Mitochondrial DNA sequence variation within and between *Thunnus* tuna species and its application to species identification

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Running title: Tuna species identification using mtDNA variation

ABSTRACT

PCR-RFLP analysis on a mitochondrial DNA segment flanking ATPase and cytochrome oxidase subunit III genes has previously been used to identify *Thunnus* tuna species. Yet a subsequent survey on a large number of specimens from different ocean basins detected a number of individuals showing significant discordance with the RFLP markers. Nucleotide sequence analysis was performed on the "inconsistent" and “consistent” individuals to the RFLP markers. Restriction analysis detected two types of bigeye tuna (α and β); the α type was in the majority in the Atlantic but nearly absent in the Indo-Pacific. Interestingly the α type shared larger number of restriction sites with some other species than the conspecific β type, but bigeye-specific nucleotide substitutions with a novel diagnostic restriction profile were found. Although nucleotide sequence difference between Atlantic and Pacific subspecies of the northern bluefin tuna was nearly the largest among species, individuals possessing the Atlantic type of mtDNA were found at very low frequency in the Pacific and *vice versa*. Previous RFLP markers were found to be diagnostic for the other five species (albacore, blackfin, longtail, southern bluefin and yellowfin tunas). The present study provides useful genetic information to discriminate all *Thunnus* tuna species regardless of their origin or to identify the ocean of capture in the northern bluefin and bigeye tunas.

Key words: tuna, species identification, mtDNA sequence, RFLP

INTRODUCTION

There are seven species and one subspecies in tunas of the genus *Thunnus* South (Collette & Nauen, 1983). The blackfin tuna *T. atlanticus* (Lesson) and longtail tuna *T. tonggol* (Bleeker) are confined to the Atlantic and Indo-Pacific, respectively. The southern bluefin tuna *T. maccoyii* (Castelnau) occurs only in the southern hemisphere, while the Atlantic northern bluefin tuna *T. thynnus thynnus* (Linnaeus) and the Pacific subspecies *T. t. orientalis* (Temminck and Schlegel) are distributed mainly in the northern hemisphere of the Atlantic and Pacific, respectively. The Atlantic and Pacific populations

of the northern bluefin tunas are geographically well isolated from one another, as they are rare in the Indian Ocean as well as in the southern hemisphere. The other three species, albacore *T. alalunga* (Bonnaterre), bigeye *T. obesus* (Lowe) and yellowfin *T. albacares* (Bonnaterre) tunas are considered worldwide panmictic species.

Species identification at early life stages such as egg, larvae and small juveniles is very important to clarify species distribution and reproductive activity. The most useful morphological diagnostic character to discriminate *Thunnus* tuna species at the larval stage is body pigment patterns (Nishikawa, 1985), but this may have limited use for some developmental stages. There is no external morphological key for species identification for small juveniles. Species identification at the adult stage has also become very important for detecting illegal fishing and trading, especially in the northern and southern bluefin tunas, but may be difficult when the few diagnostic external and internal morphological characters are removed and/or when fish are filleted.

Genetic species identification is useful through all life stages. There are several studies on restriction fragment length polymorphism (RFLP) and nucleotide sequence variation of mtDNA for *Thunnus* tuna species identification and phylogeny (Bartlett & Davidson, 1991; Chow & Inoue, 1993; Chow & Kishino, 1995; Finnerty & Block, 1995; Alvarado Bremer *et al*. 1997). However, these studies have paid little attention to comparing samples from different ocean basins. For example, Bartlett & Davidson (1991) determined 307 bp partial nucleotide sequence of mtDNA cytochrome *b* gene in albacore, bigeye, northern bluefin and yellowfin tunas. Finnerty & Block (1995) analyzed five tuna species using a longer segment of the cytochrome *b* gene. However, all tuna samples used in these studies were from the Atlantic. Chow & Inoue (1993) and Chow & Kishino (1995) analyzed mtDNA cytochrome *b* and ATPase genes of all *Thunnus* tuna species and found that the difference between Atlantic and Pacific subspecies of the northern bluefin tuna was larger than that between other species. Chow & Inoue (1993) developed PCR-based RFLP markers, by which they expected to completely identify all species. However, all samples used except three species (Atlantic northern bluefin, southern bluefin and blackfin tunas) were from the Pacific, and it was unknown whether or not their RFLP markers could be practically used for samples from different ocean basins. In yellowfin tuna, Scoles & Graves (1993) using mtDNA analysis suggested that there was appreciable gene flow between the Atlantic and Pacific yellowfin tuna populations, while Ward *et al*. ◀(1997) observed highly significant heterogeneity in *Gpi* allozyme gene frequency within and between ocean basins. Chow & Ushiama (1995) concluded that gene flow between Atlantic and Pacific populations of albacore was restricted. Furthermore, Alvarado Bremer *et al*. (1998) and Chow *et al*. (2000) found very large genetic differentiation between Atlantic and Indo-Pacific samples of bigeye tuna. Thus, even in these highly migratory tuna species, population subdivision accompanying independent genotype rearrangement history is likely. Therefore, investigation of genetic variation within species between oceans would be essential for constructing a reliable species identification protocol.

In this study, RFLP markers developed by Chow and Inoue (1993) were re-investigated using tuna specimens collected from different ocean basins in order to identify individuals as consistent or inconsistent to their species identification protocols. Nucleotide sequences of the "consistent" and "inconsistent" individuals were determined and compared. We here report the results of our RFLP and nucleotide sequence analyses to investigate genetic variation within and between *Thunnus* tuna species, which may

make reliable tuna species identification possible regardless of the origin of sample or may make identification of the origin of tunas possible.

MATERIALS AND METHODS

Tuna samples used in this study are presented in Table I. All samples were derived from the laboratory collection of the National Research Institute of Far Seas Fisheries, and detailed information is available upon request. Ocean basin of origin for these samples was indicated as A (Atlantic) or P (Pacific) attached to abbreviated species name such as ALBA (albacore from Atlantic).

Standard phenol-chloroform extraction was used to extract crude DNA from frozen or ethanol-preserved muscle tissue. The PCR primer set and amplification conditions to amplify a flanking region (ATCO) between mtDNA ATPase and cytochrome oxidase III genes are in Chow & Inoue (1993) and Chow & Kishino (1995). Eleven restriction endonucleases out of twenty used by Chow and Inoue (1993) were informative to identify *Thunnus* tuna species, among which *Alu* I, *Hinc* II, *Mse* I or Rsa I were conspicuously useful. Twenty or more individuals per species of the genus *Thunnus* were first subjected to RFLP analysis using above enzymes. Individuals showing consistent or inconsistent RFLP profiles with those described by Chow & Inoue (1993) were selected for nucleotide sequence analysis. In species where no such inconsistency was found, individuals were randomly selected from each oceanic sample. For reference, three individuals each from four other species of the Thunnini (*Auxis rochei* and *A. thazard*, *Euthynnus affinis* and *Katsuwonus pelamis*) were subjected to the RFLP analysis to show distinct restriction profiles of these Thunnini from *Thunnus* tunas.

For nucleotide sequence analysis, amplified PCR fragments were electrophoresed on 1% agarose gel, excised, and cloned into PUC18 which was subsequently used to transform *E. coli* DH5αMCR. Clones containing the target fragment were screened and sequenced using an automatic DNA sequencing machine (DSQ-1000L, Shimadzu). Sequences were aligned using the Clustal W program (Thompson *et al*. 1994).

RESULTS AND DISCUSSION

SCREENING INDIVIDUALS FOR NUCLEOTIDE SEQUENCE ANALYSIS

With *Alu* I digestion, 20 Atlantic and 20 Pacific individuals of albacore (ALB) showed species-specific restriction profiles as previously shown in Chow & Inoue (1993). Therefore, 2 individuals each from the Atlantic and Pacific (designated ALBA and ALBP, respectively) were randomly selected for nucleotide sequence analysis. Chow & Inoue (1993) reported distinct restriction profiles and nucleotide sequences between the Atlantic (NBTA) and Pacific northern bluefin (NBTP) tunas, except for one Pacific individual possessing the same restriction profiles and nucleotide sequence with NBTA in 18 NBTP examined. Further RFLP survey performed in the present study detected two more NBTA-like individuals out of 154 NBTP. One ($NBTP^{\varphi}$) of these three was selected and 8 NBTP representatives were randomly selected. In 70 NBTA, we also found four individual sharing an identical restriction profiles with a NBTP representative, and one of thess individuals ($NBTA^{\varphi}$) and 4 others randomly chosen were sequenced.

In southern bluefin tuna (SBT) samples, little variation in *Alu* I and *Mse* I digestions for the amplified ATCO fragments was observed within and between the Cape of Good Hope (n=47) and Tasmania (n=35) samples. Grewe *et al*. (1997) also found no

spatial differentiation in mtDNA genotype distributions among SBT samples collected off South Africa, north and southwestern Australia and Tasmania. Therefore, 5 individuals caught around South Africa were randomly selected for nucleotide sequencing.

In bigeye tuna (BE), Chow *et al.* (2000) observed two highly divergent mtDNA genotypes (α and β). They found only one α type out of 195 individuals collected in the Indo-Pacific, while large proportion of Atlantic bigeye tuna (BEA) sample (178 out of 244 individuals) were α type. We found here that the BE-specific restriction profiles in *Alu* I, *Mse* I and *Rsa* I digests proposed by Chow & Inoue (1993) characterised the β type and were not applicable for discriminating the α type from some other species. Four Pacific BE individuals (BEP β) were randomly selected. Among eight BE individuals selected from Atlantic sample, three were β type (BEA β) and five were α type (BEA α).

Since we analysed 395 Pacific and 20 Atlantic yellowfin tuna (YF) individuals and observed no discrepancy from the RFLP markers, 6 Pacific (YFP) and 2 Atlantic (YFA) individuals were randomly selected.

As blackfin tuna (BKT) and longtail tuna (LTT) are endemic to the Atlantic and Indo-Pacific, respectively, two individuals of each were sequenced.

NUCLEOTIDE SEQUENCE ANALYSIS

The amplified fragments were 927 bp in length. The aligned nucleotide sequences of 885 bp excluding priming sites of 47 individuals of these tuna species are shown in Fig. 1. There were no insertions or deletions, and nucleotide substitutions were observed at 95 sites. Nucleotide sequence variation between individuals within species was low in ALB, BKT, LTT, SBT and YF, where the proportion of nucleotide substitution between individuals ranged from 0 to 1.0%.

In contrast, much higher variation within species was observed in NBT and BE. In NBT, the proportion of nucleotide substitutions between NBTP and NBTA individuals ranged from 4.2 to 4.5%, comparable with or even much larger than those between tuna species. For example, the difference between ALB and NBTP ranged from 0.8 to 1.8%, and that between SBT and NBTA ranged from 0.8 to 1.2%. Nucleotide difference between NBTP$^{\varphi}$ and NBTP ranged from 4.4 to 4.6%, but that between NBTP$^{\varphi}$ and NBTA ranged from 0.3 to 0.5%. One Pacific-type individual (NBTA$^{\varphi}$) found in the Atlantic differed from NBTA with 4.0 to 4.1% of nucleotide substitution but from NBTP with 0.5 to 0.7%. Chow & Kishino (1995) reported no nucleotide difference between NBTA and NBTP$^{\varphi}$, but the present analysis with a longer sequence showed nucleotide substitutions between NBTA and NBTP$^{\varphi}$ and between NBTP and NBTA$^{\varphi}$. This suggests that NBTP$^{\varphi}$ and NBTA$^{\varphi}$ may be scions of immigrants in the not so recent past, indicating no ongoing gene flow between ocean basins.

The proportion of nucleotide substitution between the α type individuals of BEA was small ranging from 0 to 0.7%, and between BEA β and BEP β ranged from 0.1 to 0.7 %. In contrast, the difference between the α and β types was significantly larger ranging from 1.6 to 2.1 %. These values between the α and β types were comparable or even larger than those between the α type and SBT (1.1-1.5 %) and between the α type and tropical tunas (BKT, LTT and YF) (1.5-2.1 %) or NBTA (1.6-1.9 %).

RFLP MARKERS FOR SPECIES IDENTIFICATION

Chow & Inoue (1993) proposed that *Alu* I, *Mse* I and *Hinc* II or *Hin*F I digestions for the ATCO fragment were diagnostic for identifying all *Thunnu*s tuna species. Size distributions of restricted ATCO fragment by these enzymes plus *Rsa* I are shown in Appendix I. Restricted fragment sizes in *Thunnus* tuna species were based on the nucleotide sequences obtained in this study and those in the other 4 species (BUT, FRT, KAW and SKJ) of Thunnini were estimated with size standard upon gel electrophoresis. Distinct restriction profiles are evident between *Thunnus* tuna and the other 4 species in all restriction assays. These Thunnini (BUT, FRT, KAW and SKJ) shared the identical restriction profiles in *Hinc* II and *Rsa* I digestions but could be identified one another using *Alu* I or *Mse* I.

In *Thunnus* tuna species, these RFLP markers were reconfirmed to be diagnostic for species identification in ALB, BKT, LTT, SBT and YF, a conclusion substantiated by the nucleotide sequence analysis (Fig. 1). On the other hand, new variants within and between BE and NBT species found in this study necessitate re-evaluation of RFLP markers for identification. NBTA$^{\varphi}$ and NBTP$^{\varphi}$ give identical restriction profiles to their Pacific and Atlantic counterparts, meaning that RFLP markers for identifying NBTA and NBTP are not fully diagnostic. However, these inconsistent profiles occur at very low frequency in each ocean basin; so far only 4 Pacific-type out of 70 NBTA and 3 Atlantic-type out of 172 NBTP have been found. Thus the probability for mis-identifying the origin of an individual NBTA or NBTP is 2 to 6 %.

New variants found in BE may be of more concern for species identification. Interestingly, the α type shared a larger number of diagnostic restriction sites with SBT and tropical tunas than with β type. For example, Chow & Inoue (1993) proposed that BE showed characteristic restriction profiles in *Mse* I and *Rsa* I digestions. However, the present study revealed that the loss and gain of *Mse* I sites (at positions 107-110 and 458-461) and a *Rsa* I site (452-455) in the α type, making these restriction enzymes unreliable for identifying BE (especially the α type) from the other species. The present sequence analysis also revealed novel BE-specific nucleotide substitutions at positions 166, 220 and 837, and that at 220 was responsible for the site loss for the *Tsp* 509I enzyme which recognizes the AATT palindrome. Representative *Tsp* 509I restriction profiles of *Thunnus* tuna species are shown in Fig. 2. We performed further restriction assays using this enzyme and found only one individual out of 101 BEA and two of BEP shared the same restriction profile with other species, and in *Mse* I and *Rsa* I digestions these three individuals were β type, uniquely representative of BE. Three to nine individuals each of the other *Thunnus* species were analysed but so far no variation was observed. Therefore, by incorporating the *Tsp* 509I digests to an RFLP assay proposed by Chow & Inoue (1993), all *Thunnus* tuna species may be discriminated regardless the origin of samples and ocean of capture may be identified in NBT and BE.

Recently, Takeyama *et al*. (2000) developed a magnetic-capture hybridization technique employing bacterial magnetic particles (BMPs) to discriminate Atlantic and Pacific subspecies of the northern bluefin tuna. They demonstrated that the system could recognize single nucleotide difference between sequences. The magnetic-capture hybridization technique may be applied to discriminate all tuna species using the nucleotide sequence information given in the present study, which would make more high-throughput identification of tuna species or catch locality possible.

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TABLE I. Tuna species of Thunnini used in this study

Species	Common name	Abbreviation	Locality sampled
Auxis rochei	bullet tuna	BUT	Japan, Pacific
A. thazard	frigate tuna	FRT	Japan, Pacific
Euthynnus affinis	kawakawa	KAW	Japan, Pacific
Katsuwonus pelamis	skipjack tuna	SKJ	Brazil, Atlantic
Thunnus alalunga	albacore	ALBA	Biscay Bay, Atlantic
	albacore	ALBP	Hawaii, Pacific
T. albacares	yellowfin tuna	YFA	Gulf of Guinea, Atlantic
	yellowfin tuna	YFP	Philippines, Pacific
T. atlanticus	blackfin tuna	BKT	Miami, Atlantic
T. maccoyi	southern bluefin tuna	SBT	Cape of Good Hope
	southern bluefin tuna	SBT	Tasmania, Pacific
T. obesus	bigeye tuna	BEA	Gulf of Guinea, Atlantic
	bigeye tuna	BEP	Philippines, Pacific
T. thynnus orientalis	northern bluefin tuna	NBTP	Japan, Pacific
T. thynnus thynnus	northern bluefin tuna	NBTA	Northwest Atlantic
T. tonggol	longtail tuna	LTT	Japan, Pacific

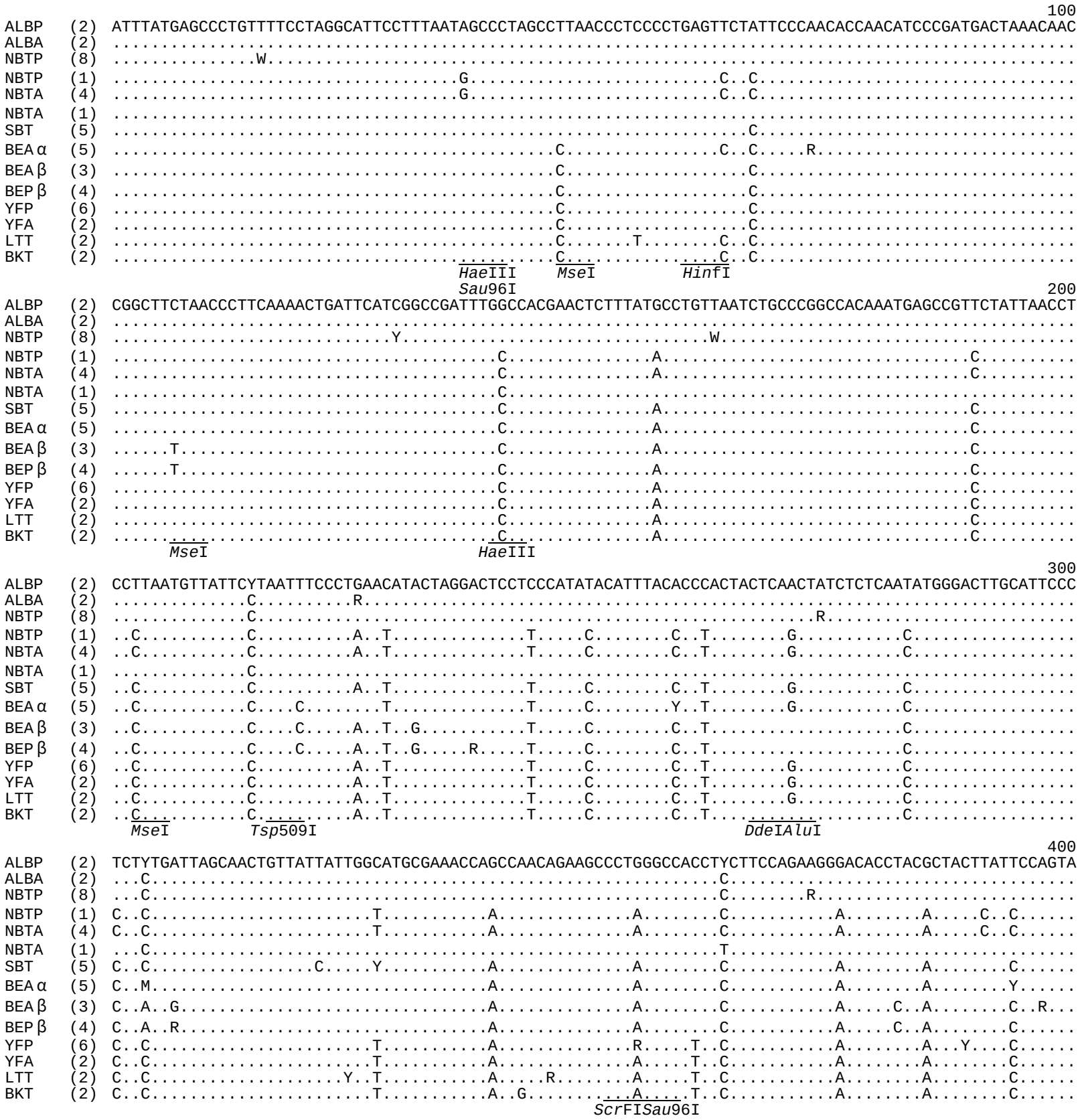

Fig. 1. MtDNA ATCO region sequences of Thunnus tuna species. Identity with top reference sequence is indicated by dot. Number of individuals analyzed was shown in the parenthesis. Diagnostic restriction sites are underlined. See Table 1 for species abbreviation. M, R, S, W and Y denote A or C, A or G, C or G, A or T, and C or T, respectively.

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                                                                                                          500
ALBP  (2) CTAATTGTCATCGAAACAATTAGCTTATTCATCCGACCCTTAGCACTTGGAGTGCGGTTAACAGCCAACCTAACGGCYGGACATCTTCTAATTCAACTAA
ALBA  (2) .............................................................................T......................
NBTP  (8) ...........T...........Y.........................................T...........T......................
NBTP  (1) ..................................................G..............A...........T.........T............
NBTA  (4) .............................................................................T.........T............
NBTA  (1) ...........T.....................................................T...........T......................
SBT   (5) ........Y..............................C.........................T...........T.........T............
BEAα  (5) ..............R........................C.........................T...........T.........T............
BEAβ  (3) .....................................................A..R..G.....T...........T..G......T............
BEPβ  (4) .....................................................A.....G.....T...........T..G......T............
YFP   (6) .......................................Y.........................T...........T.........T............
YFA   (2) .................................................................T...........T.........T............
LTT   (2) .........................................................C.......T...........T.........T............
BKT   (2) ........................................................A........T...........T.........T............
                    TaqI                     DdeI         RsaI  MseI AluI                    MseI
                                                            Fnu4HIHincII                                  600
ALBP  (2) TCGCTACAGCAGCAACTGTCCTTCTACCACTAATGCCAACTGTAGCYATCCTAACAGCAACCCTACTTTTCCTTCTAACGCTTCTAGAAGTCGCYGTCGC
ALBA  (2) ..............................................T....................................Y..........T.....
NBTP  (8) ......................C.......................T...............................................T..Y..
NBTP  (1) ...........................................G..A................................A...........G..T.....
NBTA  (4) ...........................................G..A................................A..............T.....
NBTA  (1) ......................C.......................T...............................................T.....
SBT   (5) ...........................................G..A................................A..............T.....
BEAα  (5) ...........................................G..A..Y.............................A..............T.....
BEAβ  (3) ................Y..........................G..A................................A..............T.....
BEPβ  (4) ...........................................G..A..............Y.................R...........Y..T.....
YFP   (6) ........................................C.....A.................G..............A..............T..S..
YFA   (2) ........................................C.....A.................G..............A..............T.....
LTT   (2) ............................M..............G..A.................G..............A..............T.....
BKT   (2) ............................R.................A.................G........C.....A..............T.....
                                                    AluI
                                                                                                          700
ALBP  (2) AATAATCCAAGCTTACGTATTTGTCCTACTCCTAAGCCTTTACCTACAAGAAAACGTCTAATGGCCCATCAAGCACACGCATACCACATAGTTGACCCCA
ALBA  (2) ....................................................................................................
NBTP  (8) .........R.............................C............................................................
NBTP  (1) .......................................C............................................................
NBTA  (4) .......................................C............................................................
NBTA  (1) .......................................C............................................................
SBT   (5) ........................Y..............C............................................................
BEAα  (5) .......................................C............................................................
BEAβ  (3) ........................T..............C............................................................
BEPβ  (4) ........................Y..............C............................................................
YFP   (6) .......................................C............................................................
YFA   (2) ...............Y.......................C............................................................
LTT   (2) .......................................C............................................................
BKT   (2) .....................C.................C..............................................M.............

                                                                                                          800
ALBP  (2) GCCCTTGACCATTAACAGGTGCAGTTGCTGCCCTACTAATAACGTCAGGCCTCGCTATCTGATTTCACTTCCACTCTACAACACTAATAACTGTCGGAAC
ALBA  (2) ....................................................................................................
NBTP  (8) ................R........C..........................Y...................................W...........
NBTP  (1) ....................................................................................................
NBTA  (4) ....................................................................................................
NBTA  (1) .........................C..........................................................................
SBT   (5) ....................................................................................................
BEAα  (5) ....................................................................................................
BEAβ  (3) ....................................................................................................
BEPβ  (4) ........................................................................................W...........
YFP   (6) ....................................................................................................
YFA   (2) ....................................................................................................
LTT   (2) ...................................T................................................................
BKT   (2) ....................................................................................................
                                             Mse I
                                                                                         885
ALBP  (2) AGCCCTCCTGCTCCTYACAATGTACCAATGATGACGAGACATCATCCGAGAAGGTACCTATCAAGGACACCACACCCCTCCTGTC
ALBA  (2) ...............T..............................................................A......
NBTP  (8) ...............T.....................................................................
NBTP  (1) ......T........T.....A....................T.............................T............
NBTA  (4) ......T........Y.....A....................T.............................T............
NBTA  (1) ...............T.....................................................................
SBT   (5) ......T........T....................R.....T.............................T............
BEAα  (5) ......T........T....................G.....T..........................................
BEAβ  (3) ......T........T....................G.....T..........................................
BEPβ  (4) ......T........Y....................G.....T..........................................
YFP   (6) ......T........T..........................T.............................T............
YFA   (2) ......T........T..........................T.............................T............
LTT   (2) ......T........T..........................T..........................................
BKT   (2) ......T........T..........................T..........................................
                                 RsaI
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Fig. 1. Continued.

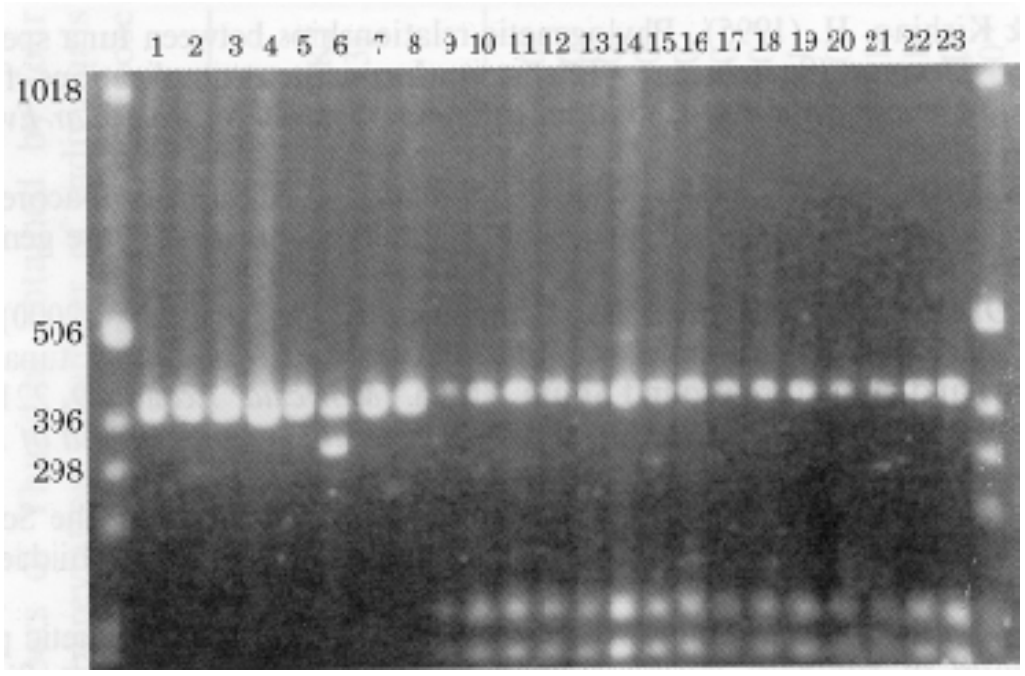

Fig. 2. New diagnostic restriction profiles obtained by *Tsp* 509I digestion to discriminate bigeye tuna from other *Thunnus* species. Left and right ends are 1kb DNA ladder (GIBCO, BRL) and sizes are indicated along the left margin. Lanes: 1-8, BE; 9-14, YF; 15, 16, NBTP; 17, 18, NBTA; 19, 20, SBT; 21, LTT; 22, BKT; 23, ALB.

Appendix I.

Distribution of restricted ATCO fragments of *Thunnus* tuna species and 4 other members of Thunnini obtained by four restriction endonucleases (*Alu* I, *Hinc* II, *Mse* I and *Rsa* I). See Table I for species abbreviation. Number of inviduals examined are shown in the parenthesis. Fragment sizes in *Thunnus* tuna species were based on the nucleotide sequences, while those in BUT, FRT, KAW and SKJ were estimated with molecular size standard. Fragments smaller than 60 bp are not listed.

Alu I (bp)	NBTA$^{\varphi}$ (3) NBTP (117)	NBTP (1)	NBTP (1)	ALBA (17) ALBP (23)	ALBP (1)	NBTA (66) NBTP$^{\varphi}$ (3)	BEPβ (41) BKT (6) YFP (1)	Other *Thunnus*[1]	BUT (3)	FRT (3)	KAW (3)	SKJ (3)
755	0	0	0	0	0	0	0	0	1	0	0	0
485	0	1	0	0	0	0	0	0	0	0	0	0
444	1	0	1	1	1	0	1	0	0	0	0	0
361	0	0	1	0	0	0	0	0	0	0	0	0
350	0	0	0	0	0	0	0	0	0	1	0	0
295	1	1	0	1	1	1	1	1	0	0	0	0
292	0	0	0	0	0	1	0	1	0	0	0	0
240	0	0	0	0	0	0	0	0	0	1	1	1
206	0	0	0	0	0	0	0	0	0	0	0	0
188	0	0	0	0	1	1	0	0	0	0	1	1
170	0	0	0	0	0	0	0	0	1	1	1	1
152	0	0	0	0	0	1	0	1	0	0	1	0
147	0	0	0	0	0	0	1	1	0	1	0	0
122	0	0	0	1	0	0	0	0	0	0	1	1
86	0	0	0	0	0	0	0	0	0	0	0	0
81	1	1	1	0	0	0	0	0	0	0	1	1
66	1	1	0	1	0	0	0	0	0	0	0	0

Hinc II (bp)	BEP (1) BKT (6) LTT (18)	Other *Thunnus*[2]	BUT FRT KAW SKJ (3)
714	1	0	0
510	0	0	1
480	0	1	0
234	0	1	0
213	1	1	1
170	0	0	1

Rsa I (bp)	NBTA (66) NBTP$^{\varphi}$ (3)	BEβ (252) SBT (2)	ALBA (33) ALBP (34) Other *Thunnus*[3]	ALBA (1) ALBP (7)	BUT FRT KAW SKJ (3)
456	1	0	0	0	1
424	0	0	1	1	0
420	1	1	1	0	0
380	0	0	0	1	0
370	0	1	0	0	1

Mse I (bp)	ALB (99) NBTA$^{\varphi}$ (1) NBTP (90)	ALB (31)	ALB (1)	NBTP (3)	NBTP (1)	NBTA (66) NBTP$^{\varphi}$ (3) SBT (81)	BEα (104) BKT (6) YFP (88)	BEβ (93)	LTT (18) SBT (1)	YFP (3)	BUT (3)	FRT (3)	KAW (3)	SKJ (3)
927	0	0	0	0	0	0	0	0	0	0	0	0	0	1
600	0	0	0	0	0	0	0	0	0	0	1	0	0	0
440	0	0	0	0	0	0	0	0	0	0	0	1	1	0
294	0	0	0	0	0	0	0	1	1	0	0	0	0	0
264	0	0	0	0	0	1	1	0	0	1	0	0	0	0
255	1	1	0	1	1	0	0	0	0	0	0	0	0	0
254	1	0	0	1	1	0	0	0	0	0	0	0	0	0
243	0	1	1	0	0	0	0	0	0	0	0	0	0	0
224	0	0	0	0	0	1	1	1	1	1	0	0	0	0
194	1	1	1	1	1	1	1	1	0	1	0	1	0	0
170	0	0	0	0	0	0	0	0	1	1	0	0	0	0
168	0	0	0	0	1	0	0	0	0	0	0	0	0	0
155	0	0	0	0	0	0	0	0	0	0	0	0	1	0
147	0	0	1	1	0	0	0	0	0	0	0	0	0	0
130	0	0	0	0	0	0	1	0	1	0	0	0	1	0
115	1	1	1	0	0	1	0	0	0	0	1	0	0	0
75	0	0	0	0	0	0	0	1	0	0	0	0	0	0

Other *Thunnus*[1]: BEα (99), LTT (18), SBT (82), YFA (2), YFP (33)
Other *Thunnus*[2]: ALBP (23), ALBA (2), BEA (8), BEP (20), NBTA (15), NBTP (18), SBT (44), YFA (2), YFP (91)
Other *Thunnus*[3]: BEα (151), BKT (6), LTT (18), NBTP (26), NBTA$^{\varphi}$ (4), SBT (42), YFA (20), YFP (327)