中層トロールによって西部太平洋で採集されたまぐろ類小型稚魚の遺伝学的種判別

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Genetic and morphological identification of larval and small juvenile tunas (Pisces: Scombridae) caught by a mid-water trawl in the western Pacific

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Abstract Diagnostic DNA markers based on the restriction fragment length polymorphism (RFLP) and nucleotide sequence analyses on two regions of the mitochondrial DNA (cytochrome *b* gene and flanking region between ATPase and COIII genes) were obtained using representative specimens of *Auxis rochei, A. thazard, Euthynnus affinis, Katsuwonus pelamis, Sarda orientalis* and all *Thunnus* species. These DNA markers were applied to a total of 936 scombrid larvae and juveniles (9 mm to 186mm in standard or fork length) collected by a mid-water trawl operated in the tropical and sub-tropical western Pacific during 1992 to 1998. Four Thunnini species (*K. pelamis, T. alalunga, T. albacares,* and *T. obesus*) were observed in the samples from the open water area (2°N-15°N, 135°E-157°E), while eight species (*A. rochei, A. thazard, E. affinis, K. pelamis, S. orientalis, T. alalunga, T. albacares* and *T. thynnus orientalis*) were observed in the samples from the island associated area (24°N-30°N, 123°E-131°E). Gill rakers were not fully developed in the juveniles smaller than 60mm in fork length, and may not be useful for identifying the species of *Thunnus* and *Auxis*.

Key words: small tuna juveniles, species identification, mtDNA, gill rakers

In the western Pacific, eight tuna species spanning four genera (*Auxis, Euthynnus, Katsuwonus*, and *Thunnus*) within the tribe Thunnini are observed (Collette and Nauen, 1983). Biological information of small juveniles of these commercially important species is deficient, because the small juveniles are agile enough to avoid small sampling nets and too small to be caught by conventional fishing gear. Recently, Tanabe and Niu (1998) designed a large mid-water trawl net (mouth opening 20m width, 20m height) that can be towed at high velocity (nearly 5 knots) and is considerably efficient in catching small juvenile tunas (up to 60mm in standard length; SL). Annual research cruise conducted by the National Research Institute of Far Seas Fisheries (NRIFSF) in the tropical and sub-tropical western Pacific during 1992 to 1998 were able to routinely collect large numbers of small juvenile scombrids with the mid-water trawl net (Tanabe and Niu, 1998; Itoh *et al.*, 1999, 2000; Tanabe, 2002).

Identification of the small juvenile of many Thunnini species based on morphological and osteological variation is difficult and often times questionable (Matsumoto *et al.*, 1972; Potthoff, 1974; Graves *et al.*, 1989). The small skipjack (*Katsuwonus pelamis*) juveniles collected from the surveys were morphologically distinguishable from other species of Thunnini

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and were used for several biological investigations (Tanabe *et al.*, 1999; Tanabe, 2001, 2002). However, studies on the other small juvenile species of Thunnini have not been possible because identification was too difficult. Gill raker count may be used to differentiate the species, but even in adult they can only marginally separate *Thunnus* species into three groups (Gibbs and Collete, 1967) and not reliable in the juveniles smaller than 30mm (Schaefer and Marr, 1948). Bullet and frigate tunas (*Auxis rochei* and *A. thazard*, respectively) can be separated by gill raker counts, but the gill rakers of small juveniles may not be fully developed (Wade, 1949). DNA analysis appears to be powerful tool for species identification throughout all life stages and may substantiate the morphological variations within and between species. The technique has become conventional by incorporating polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis or direct nucleotide sequencing. Conventional PCR-RFLP protocols have been introduced for fish species identification (Chow *et al.*, 1993; Chow and Inoue, 1993; Takeyama *et al.*, 2001; McDowell *et al.*, 2002), and the techniques are frequently used to identify tuna and billfishes (Smith *et al.*, 2001; McDowell *et al.*, 2002).

In this study, we present the diagnostic DNA markers to identify scombrid species of Thunnini and Sardini and the results of species identification for small juvenile scombrids collected in the western Pacific. The reliability of gill raker counts to differentiate the species of *Thunnus* and *Auxis* was also investigated.

MATERIALS AND METHODS

Samples

DNA samples of representative scombrid species that originated from various oceans around the world were archived at the NRIFSF and used in this study as standards (Table 1) for the identification of larval and juvenile scombrids collected during the research cruises. The DNAs of the larval and juvenile samples were extracted using the standard phenol-chloroform method (Maniatis *et al.*, 1982). Midwater trawl operations were performed in the tropical western Pacific (2°N-15°N, 135°E-157°E) during 1992 to 1994 and in the sub-tropical western Pacific (24°N-30°N, 123°E-131°E) in 1997 and 1998 (Fig. 1). Detailed informations for these operations are available elsewhere (Itoh *et al.*, 1999, 2000; Tanabe, 2002). The scombrid larvae and juveniles were frozen onboard, transferred to the laboratory, and roughly sorted according to several morphological descriptions (Schaefer and Marr, 1948; Wade, 1949, 1951; Matsumoto, 1958; Matsumoto *et al.*, 1972).

A total of 4,292 scombrids (predominantly small juveniles) were collected during the 1992-1994 research cruises by training vessels (Tanshu Maru and Omi Maru) (Tanabe, 2002). Only two morphotypes of juveniles were observed in these three cruises; one type was classified as skipjack tuna (*K. pelamis*) (SKJ) and

Table 1. Catch locality of standard tuna samples used in this study

Species	Common name	Code	Catch locality	Sample size
Auxis rochei	bullet tuna	ARO	Pacific	5
A. thazard	frigate tuna	ATH	Pacific	3
Euthynnus affinis	kawakawa	EAF	Pacific	3
Katsuwonus pelamis	skipjack tuna	SKJ	Pacific, Atlantic	5
Sarda orientalis	striped bonito	SOR	Pacific	3
Thunnus alalunga	albacore	ALB	Pacific, Atlantic	9
T. albacares	yellowfin tuna	YFT	Pacific, Atlantic	6
T. atlanticus	blackfin tuna	BKT	Atlantic	4
T. maccoyii	southern bluefin tuna	SBT	Pacific, Indian	4
T. obesus	bigeye tuna (alpha type)	BET α	Atlantic	2
	bigeye tuna (beta type)	BET β	Pacific, Indian, Atlantic	3
T. thynnus thynnus	Atlantic northern bluefin tuna	ANBT	Mediterranean, Atlantic	5
T. thynnus orientalis	Pacific northern bluefin tuna	PNBT	Pacific	6
T. tonggol	longtail tuna	LTT	Pacific	3

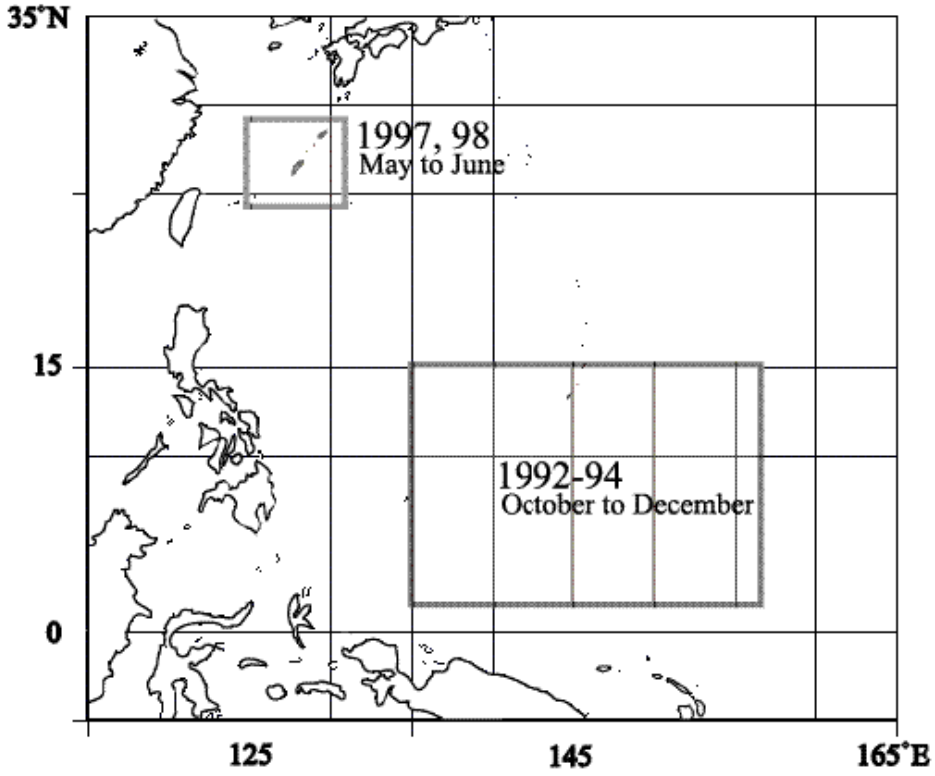

Fig. 1. A map showing the areas of mid-water trawl operation held in 1992 to 1994 and 1997 to 1998

the other as *Thunnus* sp. (Tanabe, 2002). RFLP analysis was performed on the sub-samples of each type (9 to 51mm SL). A large proportion (80%) of the scombrid juveniles (14,928 individuals in total; size range 18 to 70mm SL) collected during 1997 appeared to be bullet or frigate tuna (*Auxis* sp.) (ARO or ATH) and 7 % of those collected were SKJ (Itoh *et al.*, 1999). Subsamples of these juveniles and all other scombrid and scombrid-like juveniles were subjected to RFLP analysis. Fewer juveniles (74 juveniles in total; 12 to 186mm SL) were obtained during the1998 cruise (Itoh *et al.*, 2000), and all were subjected to RFLP analysis. Research cruises in 1997 and 1998 were performed by RV Shunyo Maru of NRIFSF, and tuna-like individuals collected were labeled as SN after the vessel.

PCR amplification

PCR-RFLP and nucleotide sequence analyses on flanking region (*ATCO*) between ATPase and cytochrome oxidase **III** genes of mtDNA have previously been used to identify all *Thunnus* tuna species (Chow and Inoue, 1993; Chow and Kishino, 1995; Takeyama *et al.*, 2001). The same primer set was used to amplify the *ATCO* regions of the other Thunnini species and *S. orientalis* (SOR). Previous restriction analysis using a short fragment (c.a. 355bp) amplified from mtDNA cytochrome *b* gene failed to discriminate between all *Thunnus* species (Chow and Inoue, 1993; Chow and Kishino, 1995), therefore, we designed another primer set to amplify a longer fragment. The primer sequences to amplify 5'-region of partial segment of cytochrome *b* gene (*CytB*) are 5'-ATGGCAAGCCTCCGAAAAAC-3' (CytB1F) and 5'-TAGGAGAAGTATGGGTGGAA-3' (CytB677R). PCR amplification for standard specimens were carried out in 25 μL reaction mixture containing 2 mM $MgCl_2$, 1 mM of each of dNTP, 1 μM of each primer, 1 unit of *Taq* polymerase and DNA template. The reaction mixtures were preheated at 95°C for 5 min followed by 30 cycles of amplification (at 95°C for

1 min, 50°C for 30s, and 72°C for 1.5min) with a final extension at 72°C for 5 min.

RFLP analysis

The PCR products were directly digested by restriction endonucleases. Four restriction enzymes (*Alu* I, *Hinc* **II**, *Mse* I and *Rsa* I) that were reported to be diagnostic for identifying all *Thunnus* species (Chow and Inoue, 1993; Takeyama *et al.*, 2001) were applied to the *ATCO* fragment in this study. Nine enzymes (*Alu* I, *Bsa* JI, *Dde* I, *Eco* NI, *Hae* **III**, *Hinf* I, *Mbo* I, *Nla* **IV**, and *Taq* I) used for smaller fragments by Chow and Inoue (1993) plus Rsa I were applied to the *CytB* fragment. PCR samples digested with these enzymes for 2 hours or longer were electrophoresed through 2.5% agarose gel (Biogel, BIO101 Inc.) for 3 to 4 hours, followed by ethidium bromide staining that was photographed.

Nucleotide sequence analysis

One to four individuals of each species used as standards (Table 1) were subjected to nucleotide sequence analysis. Amplified DNAs were electrophoresed through 2% agarose gel (NuSieve GTG, FMC BioProducts), excised and purified using a Gene Clean **II** (BIO 101). Automated sequences were generated on an automated sequencer (ABI Prism310) using the ABI Big-dye Ready Reaction kit following the standard cycle sequencing protocol. Nucleotide sequence analysis was performed on the juvenile samples that showed inconsistent RFLP profiles with those of the standard. Assignments of these juveniles were investigated by phylogenetic analysis using the neighbor-joining (NJ) method with the evolutionary distance computed by Kimura's two-parameter method as implemented in the program MEGA version 2.1 (Kumar *et al.*, 2001).

RESULTS

Restriction profiles of the ATCO fragment

The *ATCO* fragment of EAF was not amplified using the same technique described previously for the other species; therefore, the annealing temperature at the first 2 cycles was lowered to 37°C. This manipulation increased non-specific PCR products, but the restriction profiles of EAF were marginally obtained. The size of the *ATCO* fragment of *Thunnus* spp. has been determined to be 927 bp by nucleotide sequence analysis (Takeyama *et al.*, 2001), and no apparent size difference was observed among all species used in this study. All Thunnini species and SOR occurring in the western Pacific can be identified by using two enzymes (*Alu* I and Mse I) on the *ATCO* fragments as shown in Fig. 2 (also see Takeyama *et al.*, 2001).

Restriction profiles of the *CytB* fragment

The *CytB* fragments (c. a. 680 bp) of all standard species were amplified well without any difficulty using the amplification condition described above. Restriction profiles of the standards are shown in Fig. 3. Among 10 restriction endonucleases used, we observed no single enzyme by which all species could be discriminated. *Dde* I digestion could discriminate ARO, ATH, EAF and SOR, while SKJ was observed to share the identical restriction pattern with some *Thunnus* species. SKJ may be identified by incorporating any one of the restriction patterns of *Dpn* **II**, *Hae* **III**, *Nla* **IV**, *Rsa* I and *Taq* I. Five enzyme digestions (*Alu* I, *Dde* I, *Eco* NI, *Hinf* I and *Rsa* I) appeared to be necessary for discriminating all *Thunnus* species. Large intra-specific difference in the restriction patterns of the *ATCO* region observed between **α** and **β** types of bigeye tuna (*T. obesus*) (BET) (Chow *et al.*, 2000; Takeyama *et al.*, 2001) was not detected by the RFLP analysis on the *CytB* fragment.

Identification of larval and small juvenile samples

Thunnus-like larvae and juveniles that were morphologically sorted from the others were subjected to RFLP analysis of the *ATCO* region first using *Alu* I and/or *Mse* I, and the others were analyzed using the *CytB* fragments. For digesting the *CytB* fragments, *Dde* I and *Hae* **III** were chosen, because the former detected the largest number of restriction patterns and the later offered well-distinguished fragment patterns among standards (Fig. 3).

All larval and juvenile tunas collected in 1992-1994 were morphologically identified as SKJ and *Thunnus*. The *ATCO* fragments of 226 out of 230 individuals of *Thunnus*-like individuals were successfully amplified, of which 156 were YFT, 67 were BET and 3 were ALB (Table 2).

The *CytB* fragments of 68 out of 73 SKJ juveniles were amplified, of which 65 individuals shared identical restriction profile in *Hae* **III** digestion with that of the standard. The fragment of one individual appeared to have no *Hae* **III** site, and remaining two individuals showed a novel restriction pattern (data not shown).

A total of 584 juvenile scombrids were analyzed in the samples from the 1997 cruise. Successful amplification was obtained in 569 individuals, and RFLP and nucleotide sequencing (described later) analyses indicated that these juveniles comprised six Thunnini species (ARO, ATH, EAF, PNBT, SKJ and YFT), one Sardini species (SOR), and one unidentified species (Table 3). A sub-sample of 179 *Auxis* juveniles was found to consist of 177 ARO and 2 ATH. During crude morphological sorting, many EAF juveniles were erroneously classified as *Thunnus* sp. The *ATCO* fragments of these juveniles were not amplified well at first until the annealing temperature was lowered. The restriction profiles of 65 of these individuals that were subjected to the *ATCO* analysis were identical with those of the EAF standard. This result allowed us to find 14 more individuals that were morphologically different from *Thunnus* juveniles. The *CytB* analysis indicated that these individuals shared identical restriction profiles with the EAF standard. Of the 250 *Thunnus*-like juveniles collected, 79 were EAF, 165 were PNBT and 6 were YFT.

All 74 juveniles collected in 1998 were subjected to RFLP analysis of the *CytB* fragment first, and those identified as *Thunnus* sp. were subjected to the *ATCO* analysis. All individuals were successfully identified mainly by RFLP and some by nucleotide sequence analyses. The results indicated that the 1998 sample consisted of three *Thunnus* species (ALB, PNBT and YFT) and four other Thunnini species (ARO, ATH, EAF and SKJ) (Table 4).

In the *CytB* analysis, five individuals (SN 309, 318, 319, 492 and 708) of the 1997 sample and

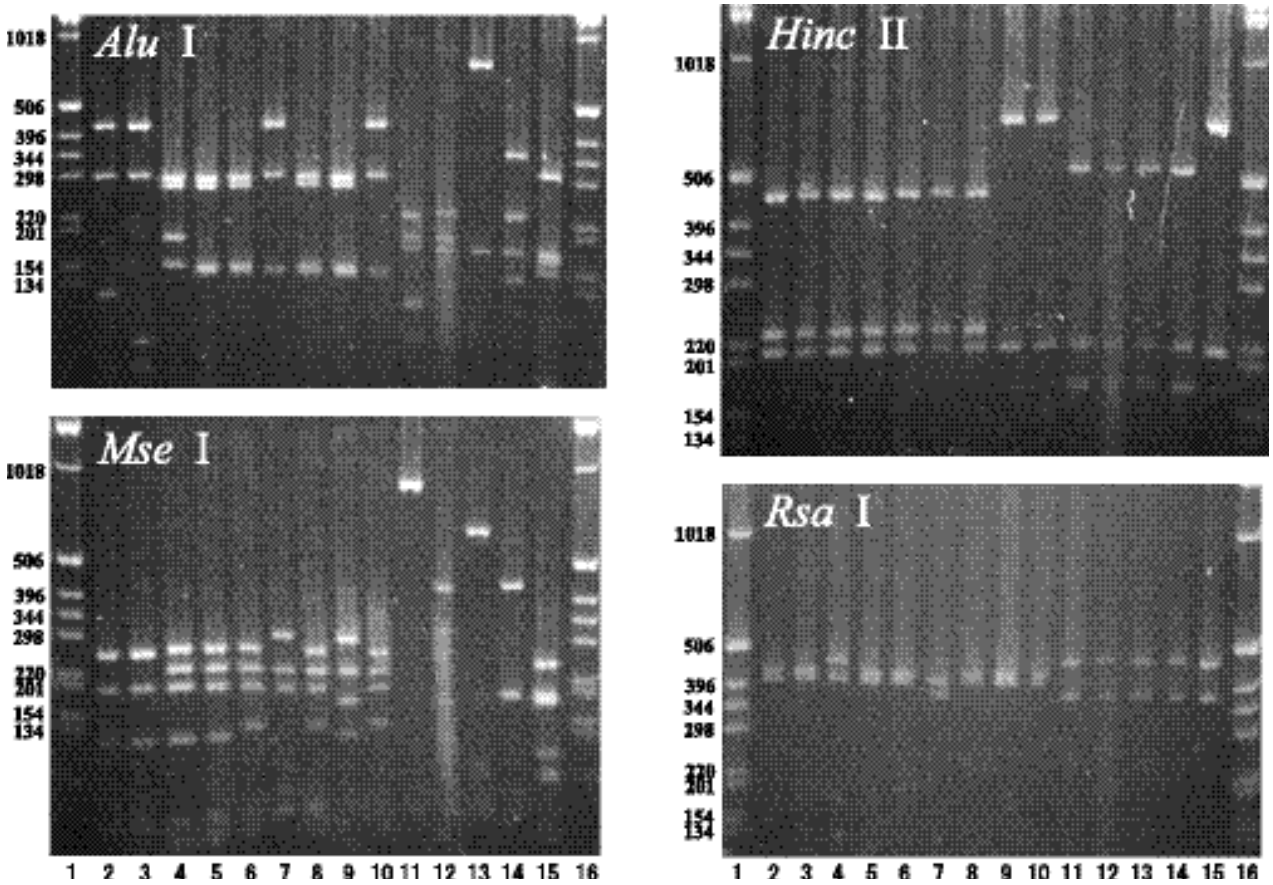

Fig. 2. Restriction profiles of the *ATCO* fragment digested by four enzymes. First and 16th lanes are molecular marker (1kb DNA ladder, GIBCO BRL) and the size are shown on left or right margin. Tuna species, lined from the second lane to the right, are ALB, PNBT, ANBT, SBT, BETa, BETb, YFT, LTT, BKT, SKJ, EAF, ARO, ATH and SOR. See Table 1 for the abbreviations.

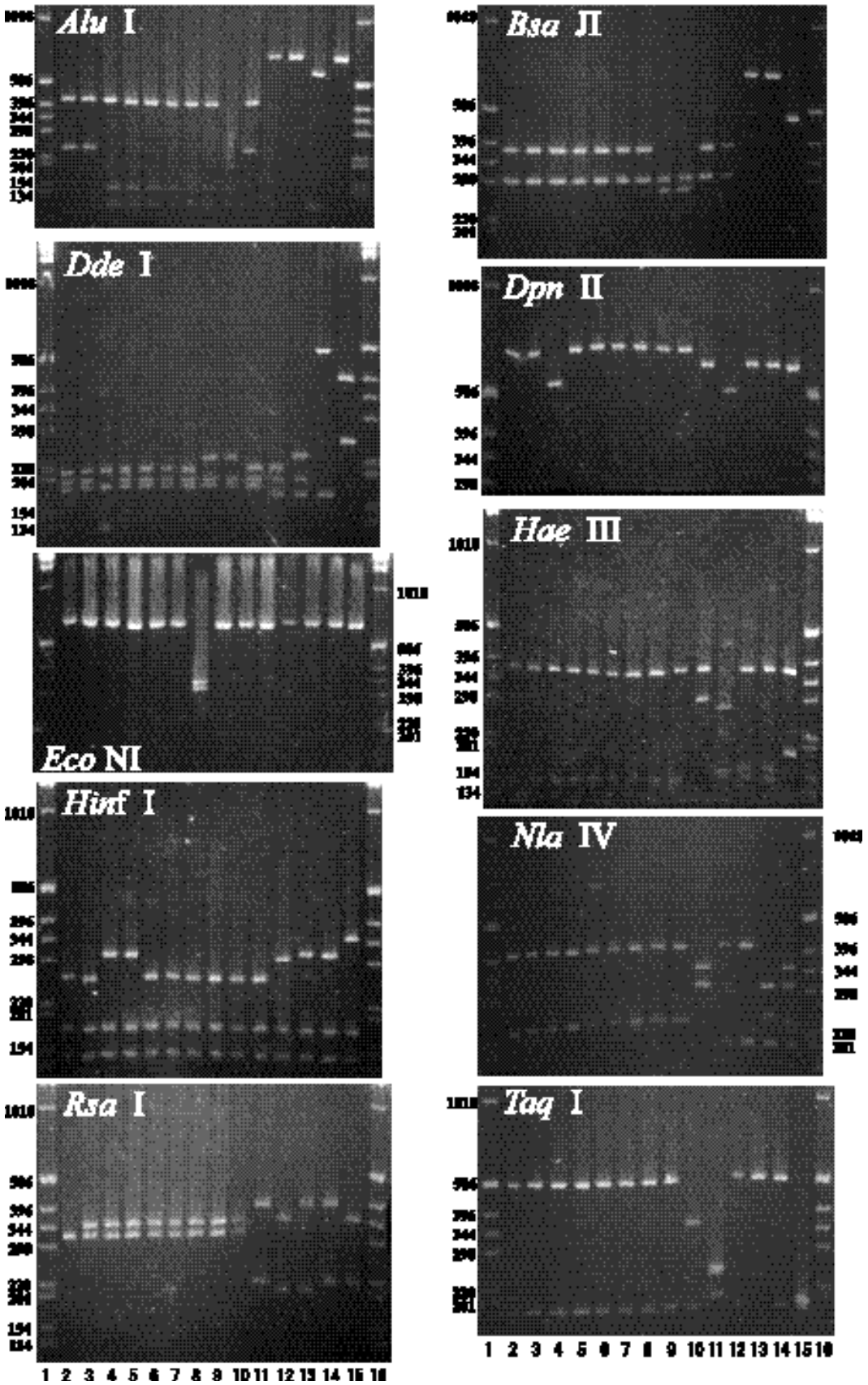

Fig. 3. Restriction profiles of the *CytB* fragment digested by four enzymes. Molecular size marker and line-up of tuna species are the same with those in Fig. 2.

two of the 1998 sample (SN 37 and 53) were found to have restriction profiles inconsistent with the standards (Fig. 4). In *Dde* I digestion, the restriction profiles of SN 309 and SN 492 matched with that of ARO, but these individuals also had novel restriction profiles in *Hae* **III** digestion. SN 319 showed SKJ-specific profile in *Hae* **III** digestion but also a novel profile in *Dde* I digestion. SN 37 had SKJ- or *Thunnus* -specific profile in *Dde* I digestion but a novel profile in *Hae* **III** which was observed in two individuals of the 1992-1994 sample. SN 708 and SN 53 showed an *Auxis*-like profile in *Hae* III but were LTT-like in *Dde* I digestion. SN 318 had novel restriction profiles in both digestions. Nucleotide sequences of the *CytB* fragment of these individuals were determined and the individuals were classified accordingly.

Nucleotide sequence analysis

Nucleotide sequence analysis on the *ATCO* fragment of EAF was not successful probably due to a large amount of non-specific PCR products. In contrast, amplification of the *CytB* fragment was considerably consistent and the nucleotide sequences were successfully determined in all standard species used. Therefore, the *CytB* fragments of juveniles having inconsistent RFLP patterns were also sequenced.

Nucleotide sequence data of the *ATCO* frag-

Table 2. Result of species identification of *Thunnus* sub-samples collected in 1992-1994 cruises. Parenthesis is standard length (mm) with standard deviation.

Species	1992	1993	1994	Total
YFT	72 (35.4±4.6)	22 (15.6±4.3)	62 (30.0±11.3)	156
BET	35 (32.7±4.2)	4 (16.7±8.6)	28 (20.5±8.7)	67
ALB	0	3 (12.2±3.4)	0	3
Total	107	29	90	226

Table 3. Result of species identification in sub-samples of tuna juveniles collected in 1997 cruise

Species Fragments	PNBT	YFT	SKJ	EAF	ARO	ATH	SOR	UI[3]
ATCO	164(4)*[1]	6	14	65				
CytB		1	132(1)*[2]	14	177(3)*[2]	2	7	1
Total	165	6	133	79	177	2	7	1
mm±SD*[4]	35.7±3.4	44.2±14.8	33.3±7.8	41.1±5	38.6±6.1	43.1±3.9	46±6	34.2

See Table 1 for the abbreviations of species.
*[1] Parenthesis is the number of individuals showing Atlantic type restriction profile (see Chow and Kishino, 1995).
*[2] Parenthesis is the number of individuals having restriction profiles inconsistent with those of standard.
*[3] Unidentified but determined as *Decapterus* sp. by nucleotide sequence analysis.
*[4] Fork length with standard deviation.

Table 4. Result of species identification for all scombrid juveniles collected in 1998 cruise

Species Fragments	PNBT	YFT	ALB	SKJ	EAF	ARO	ATH
ATCO	2	16	10	65			
CytB				39(1)*[1]	1	5(1)*[1]	1
total	2	16	10	39	1	5	1
mm±SD*[2]	18±1.4	39.8±20.5	83±49.6	67.2±53	17	80.8±49.9	36

See Table 1 for the abbreviations of species.
*[1] Parenthesis is number of individuals having restriction profiles inconsistent with those of standard.
*[2] Fork length with standard deviation.

ments of all *Thunnus* species were reported by Takeyama *et al.* (2001), and one representative sequence of each species with two types of BET were derived from GenBank data base under the accession numbers of AF 115272-AF 115278, AF 260431, and AF 260432. Nucleotide sequences of the *ATCO* region of one to two individuals from the other four species (ARO, ATH, SKJ and SOR) and those of the *CytB* from all standard species and juveniles having different RFLP patterns from the standards were deposited in DDBJ under the accession numbers of AB 098082 to AB 098121. The phylogenetic relationships among the genera inferred from the two gene segments were essentially the same, and the NJ tree based on the *CytB* sequence is shown in Fig. 5. Identification of six of the seven juveniles having inconsistent restriction profiles was unequivocal, in which two (SN 37 and 319) and four (SN 53, 309, 492 and 708) juveniles were assigned to SKJ and ARO, respectively. The homology survey using FASTA program in DDBJ indicated that the remaining individual (SN 318) had sequences similar to several carangids. The *CytB* data of two carangid species (*Selene* and *Trachurus*) derived from GenBank data base and of our archive *Decapterus* sp. data indicated that this individual was a scad mackerel belonging to the genus *Decapterus*.

Morphological investigation

Photographs of representative specimens of six species identified by DNA markers are shown in Fig. 6. As previously mentioned, some of the EAF juveniles were initially incorrectly identified as *Thunnus* sp. Once the genera were correctly identified by DNA, we observed characteristics associated with the eye that separated the two genera. The eyes of small juvenile *Thunnus* are much larger than the other; the eye diameter being equal to or larger than the distance between the snout and anterior edge of the eye orbit. According to the eye characteristic, we sorted the remaining *Thunnus*-like individuals and were able to identify 14 juveniles of

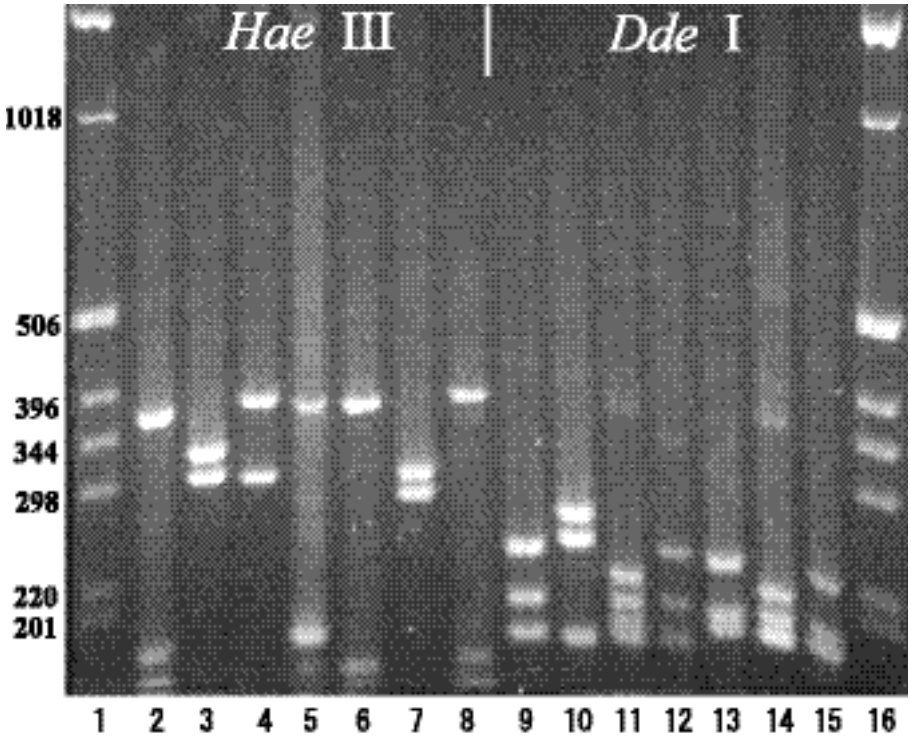

Fig. 4. Restriction profiles of the *CytB* fragments of juveniles (SN: Shunyo Maru sample in 1997 and 1998) having inconsistent RFLP patterns. First and 16th lanes are molecular marker (1kb DNA ladder, GIBCO BRL). Lanes for each samples are as follows; SN 309 (2 and 9), SN 318 (3 and 10), SN 319 (4 and 11), SN 492 (5 and 12), SN 708 (6 and 13), SN37 (7 and 14) and SN53 (8 and 15) (See text for these codes).

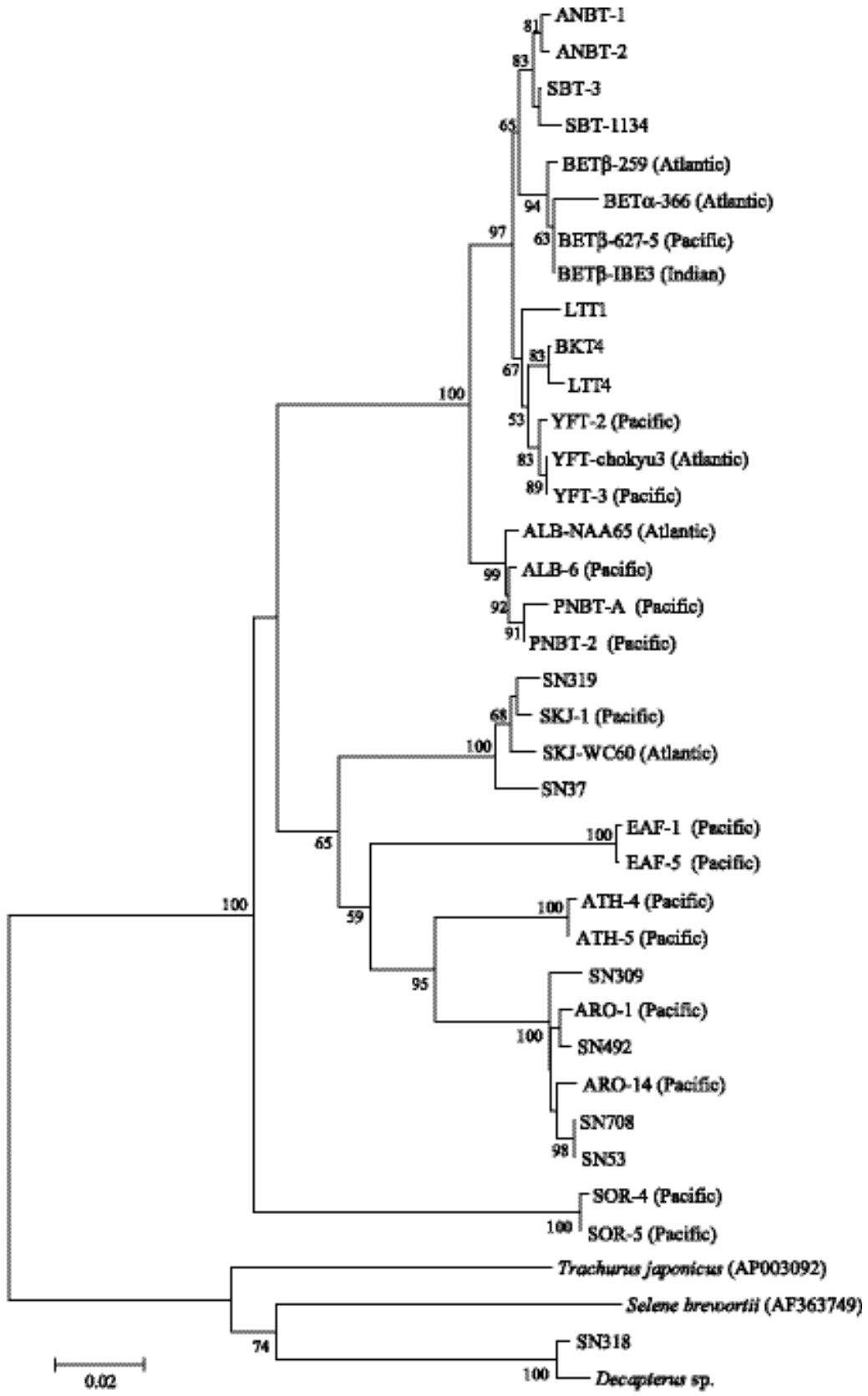

Fig. 5. Neighbour-joinng tree drawn by the *CytB* data of standard tuna species and those of juveniles (SN: Shunyo Maru sample) having inconsistent RFLP patterns. Bootstrapping was run with 1,000 replicates and values over 50% were shown at the nodes. See Table 1 for the abbreviations of species.

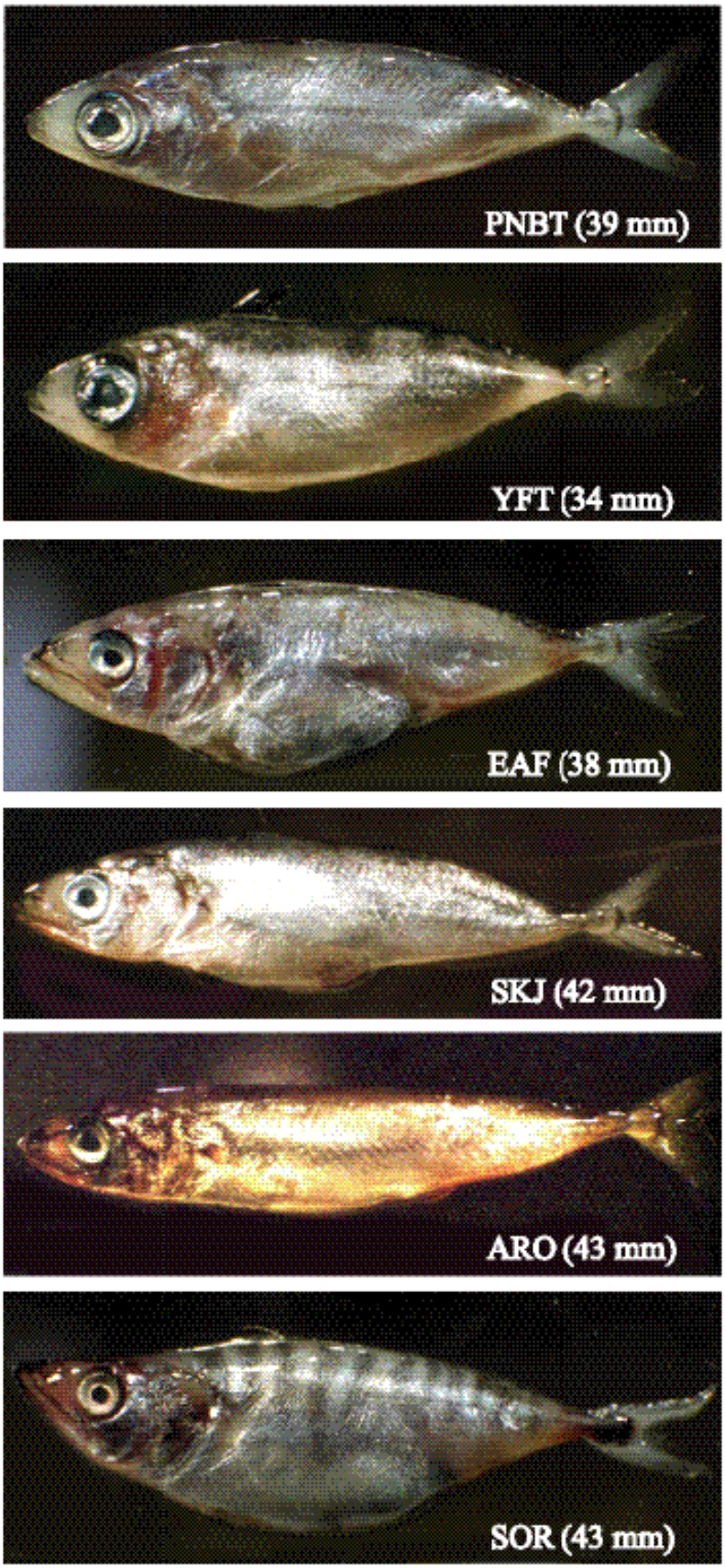

Fig. 6. Photographs of tuna juveniles. See Table 1 for the abbreviations. The fork length of each individual is shown in the parenthesis.

EAF from the 1997 samples that were confirmed as EAF by the *CytB* analysis (Table 3).

Four *Thunnus* species (ALB, BET, PNBT and YFT) were identified in this study. These and LTT are all members of the genus *Thunnus* that inhabit the western Pacific (Collette and Nauen, 1983). Adult PNBT and LTT can be differentiated from all other species of *Thunnus* by their gill raker counts, but the other three species of *Thunnus* (ALB, BET and YFT) share a similar range of gill raker counts (27-30 on average) (Gibbs and Collette, 1967). ARO and ATH can also be separated by their gill raker counts (44-47 and 37-43 gill rakers, respectively) (Wade, 1949). However, for each species, positive correlation was observed between gill raker count and fork length (FL) (Fig. 7). The gill raker counts in ARO of 27-54mm FL ranged from 28 to 42, but 49 gill rakers were observed in an individual of 91 mm FL which was comparable to that reported for juveniles and adults examined by Wade (1949). Only one ATH was available in this study, and this individual of 46 mm FL had 33 gill rakers which was less than that reported for larger juveniles and adults (Wade, 1949). Similar variation also occurred in juveniles of PNBT (32-47mm FL) and YFT (25-61mm), where the gill raker counts were significantly smaller than in the adults, ranging from 22 to 28 and from 19 to 26, respectively.

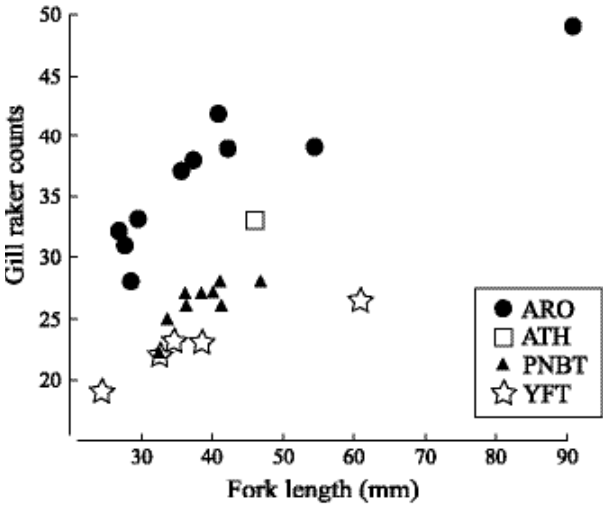

Fig. 7. Gill raker counts of small juveniles examined in this study. See Table 1 for the abbreviations.

Discussion

The reliability of PCR-RFLP analysis used in this study solely depends on the magnitude of intra-specific variation, which requires large number of wild samples. Intra-specific variation in the restriction profiles of the *ATCO* fragment was examined based on the large numbers of wild *Thunnus* species (Chow and Inoue, 1993; Takeyama *et al.*, 2001). In contrast, number of standard samples per species is small (n=3-9; see Table 1) for the *CytB* fragment. However, the species identification for juveniles using the *CytB* analysis indicated that intra-specific variation appears to be very low in ARO and SKJ, where the numbers of individuals having restriction profiles inconsistent with those of standard were 4 out of 182 and 5 out of 240, respectively. Although we need to further examine the intra-specific variation in the *CytB* fragment, especially for *Thunnus*, nucleotide substitution rate in the *CytB* fragment is lower than in the *ATCO* fragment (Chow and Kishino, 1995), and the distant locality of the standard samples in some species (Table 1) may compensate for the limited sample size. One individual (SN 318) that had completely different RFLP patterns was identified as *Decapterus* sp. by nucleotide sequence analysis. This individual may have simply been overlooked during the sorting and processing of the large numbers of samples, and this kind of error and intra-specific variation may not be significant for subsequent biological analyses.

The juvenile distribution and spatio-temporal variation of species caught during the research cruises corresponded well to the distributions of the larvae (Wade, 1951; Matsumoto, 1958; Nishikawa *et al.*, 1985). Larvae and small juveniles of ALB, BET, SKJ and YFT appeared to be widely distributed while those of PNBT and other Thunnini species were associated more with islands or coastal areas.

There have been series of morphological investigations to identify wild-caught small juveniles of Thunnini species (Schaefer and Marr, 1948; Wade, 1949, 1951; Matsumoto, 1958; Yabe *et al.*, 1966; Matsumoto *et al.*, 1972). The first dorsal fin of small juveniles in the genus *Thunnus* is densely pigmented and is one of the most prominent characteristics of this genus. This characteristic has been confirmed in labo-

ratory-reared juveniles of northern bluefin and yellowfin tunas (Mori *et al.*, 1971; Harada *et al.,* 1980; Kaji *et al.*, 1996; Miyashita, 2001). The first dorsal fin of juvenile *Euthynnus* is also heavily pigmented but usually not as dense as in the juveniles of *Thunnus*. A more slender body and less pigmentation in the first dorsal fin are observed in *Katsuwonus* and *Auxis*, and there is a large inter-space between the first and second dorsal fins in *Auxis* spp. SOR juveniles were easily identified by characteristic vertical stripes and pigmented pelvic fins. These characteristics were used for primary sorting of the juveniles in this study. Results of initial sorting of the juvenile scombrids based on their general morphology were fairly consistent with those of the DNA analysis at the generic level. We expected to observe some specific differences in morphology among the juvenile scombrids after they had been identified by DNA. In fact, DNA identification helped us to identify EAF that was initially sorted as *Thunnus*. On the other hand, no diagnostic difference in external characteristic was observed between ARO and ATH and among the species of the genus *Thunnus* (data not shown), necessitating DNA analyses.

Gill raker counts may be a useful character to identify some *Thunnus* species and ARO and ATH (Schaefer and Marr, 1948; Wade, 1949; Gibbs and Collette, 1967). However, the gill raker counts of small juveniles of ARO, ATH, PNBT and YFT used in this study were not comparable to those reported for the corresponding adults, indicating that this is not reliable for small juveniles. Wade (1949) reported that his smallest juveniles of ARO (32 mm in standard length; *A. tapeinosoma* in his study) and ATH (22mm) had gill raker counts comparable with those of adults. In contrast, our specimens had much lower counts for similar size or larger juveniles. It is possible that the sample of small juvenile ATH used by Wade (1949) may have also included ARO. Small ARO juveniles described by Wade (1949) ranged from 32 to 48mm SL and had 45-46 gill rakers. This size range corresponds to 35 to 50 mm FL in our study, where most of the individuals had gill rakers less than 40. It is not clear why there is a discrepancy of gill raker counts between the two studies. The gill raker counts of the largest ARO (91mm FL; 49 gill rakers) and YFT (61 mm FL; 26 gill rakers) in the present study were comparable to those of the adults. However, ARO smaller than 60mm FL and YFT smaller than 40mm FL had a much lower count of gill rakers than larger individuals. Schaefer and Marr (1948) and Wade (1949) indicated that species of *Thunnus* and *Auxis* could be reliably identified by gill raker counts beginning at a minimum size of 30mm SL and 20mm SL, respectively, when the gill rakers were fully developed. However, Potthoff (1974) reported that smaller *Thunnus* juveniles may have smaller number of gill rakers. Our results also indicate that the gill rakers are not fully developed until at least 60mm FL in both genera.

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中層トロールによって西部太平洋で採集されたまぐろ類小型稚魚の遺伝学的種判別

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ソウダガツオ類，スマ，カツオ，ハガツオ，マグロ属全種の基準標本についてミトコンドリアDNAの多型解析から種判別用マーカーを得，中層トロールで採集した小型稚魚（936個体：体長8～190mm）の種判別に応用した。1992年から1994年に西部熱帯太平洋海域（2°N～15°N, 135°E～157°E）で採取された標本ではカツオ，ビンナガ，キハダ，メバチの4種，1997年と1998年に亜熱帯海域（24°N～30°N, 123°E～131°E）で採取された標本ではハガツオ，マルソウダ，ヒラソウダ，スマ，カツオ，ビンナガ，キハダ，クロマグロの8種が確認された。マルソウダとヒラソウダ間及びマグロ属種間を外部形態によって分離することは困難であり，鰓耙数についても尾叉長60mm以下の稚魚では十分に発達しておらず，有効な指標ではないことが示された。

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