGTCAGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCCTATGC GGCGTATGC GGCGTATGC GGCCTATGC	GGGAAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG 310 ataggggGtt TATCCCCGAA TATCCCCGAA TATCCCCGAA
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> <li>M. gayi</li> <li>M. productus</li> </ul> Species M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. albidus M. capensis	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CC-GCAGTGCCC GC-GCAGTGCCT GC-GCAGTGCCT	$\label{eq:constraints} \begin{split} & \text{ITTCGCTGACCCT-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACTCT-GTT} \\ & \text{ITTCGCTGACTCT-GTT} \\ & \text{ITTCGCCGACTCC-GTT} \\ & \text{ITTCGCCGACTCC-GTT} \\ & \text{ITTCGCCGACTCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITCGCCGCACCCC-GTT} \\ & \text{ICCGG-GAGGCCCCTACC} \\ & \text{CCGG-GGAGGCCCTACC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCCT-CC} \\ & \text{CCGG-GGAGGCCCCC-CC} \\ & \text{CCGG-GGAGGCCCCC-CC} \\ & \text{CCGG-GGAGGCCCCCC-CC} \\ & \text{CCGG-GGAGGCCCCC-CC} \\ & \text{CCGG-GGAGGCCCCC-CC} \\ & \text{CCGG-GGAGGCCCCC-CC} \\ & \text{CCGG-GGAGGCCCC-CC} \\ & \text{CCGG-GGAGGCCCC-CC} \\ & \text{CCGG-GGAGGCCCCC-CC} \\ & \text{CCGG-GGAGGCCCCCG} \\ & \text{CCGG-GGAGGCCCCC-CG} \\ & \text{CCGG-GGAGGCCCCCG} \\ & \text{CCGG-GGAGGCCCCC-GC} \\ & \text{CCGG-GGAGGCCCCCG} \\ & CCGG-GGAGGC$	GGGCCCTCGCT GGCGC - T - GCT GGCGCCT - CT GGCGCCT - CT GGCGCC - TCGCT GGCGCC - TCGCT GGCGCC - TCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT 270 CGTCAGGA - TGA CGTCAGGA - TCAG C-TCAG - TCAG CCTCAGA - TAA	CC-GCACACC GCTCC-GCACAC GCC-GCACAC GCTCC-GCACAC GCTCC-GCACAC CCG-CACAC CCG-CACAC CC-GCACAC 	-AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -AT-TCTCCG -ATATCTCCG	AGGTCCGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGC - ATCC GGCGTATGC GGCGTATGC GGCC - ATCC	GGGAATTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG A ataggggctt TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. bilinearis</li> <li>M. bilinearis</li> <li>M. bilinearis</li> <li>M. productus</li> </ul> Species Species M. senegalensis M. polli M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. capensis M. merluccius	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G CATGACCCCC-G CATGACCCCC-G CATGACCCCC-G CC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT	$\label{eq:constraints} \begin{split} & \text{ITTCGCTGACCT-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACTCC-GTT} \\ & \text{ITTCGCCGACTCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITCGCCGACCCC-GTT} \\ & ITCGCCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC$	GGGCCCTCGCT GGCCC-T-ACTC GGCGCCTT-CT- GGCGCC-T-ACTC GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- 270 CGTCAGGA-TGGC CGTCAGGA-TGGC C-TCAG-TCAGC C-TCAG-TCAGC CGTCAGGA-TGA CGTCAGGA-TGA	CC-GCACACC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC 280 2 TTGTCTC TTGTCTCC 	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - TATCTCCG - TATCTCCG - TATCTCCCG - ATATCTCCCG - ATATCTCCG - ATATCTCCG - ATATCTCCG - ATATCTCCG - ATATCTCCG - ATATCTC	AGGTCCGAC AGGTACAGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCGTATGC GGCGTATGC GGCCTATGC GGCCTATGC GGC-ATCC	GGGAATTGCG GGAGATGCG GGAGATGCG GGAGAGTGCG GGGGAATGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GAGAGTGCG 310 ataggggctt TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA
<pre>M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bubbsi M. gayi M. productus  Species M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis </pre>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT	$\label{eq:constraint} \begin{split} & \text{ITTCGCTGACCT-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTTGGCGACCTC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITCGCCGACCCC-GTT} \\ & \text{ITCGCCGACCCC-GTT} \\ & \text{ITCGCCGACCCC-GTT} \\ & \text{ITCGCCGCCCCCCCCCC} \\ & \text{CCGG-GAGGCCCT-CC} \\ & \text{CCGG-GAGGCCCTACC} \\ & \text{CCGG-GAGGCCCT-CC} \\ & \text{CCGG-GAGGCCCTC-CC} \\ & \text{CCGG-GAGGCCCT-CC} \\ & \text{CCGG-GAGGCCCT-CC} \\ & \text{CCGG-GAGGCCCT-CC} \\ & \text{CCGG-AGAGGCCCT-CC} \\ & \text{CCGG-AGAGGCCCT-CC} \\ & \text{CCGG-GAGGCCCT-CC} \\ & \text{CCGG-CGAGGCCCT-CC} \\ & \text{CCGG-CGACGCCCT-CC} \\ & \text{CCGG-CGACGCCCT-CC} \\ & \text{CCGG-CGACGCCCT-CC} \\ & \text{CCGG-CGACGCCCT-CC} \\ & \text{CCGG-CCCCCCCCCCCCCCCC} \\ & \text{CCGG-CCCCCCCCCCCCCCCCCCCC} \\ & \text{CCCCCCCCCCCCCCCCC} \\ & \text{CCCCCCCCCCCCCCCCCCCCCCC} \\ & CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC$	GGGCCCTCGCT GGC-TC-T-ACTC GGCGCCTT-CT- GGCGCCCT-CT GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- CTCAGA-TCAG CTCAGGA-TCAG CTCAGGA-TCAG CTCAGGA-TCAG CTCAGGA-TCAG CTCAGGA-TCAG CTCAGGA-TCAG	CC-GCACAC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC CCG-CACAC CCG-CACAC CC-GCACAC 	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - ATATCTCCG - AGAGAA - CCG AGAGAA - CCG AGAGAA - CCG AGAGAA - CCG	AGGTCCAAG AGGTCAGAG AGGTACAGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCCTATGC GGCGTATGC GGCGTATGC GGCCTATGC GGCCTATGC GGCCTATGC GGCCTATGC	GGGAATTGCG GGAGATGCG GGAGATGCG GGAGATGCG GGGGAGTGCG GGGGAGTGCG GGAGATGCG GGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. bilinearis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. australis</li> <li>M. bilinearis</li> </ul>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCCG TA-GTCCCCCCCG TA-GTCCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGACCCCC-G CAGACGCCCC-G CC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT	$\label{eq:constraint} \begin{split} & \text{TTTCGCTGACCCT-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTTTGCTGACTCTTGTT} \\ & \text{TTTGGCCGACCCC-GTT} \\ & \text{TTTCGCCGACCCC-GTT} \\ & \text{TTTCGCCGACCCC-GTT} \\ & \text{TTTCGCCGACCCC-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTCGGCGACCCC-GTT} \\ & \text{TTCGGCGACCCC-GTT} \\ & \text{TCGCGGGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-C} \\ & \text{CCGGG-GGAGGCCCT-C} \\ & \text{CCGG-GGAGGCCCT-C} \\ & \text{CCGGG-GGAGGCCCT-C} \\ & \text{CCGGG-GGAGGCCCT-C} \\ & \text{CCGG-GGAGGCCCT-C} \\ & \text{CCGGG-GGAGGCCCT-C} \\ & \text{CCGGG-GGAGGCCCT-C} \\ & \text{CCGG-GGAGGCCCT-C} \\ & \text{CCGG-CGGAGGCCCT-C} \\ & \text{CCGG-CGGAGGCCCT-C} \\ & \text{CCGG-CGGAGGCCCCT-C} \\ & \text{CCGG-CGGAGGCCCCT-C} \\ & \text{CCGG-CGGAGGCCCCT-C} \\ & CC$	GGGCCCTCGCT GGCCCT-CTACT GGCCCCT-CT GGCGCCCT-CT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT CTCAGA-TCAG CTCAGG-TCTAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG	CC-GCACACC GCTCC-GCACAC GCC-GCACAC GCC-GCACAC CCG-CACAC CCG-CACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC 	- AT - TCTCCG - AT - TCTCCG CA - ATCTCCCG - AT - TCTCCG -	AGGTCCGAC AGGTCAGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCGTACGAG GGCGTATGC GGCGTATGC GGCGTATGC GGCCGTATGC GGCCGTATGC GGCCGTATGC GGCCGTATGC GGCCGTATGC	GGGAAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG 310 ataggggGtt TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> <li>M. gayi</li> <li>M. productus</li> </ul> Species M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. albidus M. capensis M. merluccius M. australis M. bilinearis	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGAGTGCGT GGTGCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT	$\label{eq:constraint} \begin{split} & \text{TTTCGCTGACCT-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTTCGCTGACTCC-GTT} \\ & \text{TTTGGCTGACTCTTGTT} \\ & \text{TTTGGCCGACTCC-GTT} \\ & \text{TTTCGCCGACTCC-GTT} \\ & \text{TTTCGCCGACTCC-GTT} \\ & \text{TTTCGCCGACCCC-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTTCGGCTGACCCC-GTT} \\ & \text{TTCGGCTGACCCC-GTT} \\ & \text{TTCGGCTGACCCC-GTT} \\ & \text{TCGGCGGACGCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-C} \\ & \text{CCGGG-GGAGGCCCT-C} \\ & \text{CCGGC-CGCGCCCT-C} \\ & \text{CCGGG-GGAGGCCCT-C} \\ & \text{CCGGCCCC} \\ & \text{CCGG-CGAGGCCCCC-C} \\ & \text{CCGG-CGAGGCCCCC-C} \\ & \text{CCGG-CGAGGCCCCC-C} \\ & \text{CCGG-CGCGCCCCC-C} \\ & \text{CCGG-CGCCCCCCCC-C} \\ & \text{CCGG-CGCCCCCCCC-C} \\ & \text{CCGG-CGCCCCCCCCC-C} \\ & \text{CCGG-CGCCCCCCCCCCCCCC} \\ & \text{CCGG-CGCCCCCCCCCCCCCCCCC} \\ & \text{CCGG-CGCCCCCCCCCCCCCCCCCCCCCCC} \\ & CCGGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC$	GGGCCCTCGCT GGCCCT-CT-CCT GGCGCCTT-CT- GGCGCCTT-CT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- 270 CGTCAGGA-TCGG CGTCAGG-TCTGG CTCAGG-TCAGG CTCAGA-TCAG CGTCAGA-TCAG CGTCAGA-TCAG CGTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG	CC-GCACACC GCTCC-GCACAC GCC-GCACAC GCC-GCACAC GCC-GCACAC CCG-CACAC CCG-CACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC TTGTCTC TTGTCTC TTGTCTC TTGTCTC TGTCTCC 	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - TAT-TCTCCG - TATCTCCCG - TATCTCCCG - TATCTCCCG - ATATCTCCCG -	AGGTCCGAC AGGTCAGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCGTACGAG GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC	GGGAGATGCG GGAGATGCG GGAGATGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGATGCG GGGAGTGCG GGGAGTGCG GGGAGTGCG GGGAGTGCG GGGAGTGCG GGGAGTGCG GGGAGTGCG GGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGAGTGCG GGGAGTGCG GGAGGGC A TATCCCCGA- TATCCCCGA- TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. bilinearis</li> <li>M. bilinearis</li> <li>M. productus</li> </ul> Species Species M. senegalensis M. polli M. polli M. peradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bilinearis M. bilinearis M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bilinearis M. bilinearis M. bilinearis M. bilinearis M. bilinearis M. bubbsi M. gayi	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCCG TA-GTCCCCCCCG TA-GTCCCCCCCG TATGACCCCC-G TATGACCCCC-G CATGACCCCC-G CATGACCCCC-G CATGACCCCC-G CC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT	$\label{eq:constraint} \begin{split} & \text{ITTCGCTGACCT-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACTCC-GTT} \\ & \text{ITTCGCCGACTCC-GTT} \\ & \text{ITTCGCCGACTCC-GTT} \\ & \text{ITTCGCCGACTCC-GTT} \\ & \text{ITTCGCCGACTCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITCGCCGACCCC-GTT} \\ & \text{ITCGCCGACCCC-GTT} \\ & \text{ITCGCGGACGCCT-CC} \\ & \text{CCGG-GGAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-C} \\ \\ & \text{CCGG-GGAGGCCCT-C} \\ & \text{CCGG-GGAGGCCCCT-C} \\ & \text{CCGG-CGGAGGCCCCT-C} \\ & \text{CCGG-CGGAGGCCCCT-C} \\ & CCGG-$	GGGCCCTCGCT GGCCC-T-GCT GGCGCCTT-CT- GGCGCC-T-ACT GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GCGCCCCCCCCCC	CC-GCACAC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC GCTCC-GCACAC CCG-CACAC CC-GCACAC 	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - ATATCTCCG - ATAT	AGGTCCGAC AGGTCAGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG 00 3' atacg GGCGTACGA GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC	GGGAATTGCG GGAGATGCG GGAGATGCG GGAGAGTGCG GGGGAATGCG GGGGAATGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGATGCG GGAGATGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGATGCG GGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG AGAGTGCG AGAGTGCG AGAGGGC AGACG AGGGAGTGCG AGAGGCG AGAGGGCG AGAGGGCG AGAGGGCG AGAGGGCG AGAGGGC AGAGGG AGAGGG AGAGGGCG AGAGGGC AGAGGAGTGCG AGAGGGC AGAGGG AGAGGG AGAGGG AGAGGG AGAGGG AGAGGG AGAGGG AGAGG AGAGGG AGAGGG AGAGG AGGG AGAGG AGGG AGAGG AG
<pre>M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bubbsi M. gayi M. productus  Species Species M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. merluccius M. australis M. bilinearis M. hubbsi M. gayi M. productus </pre>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGGCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT	$\label{eq:constraint} \begin{split} & \text{ITTCGCTGACCT-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITCGCCGACCCC-GTT} \\ & \text{ITCGCCGACCCCC-GTT} \\ & \text{ITCGCCGACCCC-GTC} \\ & \text{ITCGCCGACCCC-GTC} \\ & \text{ITCGCCGACCCC-GTC} \\ & \text{ITCGCCGACCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-C} \\ & \text{CCGGG-GGAGGCCCT-C} \\ & \text{CCGGTGGAGGCCCT-C} \\ & \text{CCGGTGGGAGGCCCT-C} \\ &$	GGGCCCTCGCT GGC-TC-T-ACTC GGCGCCTT-CT- GGCGCCCT-CT- GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- CTCAGGA-TCAG CGTCAGGA-TCAG CGTCAGGA-TCAG CGTCAGGA-TCAG CGTCAGGA-TCAG CGTCAGGA-TCAG CCTCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG	CC-GCACACC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC CCG-CACAC CC-GCACAC	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - ATATCTCCG - ATAT	AGGTCCAAGA AGGTCAGAG AGGTACAGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCT-ATCC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC	GGGAAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG 310 ataggggctt TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA
<pre>M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bilinearis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. hubbsi M. gayi M. productus </pre>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT	$\label{eq:constraint} \begin{split} & \text{TTTCGCTGACCCT-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTTTGCTGACTCTTGTT} \\ & \text{TTTGGCCGACCCC-GTT} \\ & \text{TTTCGCCGACCCC-GTT} \\ & \text{TTTCGCCGACCCC-GTT} \\ & \text{TTTCGCCGACCCC-GTT} \\ & \text{TTTCGCCGACCCC-GTT} \\ & \text{TTTCGCTGACCCC-GTT} \\ & \text{TTTCGCCGACCCC-GTT} \\ & \text{TTCGCCGACCCC-GTT} \\ & \text{TTCGCCGG-GAGGCCCT-CC} \\ & \text{CCGG-GAGGGCCCT-CC} \\ & \text{CCGG-GAGGCCCT-CC} \\ & \text{CCGG-GCAGGCCCT-CC} \\ & \text{CCGG-GCCGCCCC-CC} \\ & \text{CCGG-GCCCCC-CC} \\ & \text{CCGG-GCCGCCCCC-CC} \\ & \text{CCGG-GCCGCCCCC-CC} \\ & \text{CCGG-GCCCCC-CC} \\ & \text{CCGG-GCCCCC-CC} \\ & \text{CCGG-GCCCCC-CC} \\ & \text{CCCCC-CCC-CC} \\ & \text{CCGG-GCCCC-CC} \\ & \text{CCGG-GCCCCC-CC} \\ & \text{CCGG-GCCCCC-CC-CC} \\ & \text{CCGG-GCCCCC-CC-CC} \\ & \text{CCGG-GCCCC-CCC-CC} \\ & \text{CCGG-GCCCC-CCC-CC} \\ & \text{CCGCG-GCCCC-CC-CC} \\ & \text{CCGCCCG-GCCCC-CC-CC} \\ & \text{CCGC-GCCCCC-CC-CC} \\ & \text{CCGC-GCCCCC-CC-CC} \\ & \text{CCGC-GCCCC-CC-CC} \\ & \text{CCGCCCCC-CC-CC-CC} \\ & \text{CCCCCCCC-CC-CC-CC} \\ & \text{CCCCCCCC-CC-CC-CC} \\ & \text{CCCCCCCCC-CC-CC-CC} \\ & \text{CCCCCCCC-CC-CC-CC} \\ & \text{CCCCCCCC-CC-CC-CC} \\ & \text{CCCCCCCC-CC-CC-CC} \\ & \text{CCCCCCCC-CC-CC-CC}$	GGGCCCTCGCT- GGCGC-T-GCTG GGCGCCT-CT- GGCGCCCT-CT- GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- 270 CGTCAGGA-TGA CGTCGGG-TCTGG CGTCAGGA-TCAG CGTCAGGA-TCAG CGTCAGGA-TCAG CGTCAGGA-TCAG CGTCAGGA-TCAG CGTCAGGA-TCAG CGTCAGGA-TCAG CCTCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG	CC-GCACACC GCTCC-GCACACA GCTCC-GCACACA GCC-GCACACA CCG-CACACA CCG-CACACA CC-GCACACA CC-GCACACA CC-GCACACA CC-GCACACA 280 2 TTGTCTCC 	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - ATTCTCCG - ATATCTCCG - ATATC	AGGTCCGAC AGGTCAGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG 00 3' atagg GGCATCC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC	GGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGGGAGTGCG GGGGAGTGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG 310 ataggggctt TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> <li>M. gayi</li> <li>M. productus</li> </ul> Species M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bilinearis M. bilinearis M. bilinearis M. bilinearis M. productus	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGAGTGCGT GC-GCAGTGCGT GC-G	$\begin{array}{c} \mathrm{TTCGCTGACTCT-GTT}\\ \mathrm{TTTCGCTGACCCT-GTT}\\ \mathrm{TTTCGCTGACCCC-GTT}\\ \mathrm{TTTCGCTGACTCC-GTT}\\ \mathrm{TTTGGCTGACTCT-GTT}\\ \mathrm{TTTGGCCGACTCC-GTT}\\ \mathrm{TTTCGCCGACTCC-GTT}\\ \mathrm{TTTCGCCGACTCC-GTT}\\ \mathrm{TTTCGCCGACCCC-GTT}\\ \mathrm{TTTCGCTGACCCC-GTT}\\ \mathrm{TTTCGCTGACCCC-GTT}\\ \mathrm{TTCGGCTGACCCC-GTT}\\ \mathrm{TTCGGCTGACCCC-GTT}\\ \mathrm{TCGGCGAGGCCCTACC}\\ \mathrm{CCGG-GGAGGCCCTACC}\\ \mathrm{CCGG-GGAGGCCCT-CC}\\ \mathrm{CCGG-GGAGGCCCT-C}\\ \mathrm{CCGG-GGAGGCCCT-C}\\ \mathrm{CCGG-GGAGGCCCT-C}\\ \mathrm{CCGG-GGAGGCCCT-C}\\ \mathrm{CCGG-GGAGGCCCT-C}\\ \mathrm{CCGG-GGAGGCCCT-C}\\ \mathrm{CCGG-GGAGGCCCT-C}\\ \mathrm{CCGGG-GGAGGCCCT-C}\\ \mathrm{CCGG-GGAGGCCCT-C}\\ \mathrm{CCGG-GGAGGCCCCCCC-C}\\ \mathrm{CCGG-GGAGGCCCCCC-C}\\ \mathrm{CCGG-GGAGGCCCCCC-C}\\ \mathrm{CCGG-GGAGGCCCCC-C}\\ \mathrm{CCGG-GGAGGCCCCC-C}\\ \mathrm{CCGG-GGAGGCCCCC-C}\\ \mathrm{CCGG-GGAGGCCCC-C}\\ \mathrm{CCGG-GGAGGCCCC-C}\\ \mathrm{CCGG-GGAGGCCCC-C}\\ \mathrm{CCGG-GGAGGCCCC-C}\\ \mathrm{CCGG-GGAGGCCCC-C}\\ \mathrm{CCGG-GGAGGCCCC-C-C}\\ \mathrm{CCGG-GGAGGCCC-C-C}\\ \mathrm{CCGGCCC-C}\\ \mathrm{CCGCC-C-C}\\ \mathrm{CCGG-GGAGGCCC-C-C}\\ \mathrm{CCGG-GGCCC-C-C}\\ $	GGGCCCTCGCT GGCCCT-CT-GCT GGCGCCTT-CT- GGCGCCTT-CT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GCGCCCCCGCT- GCGCCCCCGCT- GCGCCCCCGC- CCTCAGA-TCAG CCTCAGG-TCTGG CCTCAGA-TCAG CCTCAGA-TCAG CCTCAGA-TCAG CCTCAGA-TCAG CCTCAGA-TCAG CCTCAGA-TCAG CCTCAGA-TCAG CCTCAGA-TCAG CCTCAG CCTCAG-TCAG CCTCAG	CC-GCACACC GCTCC-GCACAC GCTCC-GCACAC GC-CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC 280 2 TTGTCTCC TGTCTCC TGTCTCC TGTCTCC 	- AT - TCTCCG - AT - TCTCCG - AT - TCTCCG - AT AT - TCTCCG - AT - TCTCCG - AT - TCTCCG - TAT - TCTCCG - TAT - TCTCCG - TATCTCCCG - ATATCTCCCG - ATATCTCCCG - ATATCTCCCG 90 3 - AGAGAA - CCG AGAGAA - CCG	AGGTCCGAC AGGTCAGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCGTACGAG GGCGTATGC	GGGAGATTGCG GGAGATGCG GGAGAGTGCG GGGGAGTGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG 310 ataggggctt TATCCCCGA- TATCCCCGA- TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA
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<pre>M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. australis M. bilinearis M. bilinearis M. hubbsi M. gayi M. productus Species M. senegalensis M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. hubbsi M. gayi M. productus Species M. senegalensis M. senegalensis M. senegalensis M. senegalensis</pre>	TA-GTCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGACCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACCCCC-G CC-GCAGTGCGT GC-GCAGTG	TTTCGCTGACTCT-GTT           TTTCGCTGACCCT-GTT           TTTCGCTGACCCC-GTT           TTTCGCTGACCCC-GTT           TTTCGCCGACCCC-GTT           TTTCGCCGACCCC-GTT           TTTCGCCGACCCC-GTT           TTTCGCCGACCCC-GTT           TTTCGCCGACCCC-GTT           TTTCGCCGACCCC-GTT           TTTCGCCGACCCC-GTT           TTTCGCCGACCCC-GTT           250         260           CCGGC-GAGGCCCT-CC           CAGG-GAAGGCCCT-CC           CCGG-GAAGGCCCT-CC           CCGG-GCAGGCCCT-CC           CCGG-GCGAGGCCCT-CC           0         340           TTSINes2"           AATGTA-CCGCAGCACCCC	GGGGCCTCGGT- GGCGC-T-GCTG GGGCCCT-CT- GGGGCCCT-CT- GGCGCC-T-GCT GGCGCC-TCGCT- GGCGCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- 270 CGTCAGGA-TGA CGTCGGG-TCTGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG	CC-GCACACC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC 	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - ATATCTCCG - ATATCTCCCG - ATATCTCCCG - ATATCTCCCG - ATATCTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	AGGTCCGAC AGGTCAGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG 00 3' atacg GGCATCC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC	GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGGGAATGCG GGGGAATGCG GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG 310 ataggggctt TATCCCCGA
<pre>M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bubbsi M. gayi M. productus  Species M. senegalensis M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. hubbsi M. gayi M. productus  Species M. senegalensis M. senegalensis M. polli M. paradoww </pre>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCCG TA-GTCCCCCCCG TA-GTCCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGAGTGCGT GGTGCAGTGCGT GC-GCAGTGCGC GC-GCAGTGCGT GC-GCAGTGCGC GC-GCAGTGCGT GC-GCAGTGCGC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC GC-GCAGTGCC G	TTTCGCTGACCT-GTT         TTTCGCTGACCC-GTT         TTTCGCTGACCCC-GTT         TTTCGCTGACCCC-GTT         TTTCGCCGACCC-GTT         TTTCGCCGACTCT-GTT         TTTCGCCGACTCC-GTT         TTTCGCCGACCCC-GTT         TTTCGCCGACCCC-GTT         TTTCGCCGACCCC-GTT         TTTCGCCGACCCC-GTT         TTTCGCCGACCCC-GTT         250       260         CCGG-GGAGGCCCT-CC         CCGG-GGAGGCCCT-CC         CCGG-GGAGGCCCT-C         0       340         TTSINes2"         ATGTAA-GCGCAGCGCCC	GGGCCCTCGCT GGCCCT-GCT GGCCCT-CT-GCT GGCGCCTT-CT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GCGCCCCCGCT- GCGCCCCCGC- CGTCAGG-TCTGG CGTCAGG-TCTGG CCTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAGA-TCAGG C-TCAGC-TCAGG C-TCAGGCTCG-AC	CC-GCACACC GCTCC-GCACAC GCTCC-GCACAC GC-CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TTGTCTCC TGTCTCC TGTCTCC 	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - TAT-TCTCCG - TAT-TCTCCG - TATCTCCCG - TATCTCCCG - ATATCTCCCG - ATATCTCCCG 90 3 - AGAGAA-CCG	AGGTCCGAC AGGTCAGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCGTACGAG GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTACGC	GGGGAATTGCG GGAGATGCG GGAGATGCG GGAGAGTGCG GGGGAATGCG GGGGAATGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG A10 ataggggctt TATCCCCGA- TATCCCCGA- TATCCCCGAA
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<pre>M. senegalensis M. polli paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bubbsi M. gayi M. productus  Species M. senegalensis M. polli M. paradoxus M. hernandezi M. bilinearis M. bubbsi M. gayi M. productus  Species Species Species M. senegalensis M. hubbsi M. gayi M. productus  Species M. senegalensis M. hubbsi M. gayi M. productus  Species M. senegalensis M. polli M. paradoxus M. hernandezi M. her</pre>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G Z40 GC-GCAGTGCGT GC-GCAGTGCCGC GC-GCAGTGCGC GC-GCAGTGCGC GC-GCAGTGCGC GC-GCAGTGCGC GC-GCAGTGCCGC GC-GCAGTGCCGC GC-GCAGTGCCGC GC-GCAGTGCCGC GC-GCAGTGCCGC GC-GCAGTGCCGC GC-GCAGTGCCC GC-GCAGTGCCCCCCGC GC-GCAGTGCCC GC-GCAGTGCCC GC-GCAGTGCCC GC-GCAGTGCCCC	$\label{eq:constraint} \begin{split} & \text{ITTCGCTGACCT-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITCGCCGACCCC-GTT} \\ & \text{ITCGCCGACCCC-GTC} \\ & \text{ITCCGCGG-GAGGCCCT-CC} \\ & \text{CCGG-GGAGGCCCT-C} \\ & \text{ITSINes2"} \\ & \text{ATGTAA-GCGCGGCGC} \\ & \text{ACCCA-GCTCACGCGC} \\ & \text{ACCCA-GCTCACGCGC} \\ & \text{ACCCA-GCTCACGCGC} \\ & \text{ACCCA-GCTCACGCCC} \\ & \text{ACCCA-GCTCACCCCC} \\ & \text{ACCCA-GCTCACCCCC} \\ & \text{ACCCA-GCTCACCCC} \\ & \text{ACCCA-GCTCACCCCC} \\ & \text{ACCCA-GCTCACCCCCCC} \\ & ACCCA-GCTCACCCCCCCCCCCCCCCCCCCCCCCCCCCCC$	GGGGCCTCGGT GGC3C-T-GCTG GGC3C-T-GCT GGC3CCTT-CT- GGC3CC-T-GCT GGC3CC-TCGCT- GGC3CCTCGCT- GGC3CCCTCGCT- GGC3CCCTCGCT- 270 CGTCAGGA-TGA CGTCAGGA-TGA CGTCAGGA-TCTAG CGTCAGGA-TCTAG CGTCAGGA-TCAGG C-TCAGC-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAG-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGG-TCAGG C-TCAGC C-TCAGC-TCAGG C-TCAGC	CC-GCACACC GCTCCC-GCACAC GCTCC-GCACAC GCC-GCACAC 	- AT - TCTCCG - TAT - TCTCCG - TAT - TCTCCG - TAT - TCTCCG - AT - TCTCCG AGAGAA - CCG AGAGAA - CCG - TTGCGCT - TTGCG-TC - TTGCG-TC - TTGCG-TC - TCCC - TCCC - TCCC	AGGTCCGAC AGGTCAGAG AGGTACAGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCCTACGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTACCTG GCCGCCCTG GCCGCCCTG GCCGCCCTG GCCGCCCTG GCCGCCCTG GCCGCCCCG	GGGGAATIGCG GGAGATGCG GGAGATGCG GGAGATGCG GGGGAATGCG GGGGAATGCG GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGAGTGCG 310 ataggggctt TATCCCCGA- TATCCCCGA- TATCCCCGAA
<pre>M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bilinearis M. productus  Species M. senegalensis M. merluccius M. australis M. hubbsi M. capensis M. merluccius M. australis M. bilinearis M. hubbsi M. gayi M. productus  Species Species M. senegalensis M. polli M. senegalensis M. polli M. paradoxus M. hernandezi M. hernandezi M. hernandezi M. polli M. gayi M. productus </pre>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGACCCC-G CAGACCCC-G CC-GCAGTGCGT GC-GCAGTGCCGA GC-GCAGTGCCCGA GC-GCAGTGCCCCA GC-GCAGTGCCCGA GC-GCAG	$\label{eq:constraint} \begin{split} & \text{TTTCGCTGACCT-GTT}\\ & \text{TTTCGCTGACCCC-GTT}\\ & \text{TTTCGCTGACCCC-GTT}\\ & \text{TTTCGCTGACCCC-GTT}\\ & \text{TTTCGCCGACCCC-GTT}\\ & \text{TTTCGCCGACCCC-GTT}\\ & \text{TTTCGCCGACCCC-GTT}\\ & \text{TTTCGCCGACCCC-GTT}\\ & \text{TTTCGCCGACCCC-GTT}\\ & \text{TTTCGCTGACCCC-GTT}\\ & \text{TTTCGCTGACCCC-GTT}\\ & \text{TTCGCCGACCCC-GTT}\\ & \text{CCGG-GAGGCCCT-CC}\\ & \text{CCGG-GAAGGCCCT-CC}\\ & \text{CCGG-GAAGGCCCT-CC}\\ & \text{CCGG-GAAGGCCCT-CC}\\ & \text{CCGG-GGAGGCCCT-CC}\\ & \text{CCGG-GGAGGCCCCC-CC}\\ & \text{CCGG-GGAGGCCCCC-CC}\\ & \text{CCGG-GGAGGCCCCC-CC}\\ & \text{CCGG-GGAGGCCCCC-CC}\\ & \text{CCGG-GGAGGCCCCC-CC}\\ & \text{CCGG-GGAGGCCCC-CC}\\ & CCGG$	GGGGCCTCGCT GGCGC-T-GCT GGCGCCT-CT- GGCGCCT-CT- GGCGCCT-CT- GGCGCCTCGCT- GGCGCCTCGCT- GGCGCCTCGCT- GGCGCCTCGCT- GGCGCCTCGCT- GGCGCCTCGCT- GGCGCCCTCGCT- CTCAG-TCAGG CTCAGG-TCTGG CTCAGG-TCAGG CTCAG-TCAGG CTCAG-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCAGC-TCAGG C-TCCGGTCTCT-AC GCCGGCCTCT-AC GCCGGCTCT-AC	CC-GCACACC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC 	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - ATATCTCCG - TTGCGCTC - TTGCGCTC - TTGCGCTC	AGGTCCGAC AGGTCCGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCCTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTACCTG GCCGCCCTG GCCGCCCTG GCCGCCCTG GCCGCCCTG	GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGGGAATGCG GGGGAGTGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG 310 ataggggctt TATCCCCGA TATCCCCCCC CCGGTACCC CGGGTACCC CGGGTACCC
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> <li>M. gayi</li> <li>M. productus</li> </ul> Species <ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. dubbsi</li> <li>M. australis</li> <li>M. hernandezi</li> <li>M. jaryi</li> <li>M. productus</li> </ul> Species <ul> <li>M. senegalensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> <li>M. gayi</li> <li>M. productus</li> </ul> Species <ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> </ul>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCCG TA-GTCCCCCCCG TA-GTCCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGACGCCC-G CAGACGCCC-G CAGACGCCC-G CC-GCAGTGCGT GC-GCAGTGCCGA GC-GCAGTGCCCGA GC-GCAGTGCCCGA GC-GCAGTGCCCGA GC-GCAGTGCCCGA GC-GCAGTGCC	ITTCGCTGACCT-GTT           ITTCGCTGACCCT-GTT           ITTCGCTGACCCC-GTT           ITTCGCTGACCCC-GTT           ITTCGCCGACCCT-GTT           ITTCGCCGACCCC-GTT           ITTCGCCGACCCC-GTT           ITTCGCCGACCCC-GTT           ITTCGCCGACCCC-GTT           ITTCGCCGACCCC-GTT           ITTCGCCGACCCC-GTT           ITTCGCCGACCCC-GTT           ITTCGCCGACCCC-GTT           ITTCGCCGACCCC-GTT           250         260           CCGG-GGAGGCCCT-CC           CCGG-GGAGGCCCTACC           CCGG-GGAGGCCCT-C           O           340           ITSINes2"           AATGTAA-GCCCAGCGCGC           AATGTAA, CCCCAGCCC	GGGCCCTCGCT GGCCC-T-ACTC GGCCC-T-ACTC GGCCCCT-CT- GGCCCCTCGCT- GGCCCCTCGCT- GGCCCCTCGCT- GGCCCCTCGCT- GGCCCCTCGCT- GCCCCCCCGCT- GCCCCCCCGCT- GCCCCCCCGC- CTCAG-TCTGC CCTCAG-TCTGC CCTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAG-TCAGC CTCAGC-TCAGC CTCAGCTCC-AC TCCGGTCTCC-AC TCCGGTCTCT-AC GCCGCCCCCCCC	CC-GCACAC GCTCC-GCACAC GCTCC-GCACAC GCTCC-GCACAC GCTCC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC 280 2 TTGTCTC TTGTCTCC TTGTCTCC 	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - TAT-TCTCCG - TAT-TCTCCG - TATCTCCCG - TATCTCCCG - ATATCTCCCG - TTGCGCTC- - TTGCGCTC- - TTGCGCTC- - TTGCCGCT-	AGGTCCGAC AGGTCCGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCGTACGAG GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GCGTGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG	GGGGAATGCG GGAGATGCG GGAGATGCG GGAGAGTGCG GGGGAATGCG GGGGAATGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG 310 ataggggctt TATCCCCGA CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC
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<pre>M. senegalensis M. polli paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bubbsi M. gayi M. productus  Species M. senegalensis M. merluccius M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bilinearis M. bubbsi M. gayi M. productus  Species Species M. senegalensis M. senegalensis M. polli M. paradoxus M. hernandezi M. hernandez</pre>	TA-GTCCCCCCG          GTCCC-CCG          GTCCC-CG           TATGACCCCC-G           Z40           GC-GCAGTGCGT           GC-GCAGTGCGT </td <td><math display="block">\label{eq:constraint} \begin{tabular}{lllllllllllllllllllllllllllllllllll</math></td> <td>GGGGCCTCGGT GGC3C-T-GCTG GGC3C-T-GCT GGC3CCTT-CT- GGC3CCTT-CT- GGC3CCTCGCT- GGC3CCTCGCT- GGC3CCTCGCT- GGC3CCCTCGCT- 270 CGTCAGGA-TGA CGTCAGGA-TGA CGTCAGGA-TCTGG C-TCAG-TCTAG CGTCAGGA-TCTAG CGTCAGGA-TCAG C-TCAG-TCAGG C-TCAGCTCG-AC GCCGGCTCG-AC GCCGGCTCG-AC</td> <td>CC-GCACAC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC </td> <td>- AT - TCTCCG - TAT - TCTCCG - TAT - TCTCCG - AT - TCTCCG AGAGAA - CCG AGAGAA - CCG - CG - TCGCG - TC - TTGCGCT - - TTGCGCT -</td> <td>AGGTCCGAC AGGTCCGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCT-ATCC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG</td> <td>GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGGGAATGCG GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG A 310 ataggggctt TATCCCCGAA CCGGTACCC - CGGGTACCC - CGGGTACCC - CGGGTACCC - CGGGTACCC</td>	$\label{eq:constraint} \begin{tabular}{lllllllllllllllllllllllllllllllllll$	GGGGCCTCGGT GGC3C-T-GCTG GGC3C-T-GCT GGC3CCTT-CT- GGC3CCTT-CT- GGC3CCTCGCT- GGC3CCTCGCT- GGC3CCTCGCT- GGC3CCCTCGCT- 270 CGTCAGGA-TGA CGTCAGGA-TGA CGTCAGGA-TCTGG C-TCAG-TCTAG CGTCAGGA-TCTAG CGTCAGGA-TCAG C-TCAG-TCAGG C-TCAGCTCG-AC GCCGGCTCG-AC GCCGGCTCG-AC	CC-GCACAC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC 	- AT - TCTCCG - TAT - TCTCCG - TAT - TCTCCG - AT - TCTCCG AGAGAA - CCG AGAGAA - CCG - CG - TCGCG - TC - TTGCGCT - - TTGCGCT -	AGGTCCGAC AGGTCCGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCT-ATCC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG	GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGGGAATGCG GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG A 310 ataggggctt TATCCCCGAA CCGGTACCC - CGGGTACCC - CGGGTACCC - CGGGTACCC - CGGGTACCC
<pre>M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. australis M. bilinearis M. bilinearis M. poluctus Species M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. poductus Species M. senegalensis M. poluctus Species M. senegalensis M. polli M. poluctus Species M. senegalensis M. hernandezi M. hernandezi M. hernandezi M. albidus M. capensis M. polli M. polli M. paradoxus M. hernandezi M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bilinearis</pre>	TA-GTCCCCCCG GTCCC-CCG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGACCCC-G CCCCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT GC-GCAGTGCGT TGCTCACCGA ATTGTCCACCGA ATTGCTCACCGA ATTGCTCACCGA ACTGTCCCCCGA	$\label{eq:constraint} \begin{split} & \text{ITTCGCTGACCT-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITTCGCTGACTCTTGTT} \\ & \text{ITTCGCCGACTCC-GTT} \\ & \text{ITTCGCCGACTCC-GTT} \\ & \text{ITTCGCCGACCCC-GTT} \\ & \text{ITTCGCTGACCCC-GTT} \\ & \text{ITCGCCGACCCC-GTT} \\ & \text{ITCGCGGAGCCCT-CC} \\ & \text{CCGG-GGAGGCCCTACC} \\ & \text{CCGG-GGAGGCCCTACC} \\ & \text{CCGG-GGAGGCCCT-C} \\ & \text{ATGTAA-GCGCGGGCG} \\ & \text{ATGTAA-GCCCAGCGC} \\ & \text{ATGTAA-GCCCAGCGCC} \\ & \text{ACCAA-GCCCAGCGC} \\ & \text{ACCAA-GCCCAGCGCC} \\ & \text{ACCAA-GCCCAGCGCC} \\ & \text{ACCAA-GCCCAGCGC} \\ & \text{ACCAA-GCCCAGCGC} \\ & \text{ACCAA-GCCCAGCGCC} \\ & \text{ACCAA-GCCCAGCGCC} \\ & \text{ACCAA-GCCCAGCGC} \\ & \text{ACCAA-GCCCAGCGC} \\ & \text{ACCAA-GCCCAGCGC} \\ & \text{ACCAA-GCCCAGCGCC} \\ & ACCAA-GCCCAGCCCCCCCCCCCCCCCCCCCCCCCCCCC$	GGGGCCTCGCT GGCGC-T-GCT GGCGCCT-CT- GGCGCCT-CT- GGCGCCTCGCT- GGCGCCTCGCT- GGCGCCTCGCT- GGCGCCTCGCT- GGCGCCTCGCT- GGCGCCTCGCT- GGCGCCTCGCT- GGCGCCTCGCT- CTCAG-TCAGG CTCAGG-TCTGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAGC-TCAGG CTCAGC-TCAGG CTCAGCTCCAC CCGGCCTCC-AC GCCGGCCTCT-AC GCCGGCCTCT-AC GCCGGCCTCT-AC GCCGGCCTCT-AC	CC-GCACACC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC CC-GCACAC TTGTCTC TTGTCTC TTGTCTC TTGTCTC TTGTCTC TTGTCTC TTGTCTC TGTCTC TGTCTC TGTCTC TGTCTC TGTCTC TGTCTC TGTCTC TGTCTC TGTCTC TGTCTC TGTCTC TGTCTC 	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - ATATCTCCG - ATATCTCCG - ATATCTCCCG - ATACCCCG - ATACCCCG - ATACCCCG - ATACCCCG - ATACCCCG - ATACCCCG - AGAGAA - CCG AGAGAA - CCG AGAGAA - CCG AGAGAA - CCG AGAGAA - CCG - TTGCGCT- - TTGCGCT- - TTGCGCT- - TTGCGCT- - TTGCGCT- - TTGCGCT- - TTGCGCT- - TTGCGCT-	AGGTCCGAC AGGTCCGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCGTACGAG GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GCGTGCCTG GCGTGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG	GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGGGAGTGCG GGGGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG GGAGAGTGCG 310 ataggggctt TATCCCCGA- TATCCCCGA- TATCCCCGA- TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA TATCCCCGAA CCGGTACCC - CGGGTACCC - CGGGTACCC - CGGGTACCC - CGGGTACCC - CGGGTACCC - CGGGTACCC - CGGGTACCC - CGGGTACCC
<ul> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> <li>M. gayi</li> <li>M. productus</li> </ul> Species M. senegalensis <ul> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> </ul> Species M. senegalensis <ul> <li>M. merluccius</li> <li>M. australis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> <li>M. gayi</li> <li>M. productus</li> </ul> Species M. senegalensis <ul> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. senegalensis</li> <li>M. polli</li> <li>M. paradoxus</li> <li>M. hernandezi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. merluccius</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. hubbsi</li> <li>M. albidus</li> <li>M. capensis</li> <li>M. hublai</li> <li>M. bilinearis</li> <li>M. bilinearis</li> <li>M. hubbsi</li> </ul>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCCG TA-GTCCCCCCCG TA-GTCCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G CAGACCCCC-G CAGACCCCC-G CAGACGCCCCCG CC-GCAGTGCGT GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCCGA GC-GCAGTGCCCCGA GC-GCAGTGCCCCCACCGA GC-GCAGTGCCCCCCCGA GC-GCAGTGCCCCCACCGA GC-GCAG	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	GGGGCCTCGGT GGCCCT-GCT GGCGC-T-ACT GGCGCCTT-CT- GGCGCC-T-ACT GGCGCC-TCGCT- GGCGCC-TCGCT- GGCGCCTCGCT- GGCGCCCTCGCT- GGCGCCCTCGCT- GCGCCCCCGCT- GGCGCCCTCGCT- CGTCAGG-TCTGG CGTCAGG-TCTGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG CTCAG-TCAGG GCTGGCCTCG-AC GCCGGCCTCT-AC GCCGGCCTCT-AC GCCGGCCTCT-AC GCCGGCCTCT-AC GCCGGCCTCT-AC	CC-GCACACC GCTCC-GCACAC GCTCC-GCACAC GCTCC-GCACAC 	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - TAT-TCTCCG - TATCTCCG - TATCTCCG - ATATCTCCG - TTGCGCT- - TTGCGCT- - TTGCGCT- - TCGCGCT- - TCGCCCT- - TCGCGCT- - TCGCGCT- - TCGCCCT- - TCGCCCT- - TCGCCCT- - TCGCCCCC- - TCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	AGGTCCGAC AGGTCCGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG GGCGTACGAG GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GCGTGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTA	GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGGGAATGCG GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGACGCG GGAGACGCG GGAGACGCG GGAGACGCG A 10 ataggggctt TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC
<pre>M. senegalensis M. polli paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bubbsi M. gayi M. productus  Species M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bilinearis M. hubbsi M. gayi M. productus  Species M. senegalensis M. hernandezi M. hubbsi M. gayi M. productus  M. albidus M. capensis M. merluccius M. albidus M. data albidus M. data albidus M. data albidus M. hernandezi M. her</pre>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G 240 GC-GCAGTGCGT GC-GCAGTGCGA GC-GCAGTGCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCCGA GC-GCAGTGCACCGA GC-GCAG	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	GGGGCCTCGGT GGCCCT-T-ACTC GGCGCCTT-CT GGCGCCCT-CT GGCGCCCTCGCT GGCGCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT GGCGCCCTCGCT CTCAGA-TCAG CTCAGGA-TCTGG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGA-TCAG CTCAGCTCCAA GCCGGCCTCT-AC GCCGGCCTCT-AC GCCGGCCTCT-AC GCCGGCCTCT-AC	CC-GCACACC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC CC-GCCACAC CC-GCCCCC CCGCC CCGGCC CCGGCC CCGGCC CCGGCC	- AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - AT-TCTCCG - ATATCTCCG - AGAGAA - CCG AGAGAA - CCG AGAGAA - CCG AGAGAA - CCG AGAGAA - CCG AGAGAA - CCG - AGAGAA - CCG - AGAGAA - CCG - AGAGAA - CCG - AGAGAA - CCG - TTGCGCT - TTGCGCT - TTGCGCT - TTGCGCT - TTGCGCT - TTGCGCT - TTGCGCT - TTGCGCT	AGGTCCGAC AGGTCAGAG AGGTACAGAG AGGTACGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCCTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GCGTACCTG GCGTGCCTG GCGTGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG GCGCCCCTG	GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG A 10 ataggggctt TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA TATCCCCGA CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC -CGGGTACCC
<pre>M. senegalensis M. polli M. paradoxus M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bubbsi M. gayi M. productus Species M. senegalensis M. hernandezi M. albidus M. capensis M. merluccius M. australis M. bilinearis M. bilinearis M. bilinearis M. polli M. gayi M. productus Species M. senegalensis M. hernandezi M. hubbsi M. gayi M. productus</pre>	TA-GTCCCCCCG GTCCC-ACG TATGACCCCC-G TATGACCCCC-G TA-GTCCCCCCG TA-GTCCCCCCG TA-GTCCCCCCG TATGACCCCC-G TATGACCCCC-G TATGACCCCC-G Z40 GC-GCAGTGCGT GC-GCAGTGCG	$\label{eq:constraint} \begin{tabular}{lllllllllllllllllllllllllllllllllll$	GGGGCCTCGGT GGC3C-T-GCTG GGC3C-T-GCT GGC3CCTT-CT- GGC3CCTCGCT- GGC3CCTCGCT- GGC3CCTCGCT- GGC3CCCTCGCT- GGC3CCCTCGCT- GGC3CCCTCGCT- GGC3CCCTCGCT- CTCAGGTCTGG CTCAGG-TCTGG CTCAGG-TCTGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGG-TCAGG CTCAGGCTCC-AC TCCGGTCTCT-AC GCC3GCCTCT-AC GCC3GCCTCT-AC GCC3GCCTCT-AC	CC-GCACAC GCTCC-GCACAC GCTCC-GCACAC GCC-GCACAC CC-GCC-CCAC CC-GCC-CCC CCGCC-C	- AT - TCTCCG - AGAGAA - CCG AGAGAA - CCG - TGCGCT TTGCGCT - TTGCGCT - TTGCGCT - TTGCGCT - TTGCGCT - TTGCGCT - TTGCGCT -	AGGTCCGAC AGGTCAGAG AGGTACAGAG AGGTACGAG AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAC AGGTACGAG AGGTACGAG AGGTACGAG AGGTACGAG GGCTACGAG GGCGTACGAG GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GGCGTATGC GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG GCGCGCCTG	GGGGAATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGGGAATGCG GGGGAGTGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG GGAGATGCG 310 ataggggctt TATCCCCGA TATCCCCCA TATCCCCCCA TATCCCCCA TATCCCCCA TATCCCCCA TATCCCCCA TATCCCCCA TATCCCCCA TATCCCCCA TATCCCCCA TATCCCCCA TATCCCCCA TATCCCCCA TATCCCCCA TATCCCCCA TATCCCCCCCC

**Species** M. senegalensis M. polli

J. Aaric.	Food	Chem.	Vol.	53.	No.	13.	2005
J. Ayrıc.	1000	onem.,	v 01.	55,	140.	10,	2000

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M paradoxus	TTCCACACT	~TCCGGGTCT	GCTTACGCAC	adecadadaa	GGGGTTCAA	TGACCGCG-GT	GT-CACGGG	CTTCGGTCAC	GTGGT
M. hernandezi	TGTCACACT	CTCCGGGTCT	CG-TACGCAC	GCCGGGGGA	TGGGTTCAA	TGACCGCG-GT	GT-CACGGG	CTTCGGTCAC	GTGGT
M. albidus	TGTCACACT	TCCGGGTCT	CG-TACGCAC	GCCGGGGGA	GGGGTTCAA	TGACCGCG-GT	GT-CACGGG	CTTCGGTCAC	GTGGT
M. capensis	TGCCACACT	CTCCGCGTCT	GCATACGCAC	GCGGGGGGA	GGGGTTCAA	TGACCGCG-GT	GT-CACGGG	CTTCGGTCAC	GTGGT
M. merluccius	TGCCACACT'	TCCGCGTCT	GCTTACGCA	GCGGGGGGG	GGGGTTCAA	TGACCGCG-GT	GT-CACGGG	CTTCGGTCAC	GTGGT
M. australis	TGTCACACT	CTCCGGGTCT	GCTTACGCA	GCCGGGGGA	GGGGTTCAA	TGACCGCG-GT	GT-CACGGG	CTTCGGTCAC	GTGGT
M hilinearis	TGTCACACT	TCCGGGTCT	GCTTACGCA	GCCGGGGGA	GGGGTTCAA	TGACCGCG-GT	GT-CACGGG	CTTCGGTCAC	GTGGT
M kubbai	TGTCACACT	TTCCGGGTCT	CCTTACCCA	CCCCCCCCCCCC	GGGGGTTCAA	TGACCGCGAAC	CATACACCCC	CTTCGGTCAC	GTGGT
M gavi	TGTCACACT	TCCGGGTCT	GCTTACGCAC	GCCCGGGGG	TGGGTTCAA	TGACCGCG-GT	GT-CACGGG	CTTCGGTCAC	GTGGT
M. productus	TGTCACACT	CTCCGGGGTCI	GCTTACGCA	GCCGGGGGGA	TGGGTTCAA	TGACCGCG-GT(	GT-CACGGG	CTTCGGTCAC	GTGGT
	490	500	510	520	530	540		560	
Species	150	500	510	520	550	540	200	200	
M. senegalensis	CCAGCGCGG	GCCCGTAAG	ютс т <u>л</u>	rgtC-AA	AAA		CCCTC-A	ATCGTCGAAG	CTTTG
M polli	CCAGCGCGG	GCCCGTAAG	юто катт	rgtcca-		GTTGATAA	AAACCCTC-A	ATCGTCGAAC	CTTTG
M paradoxus	CCAGCGCGG	GCCCGTAAG	GTT	гот С-АА		GTTGA-AA	AA - CCCTCCA	ATCATCGAAAA	CTTTG
M bernandezi	CCAGCGCGG	CCCCCGTCAG	33TT	rammamera a			AA - CC - ATTG	STCGTCGAAC	CTTTG
M albidus	CCAGCGCGG	GCCCGTCAG	39776-07777 197779-07777	CGTTGTC-AA	A		CC-ATTG	ATCGTCGAAC	CTTTG
M capensis	CCAGCGCGG	GCCCGTAAG	GTCT	CGTC-AA			CCCTC-A	ATCGTCGAAG	CTTTG
M. merluccius	CCAGCGCGG	GCCCGTAAG	GTCT1	rgtC-AA		GTTGA-AA	AA-CCCTC-A	ATCATCGAAG	CTTTG
M australis	CCAGCGCGG	GCCCGTCAG	KTT			Ai	AA-CC-ATTG	ATCGTCGAAC	CTTTG
M. bilinearis	CCAGCGCGG	GCCCGTCAG	GTT	rgtC-AA	ACCCCCC	CCCGA	AA-CC-TTTG	ATCGTCGAAG	CTTTG
M hubbsi	CCGGCGCGG	GCCCGTCCG	KITTATCTGT1	CATTOTC-AA	ACC		TTTG	ATCGTCGAAC	CTTTG
M gavi	CCAGCGCGG	GCCCGTCCG	GTT	CGTTGTCCAA		A	AA-CC-ATTG	ATCGTCGAAC	CTTTG
M productus	CCAGCGCGG	GCCCGTCAG	GTT	CGTTGTCCAA		Ai	AA - CC - ATTG	ATCGTCGAAC	CTTTG
III producedo									01110
	570	580	590	600	610	620	630	640	
Species			←	— ITS1-r	DNA-3 '				
M. senegalensis	TCC-AAAAA	CCAAA	CC-AAA	AAAAC	AAATA				
M. polli	TCC-AAA	CCAAACAA	CAAA-	CCAAAC	AAATA				
M. paradoxus	TCC-AAA	CCAAAAA	CC-AAA-	C-AAA-	AA-TA				
M. hernandezi	TCC-AAAA-	CCAAACAA	AAACAAAA	AAG	AAATA				
M. albidus	TCC-AAAA-	CCAAACAA	AAACC-AAA	4A0	AAATA				
M. capensis	TCC-AAAAA	CCAAA	CCCAAAA	AAC	AAATA				
M. merluccius	TCC-AAAAA	CCAAAA	CC-AAA-	C	AAATA				
M. australis	TCC-AAAA-	CCAAACAA	AAACC-AAAA	4	AAATA				
M. bilinearis	TCC-AAAA-	CCAAACAA	AAACC-AAAA	4C	AAATA				
M. hubbsi	TCCCAAA(	CCAAAA-CAA	ACAAAA	¥	TA				
M. gavi	TCC-AAAA-	CCAAACAA	AAACAAAA	4@	AAATA				
M. productus	TCC-AAAA-	CCAAACAA	AAAC AAAA	4AG	AAATA				
	650 61	50 6			690				

Figure 1. Alignment of ITS1-rDNA sequences from 12 hake species. The nested PCR primers *MerITS1Nes1* and *MerITS1Nes2* used to detect the presence of DNA from *Merluccius* spp. and *G. morhua* (GenBank accession number AY323948) are shown in bold above their annealing positions.

Table 2. Restriction Fragments (in bp) Obtained after Digestion of PCR-ITS1 Products from *Merluccius* spp. and *G. morhua* with the Enzymes Afa I, Nar I, M/u NI, and Mn/ I<sup>a</sup>

		Afa I		Narl		<i>Mlu</i> N	11	Mn/ I		
hake	ITS1	fragment		fragment		fragment		fragment		
species	length	sizes	type	sizes	type	sizes	type	sizes	type	pattern
M. merluccius	629	189, 440	А	92, 265, 272	А	629	А	5, 8, 22, 26, 36, 47, 61, 67, 74, 79, 84, 120	А	A <b>A</b> AA
M. senegalensis	618	186, 432	А	89, 255, 274	В	618	Α	5, 8, 22, 26, 36, 47, 61, 67, 74, 77, 80, 115	А	ABAA
M. polli	659	27, 166, 466	В	97, 562	С	659	Α	7, 16, 30, 31, 55, 65, 84, 91, 112, 168	В	B <b>C</b> AB
M. capensis	614	185, 429	А	88, 526	С	614	Α	8, 26, 36, 38, 63, 74, 78, 85, 91, 115	А	ACAA
M. paradoxus	630	190, 440	А	93, 537	D	630	Α	7, 27, 29, 55, 63, 83, 89, 113, 164	В	ADAB
M. productus	603	156, 193, 254	С	96, 226, 281	Е	603	Α	5, 7, 23, 25, 29, 30, 69, 86, 106, 223	С	CEAC
M. gayi	604	158, 192, 254	С	95, 226, 283	Е	216, 388	В	5, 7, 24, 30, 30, 69, 106, 110, 223	С	CE <b>BC</b>
M. australis	610	155, 194, 261	С	97, 234, 279	F	610	Α	5, 7, 22, 26, 29, 31, 70, 74, 85, 111, 150	D	CFAD
M. hubbsi	602	65, 125, 158, 254	D	96, 227, 284	Е	602	Α	5, 7, 22, 23, 28, 30, 70, 75, 86, 110, 146	D	DEAD
M. albidus	610	193, 417	А	97, 239, 274	F	610	Α	5, 24, 28, 29, 38, 59, 112, 150, 155	Е	AFAE
M. hernandezi	602	156, 192, 254	С	96, 226, 280	Е	214, 388	В	5, 7, 30, 69, 106, 164, 221	F	CEBF
M. bilinearis	607	76, 123, 194, 214	Е	97, 510	Н	219, 388	В	5, 28, 29, 32, 40, 59, 64, 98, 102, 150	D	EHBD
G. morhua	605	72, 173, 360	F	88, 517	I	605	А	35, 45, 98, 113, 156, 158	G	FIAG

<sup>a</sup> The fragment sizes shown are exact as determined by sequencing. Bold letters indicate the restriction pattern of the species identified with that enzyme.

species with four restriction enzymes is given in **Table 3**. All conspecific samples presented identical restriction patterns. The consensus tree that summarizes the pool of 110 parsimonious trees recovered showed a full nodal resolution (100%) for each species' cluster (**Figure 5**).

ITS1-rDNA-Based Methodology

The phylogenetic reconstruction using either the ITS1 amplicon or the ITS1 nested fragment of 193 bp of 10-45 individuals per species (FINS) showed an unambiguous grouping of all test samples in their expected cluster defined by samples types. The same full species assignment (data not shown) was obtained using the BLAST engine to match test samples to GenBank entries (**Figure 1**) of *Merluccius* spp.

#### DISCUSSION

The *a priori* morphological identification of the sample types used to calibrate any molecular identification method is indispensable for the creation of a reliable key (*33*). Therefore, all specimens were assigned to 1 of 12 well-recognized hake species. The exceptions were *M. hernandezi* and *M. albidus*, from which no entire specimens were available. Tissue samples from these two species were identified following four criteria, (i) the records of the trawling surveys during which they were captured, (ii) the latitude and longitude describing the exact oceanographic catching point, (iii) previous allozyme data





Figure 2. Agarose gel showing restriction fragments (in bp) from digestion of the ITS1–PCR product of hakes with Afa I (top panel), Nar I (middle panel; here, the third lane corresponds to *M. polli* and the fourth to a *M. polli/M. senegalensis* hybrid individual included only in this panel), and M/u NI (bottom panel). The first lane corresponds to the molecular marker pGEM (Promega) and the remaining lanes are identified using the codes of the species described in **Table 1**. The exact fragment sizes are given in **Table 2**.



**Figure 3.** Agarose gel showing the restriction fragments (in bp) from digestion of the ITS1–PCR product of *G. morhua* with Afa I, Nar I, and *Mlu* NI. The first lane corresponds to the molecular marker pGEM (Promega). Exact fragment sizes are given in **Table 2**.

obtained on the same individuals of *M. albidus* (M. Roldán, personal communication), and (iv) the morphology of the scales of *M. hernandezi* that serves as unambiguously differentiating this species from the neighboring hake *M. angustimanus* (C. P. Mathews, personal communication). Although the 10 species of commercial relevance are included in this study, there are two additional species from this genus which were not obtainable for this study, i.e., *M. angustimanus* or Panama hake, distributed from Baja California to Colombia (*34*), and the recently discovered *M. patagonicus* (2). Previous studies have attempted to differentiate 11 hake species using a PCR–RFLP



Figure 4. Agarose gel showing the 164-bp fragment that distinguishes *M. gayi* (GA) from *M. hernandezi* (HE), after digestion of their ITS1–PCR products with *MnI* I. GM, *G. morhua*; ITS1, undigested ITS1 sequence of *M. gayi*; pGEM, molecular marker (Promega).

protocol that used four and seven enzymes to digest the left domain of the mitochondrial DNA control region (12) and a cytochrome b gene fragment (13), respectively. While both methods perform well at identifying some species, they are devoid of several indispensable properties, i.e., (1) a full genetic discrimination between the nine species of hake analyzed (see Table 2 of ref 13), (2) an *a priori* morphological identification of species and origin of the sample types analyzed, (3) an assessment of the usual intraspecific variation of mtDNA markers (14), which might lead to sample misidentifications, (4) a sufficient number of sample types from the distribution range of the species, and (5) a final validation step on the large number of individuals per species.

Table 3. Ma	trix o	PI PI	resen	ce and A	bsence	e of R	testric	tion F	Fragm	ients (	(in bp	) Obta	ined v	with F	our En:	zyme	s on t	ne ITS1	-rDNA	Am	plicor	of H	akes	and Gav	dus Mo	rhua							
							Afal										Na	rI					M/u	7					InN	_			
species	N <sup>a</sup> 2	7 65	72-7(	6 123-125	155-16	6 173	185-1	94 214	1 254–2	261 36(	0 417	429-44C	466 8	88-97	226-239	255	265 272	-284 510	)-517 5	526 53	37 562	214-2	219 386	602-65	9 25-32	36-45	4755	59-70	74-80 8	33-91 9	8-120 1	46-168	221–223
M. merluccius	45 0	0 0	0	0	0	0	-	0	0	0	0	-	0	-	0	0	-	-	0	0	0	0	0	-	-	-	-	-	-	-	-	0	0
M. senegalensis	34 0	0 C	0	0	0	0	-	0	0	0	0	-	0	-	0	~	0	+	0	0	0	0	0	-	-	-	-	-	-	0	-	0	0
M. polli	33 1	1	0	0	-	0	0	0	0	0	0	0	-	-	0	0	0	0	0	0	1	0	0	-	-	0	-	-	0	-	-	-	0
M. capensis	45 C	0 C	0	0	0	0	-	0	0	0	0	-	0	-	0	0	0	0	0	-	0	0	0	-	-	-	0	-	-	-	-	0	0
M. paradoxus	3000	0 C	0	0	0	0	-	0	0	0	0	-	0	-	0	0	0	0	0	ò	1	0	0	-	-	0	-	-	0	-	-	-	0
M. productus	45 C	0 C	0	0	-	0	-	0	-	0	0	0	0	-	-	0	0	+	0	0	0	0	0	-	-	0	0	-	0	-	-	0	-
M. gayi	45 C	0 C	0	0	-	0	-	0	-	0	0	0	0	-	-	0	0	+	0	0	0	~	-	0	-	0	0	-	0	0	-	0	-
M. australis	45 C	0 C	0	0	-	0	-	0	~	0	0	0	0	-	-	0	0	-	0	0	0	0	0	-	~	0	0	-	-	-	-	-	0
M. hubbsi	45 C	1	0	-	-	0	0	0	-	0	0	0	0	-	-	0	0	+	0	0	0	0	0	-	-	0	0	-	-	-	-	-	0
M. albidus	20	0 C	0	0	0	0	-	0	0	0	-	0	0	-	-	0	0	-	0	0	0	0	0	-	~	-	0	-	0	0	-	-	0
M. hernandezi	10 0	0 C	0	0	-	0	-	0	~	0	0	0	0	-	-	0	0	-	0	0	0	-	~	0	~	0	0	-	0	0	-	-	-
M. bilinearis	45 C	0 C	-	-	0	0	-	-	0	0	0	0	0	-	0	0	0	0	-	0	0	-	~	0	~	-	0	-	0	0	-	-	0
G. morhua	25 C	000	-	0	0	-	0	0	0	-	0	0	0	-	0	0	0	0	-	0	0	0	0	-	-	-	0	0	0	0	-	-	0
<sup>a</sup> N is the s	amply	le siz	ze use	⇒d to valid	ate the	identif	ication	test	(1–3 s	ample	s per ;	species	). Len	gth fra	gments	not si	traightfo	orwardly	disting	uishe	d fror	n each	other	in agaro	se gels	are gro	puped	into the	e same	length	class (e	e.g., 72-	-76 bp).
	-									-	-	-		0	0		0		)					)	)	)	-			>	-	0	



Figure 5. Consensus diagram of 110 equally parsimonious trees recovered with the polymorphism parsimony method. Because all conspecific samples showed the same restriction pattern, only one representative individual from each species was used in this reconstruction. (\*) Branching topology was recovered in 100% of trees.

In this study, we have designed a preliminary test to shortcut the development of tedious and expensive identification protocols when there is no hake in the sample. It consists of PCR amplification of a ITS1-rDNA fragment using ITS1 nested primers (*MemeITS1Nes1* and *MemeITS1Nes2*) that are interspecifically conserved in hakes. This exclusion test is very robust because the amplified fragment spans only 193 bp, a DNA length usually recovered in most cases, including ancient DNA samples (*35*). While Atlantic cod was not excluded from the hake-only DNA test, no PCR amplification was observed from Gadidae species, such as grenadiers, or from distant species such as salmonids, flatfishes, and mollusks.

The second step of the identification protocol consisted of three equally performant approaches, (i) species-specific PCR-RFLPs on the whole ITS1 amplicon, (ii) nucleotide sequence comparison using BLAST against the GenBank nucleotide database, and (iii) molecular phylodiagnosis using FINS. The universal primers selected at the conserved ends of the ITS1flanking 18S (XelaITS1.1) and 5.8S genes (OnmyITS1.2) produced a PCR fragment from each species that spanned 53 bp of the 3' end of the 18S ribosomal gene, a 602-659 bp fragment of the specific ITS1 sequence, and 20 bp of the 5' end of the 5.8S gene. Noteworthy, this primer pair amplifies the ITS1 regions of all eukaryotic taxa so far screened in our laboratory. The application of an array of four restriction enzymes with restriction sites tied to interspecifically nonconserved regions of the ITS1 permitted the unequivocal identification of 12 hake species, including the never-before genetically

studied *M. hernandezi* (*36*) and the Atlantic cod *G. morhua*. The restriction patterns were differentiated in agarose gels and matched those predicted with the restriction maps of the ITS1 sequences. Because a single enzyme identifies three species of hake and the Atlantic cod, two enzymes distinguish 9 species, three enzymes identify 11 species, and the four enzymes together discriminate the 12 species of hake and the Atlantic cod; it may not be necessary to apply all four enzymes to identify unknown hake samples. This PCR–RFLP method would also serve to identify hakes in seafood products where more than one species is present because of a partial substitution (M. Pérez, unpublished data). For this purpose, one should apply the three first restriction enzymes to detect the expected composite patterns between species for each enzyme.

Two important properties of the ITS1 region facilitated designing the species-specific restriction patterns. First, nucleotide divergence between species in portions of the ITS1 region allowed us to select sequence-specific restriction targets. Second, the presence of a consensus ITS1 sequence within species (18) allowed for reproducibility among the species-specific restriction patterns. These two properties make ITS1 one of the most valuable regions for development of nuclear DNA markers to easily identify species by PCR-RFLP (20), FINS (31), and BLAST (32).

The intraspecific polymorphism of the ITS1 region is very low in vertebrates (18) and is apparent in some restriction patterns of hakes, e.g., Nar I (**Figure 2**). These weak fragments are not due to cross-species contamination, as concluded from replicated experiments, and are most likely due to a low ITS1rDNA intragenic variability that does not weaken the reproducibility of the diagnostic patterns, which have been worked out on the consensus ITS1 sequence present in all conspecific individuals.

The confidence intervals of genetic distances used to identify commercial fishes usually overlap within and between species, rendering this methodology useless for an exact identification of species. Also the bootstrap method used to calculate a "quantitative estimate of the degree of confidence" of the species' assignment (37) usually gives nodal values smaller than 100%, which do not provide the level of confidence required for identification purposes. In this study, we have applied a polymorphism parsimony method of restriction fragment data (29) to assess the diagnostic power of our methodology. The correct grouping of all individuals from the same species and the maximal nodal scores of the tree demonstrated the full identification power of this method. The phylogenetic tree recovered with the ITS1 nucleotide sequence allows for the correct assignment of unknown samples to 1 of the 12 hake species (FINS). Complementary to the FINS method, the BLAST comparison of the ITS1 nucleotide sequence (the 193 bp ITS1 nested fragment as well as the full ITS1 sequence) from an unknown hake sample also permitted its quick assignment to species provided that the ITS1 sequences of the 12 hakes have been made available at the GenBank nucleotide database.

The present identification key has interesting applications in various tasks such as the conservation and management of hake species, the study of their ecological interactions, and the commercial tracking of hake products. For instance, fishing effort in mixed fisheries of hakes could be conveniently measured for each hake by analyzing the species composition of the catches. This method can also be used to discourage fraudulent fishing (*38*) in protected areas and to detect illegal trade of hake products. From an industrial perspective, the

methodology developed could be of great help at identifying commercial products of hake at different stages of the food chain. After the exact hake species in mixed and diversified products was determined, the fish industry could use this key as a marketing tool to guarantee the quality and authenticity of hake-based products for sale (*39*). From a legal perspective, there is need for analytical approaches to enforce labeling regulations and to authenticate imports and exports of hakes. In particular, fishery forensics, in cases of food alarms or fishing conflicts, can benefit from a reliable genetic diagnose.

The diagnostic method described herein is one of the most reliable tools so far developed for the identification of hakes from the genus *Merluccius*, because of its ease of execution and high reproducibility. In addition, the total time required to achieve a reliable diagnosis is approximately a working day for a large number of samples, making this methodology suitable for routine analysis. These properties have also been tested by independent researches of the seafood quality control reference laboratory of CECOPESCA (Centro Técnico Nacional de Conservación de Productos de la Pesca). Several case studies have shown that most industrial processes applied to hake meat, such as heating, cooking, and food additives, neither degraded the DNA nor inhibited the PCR reaction to such an extent as to prevent the amplification of the two diagnostic fragments of the ITS1-rDNA (20, 40).

#### ABBREVIATIONS USED

EDTA, ethylenediaminetetraacetic acid; bp, base pair(s); FINS, forensically informative nucleotide sequencing; GPS, global positioning system; ITS1, internal transcribed spacer 1; rDNA, ribosomal DNA; SDS, sodium dodecyl sulfate; SDS– PAGE, sodium dodecyl sulfate—polyacrylamide gel electrophoresis; TBE, Tris-borate-EDTA.

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