



# Little population structuring and recent evolution of the Pacific saury (*Cololabis saira*) as indicated by mitochondrial and nuclear DNA sequence data

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## ABSTRACT

Genetic population structure of the Pacific saury (*Cololabis saira*) was investigated using nucleotide sequence analysis on the mitochondrial DNA control region (355–361 bp). Although the left domain of the control region is known to be highly variable in many species, extremely low nucleotide and haplotype diversities ( $\pi=0.17\%$  and  $h=0.418$ , respectively) were observed in a total of 141 individuals collected from five distant locales (East China Sea, Sea of Okhotsk, northwest Pacific, central North Pacific and northeast Pacific). No significant haplotype frequency differences were detected among widely separated samples, therefore we were unable to reject the null hypothesis of no genetic structuring in the Pacific saury population. Moderate levels of nucleotide substitution ( $p$ -distance) were observed between the Pacific saury and its Atlantic counterpart (*Scomberesox saurus*) in the control region (7.44%), cytochrome *b* gene (4.64%), and internal transcribed spacer (ITS1) (11.49%), indicating that the low sequence diversity of the control region in the Pacific saury is not due to the slow mutation rate. The molecular data suggest the Pacific saury may be a relatively recent offshoot among the extant members in the family Scomberesocidae.

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## 1. Introduction

The Pacific saury (*Cololabis saira*) is one of the dominant epipelagic nekton species in the North Pacific (Brodeur, 1988) and is among the most abundant species caught in the North Pacific Transition Region (Percy, 1991; Percy et al., 1996; Brodeur et al., 1999). It is also commonly collected in coastal fish collections from both sides of the Pacific (Ueno et al., 1990; Brodeur et al., 2003). This species is widely distributed in the north Pacific from Baja California to Alaska and western Pacific including Sea of Okhotsk, Japan Sea, East and South China Sea (Schaefer and Reintjes, 1950; Ahlstrom and Casey, 1956; Parin, 1970). This species has been the mainstay of a directed fishery concentrated in the northwestern Pacific with an annual catch exceeding 400 thousand tonnes (FAO Fisheries Statistics: <http://www.fao.org/>).

Understanding stock structure is important to manage fishery resources and to maintain the stock at desirable levels, and the stock structure of the Pacific saury has been investigated using different approaches. Odate (1956) found differences in vertebrate counts between juvenile samples from the large (more than 29 cm in knob length) and the medium (24–29 cm) sized saury, concluding that there are at least two stocks in the north Pacific. Sugama (1959) and Hotta (1960), using otolith structure observation, agreed with the stock

structure proposed by Odate (1956). Heterogeneous growth profiles were observed between samples from the northwest and northeast Pacific (Watanabe et al., 1988). On the other hand, Fukushima et al. (1990) suggested that two size groups were derived from single stock with different hatching seasons, based on the relationships between the size distribution of saury and spawning season. Spawning locations and intensity change from season to season, although spawning activity can be seen year round in the northwest Pacific (Hatanaka, 1955; Iwahashi et al., 2006).

Numachi (1973) used enzyme electrophoresis analysis to investigate the genetic population structure of the Pacific saury, in which allele frequencies at two allozyme loci encoding malate dehydrogenase (MDH) and  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ -GDH) were compared among 199 local samples comprising 21,000 individuals collected from western to eastern North Pacific. He found no allele-frequency heterogeneity among local samples or between size or year classes and concluded that there must be a high level of gene flow across the North Pacific. A decade later, additional genetic markers, encoding isocitrate dehydrogenase (IDH) and phosphoglucosmutase (PGM), were used to examine saury samples collected from central to western North Pacific (Hara et al., 1982a,b; Kijima et al., 1984), which suggested that the Pacific saury populations were not genetically homogeneous.

The present study was undertaken to evaluate the levels of intraspecific mitochondrial (mt) DNA diversity and genetic differentiation among saury

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samples across the North Pacific. We found extremely low variability in the mtDNA control region of the Pacific saury. Thus, to determine the cause of the low intraspecific sequence diversity in addition to the control region, sequences of mtDNA cytochrome *b* gene and nuclear internal transcribed spacer 1 (ITS1) were also analyzed and compared with those of the Atlantic counterpart (*Scomberesox saurus*).

## 2. Materials and methods

### 2.1. Fish samples

A total of 141 Pacific saury were collected from the East China Sea (EC)(*n*=31), the Okhotsk Sea (OH)(29), the northwest Pacific (WP)(33), the North-central Pacific (CP)(22) and northeastern Pacific (EP)(26) during 2002 and 2003 (Table 1). Whole frozen fish or dissected muscle tissues preserved in ethanol onboard were transferred to the laboratory. Ethanol-preserved muscle tissues from five Atlantic saury (*Scomberesox saurus*) were collected in the northwest Atlantic and provided by Dr. B. B. Collette (Smithsonian Institute, Washington DC) and Drs. P. Bentzen and S. Clifford (Dalhousie University, Halifax, Nova Scotia, Canada).

### 2.2. DNA analysis

Crude DNA was extracted using a DNA extraction kit (GenomicPrep Cells and Tissue DNA Isolation Kit, Amersham Bioscience). The primers used to amplify a mitochondrial DNA segment containing left domain of control region via the polymerase chain reaction (PCR) were CB3RLS (5′-CACATTAAACCAGAATGATA-3′) and CSBDHLS (5′-ATGGACCT-GAAATAGGAACC-3′), and those used to amplify 5′ region of the cytochrome *b* gene were MtDNAGlu-F (5′-GCTTGAAAAACCCGTTGT-3′) and CytB677R (5′-TAGGAGAAGTATGGGTGAA-3′)(Chow et al., 2003). The first internal transcribed spacer region (ITS1) of the ribosomal RNA gene cluster was amplified using a conserved primer pair (ITS1 and 5.8S) (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>). Cloning of PCR products was performed for ITS1 using a pGEM-T Easy Vector System I (Promega), and single clone from an individual was arbitrarily chosen for subsequent nucleotide sequence analysis.

PCR amplification was carried out in a 15 µl reaction mixture using an initial denaturation at 95 °C for 2 min, followed by 30 cycles of amplification (denaturation at 95 °C for 0.5 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min) with a final extension at 72 °C for 10 min. PCR products were treated with ExoSAP-IT (USB Corp., Cleveland) to decompose unincorporated oligonucleotide primers. Both strands were sequenced using ABI Big-dye Ready Reaction kit (Applied Biosystems, Foster City) and forward and reverse PCR primers, and run on an automated sequencer (ABI Prism310). Alignment of the nucleotide sequences with the ClustalW algorithm and calculation of nucleotide substitution between sequences were performed using MEGA 3.1 (Kumar et al., 2004). We used percent nucleotide difference (*p*-distance) between sequences in the Pacific saury without weighting between substitutions including indels treated as a fifth substitution. An unrooted neighbor-joining (NJ) phylogenetic tree (Saitou and Nei, 1987) was constructed using MEGA 3.1 based on nucleotide substitutions in the control region of haplotypes.

**Table 1**  
Catch locality, date and mean body length of the Pacific saury samples used in this study

code	locality	Year	Month	Latitude	Longitude	BL* (cm)±SD
EC	East China Sea	2003	January	32.4 (N)	128.4 (E)	32.0±1.6 (31) <sup>§</sup>
OH	Sea of Okhotsk	2003	September	44.3 (N)	144.5 (E)	20.0±1.1 (29)
WP	Northwest Pacific	2002	July	42.4 (N)	145.1 (E)	20.1±1.1 (33)
CP	central north Pacific	2002	June	37.2 (N)	176.6 (E)	17.5±2.3 (22)
EP	Northeast Pacific	2002	August	44.0 (N)	125.0 (W)	28.1±1.5 (26)

\*BL: knob length; <sup>§</sup>sample size.

**Table 2**  
Polymorphic nucleotide sites in the mitochondrial DNA control region from 141 Pacific saury, and haplotype distribution in five local samples

types	variable sites	samples					total
	111111112222 11222226467788990599 459012347520979174101	EC	OH	WP	CP	EP	
1	–T-----GTAGTAATTACGA	27	18	27	16	19	107
2	–.-----.....C.	0	5	2	2	3	12
3	–.-----.....G.....	2	0	0	1	1	4
4	–.-----.....A.....	0	1	1	1	0	3
5	–.-----.....T..	0	0	1	1	0	2
6	–.-----.....G	0	1	0	0	0	1
7	–.-----.....T..	1	0	0	0	0	1
8	–.-----.....C.....	0	0	1	0	0	1
9	–.-----.....C.....	0	0	1	0	0	1
10	–.-----..G.....	0	0	0	0	1	1
11	–.-----..C.....	0	0	0	0	1	1
12	–.-----..A.....	0	0	0	0	1	1
13	A.-----.....	0	1	0	0	0	1
14	–.-----.....GT..	0	1	0	0	0	1
15	–.-----.....A.T..	0	1	0	0	0	1
16	–.-----.....G....A.	1	0	0	0	0	1
17	–.-----.....GG.....	0	0	0	1	0	1
18	–CTATATA.....	0	1	0	0	0	1
total		31	29	33	22	26	141
<i>h</i>		0.243	0.599	0.333	0.476	0.465	0.418

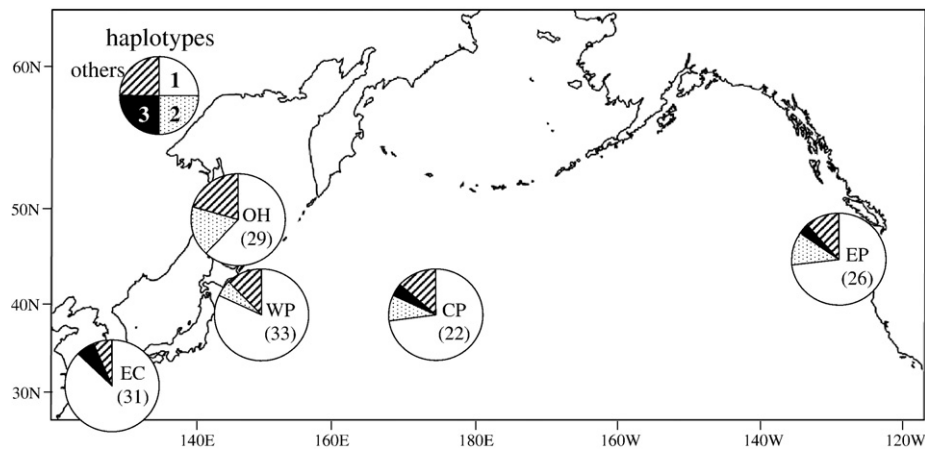
Chi-square analysis was performed to test the heterogeneity of the haplotype frequency distributions between samples of the Pacific saury using the Monte Carlo simulation method of Roff and Bentzen (1989) using 1000 randomizations of the data. Spatial genetic variation was also quantified by estimating analog of *F*<sub>ST</sub> (*Φ*<sub>ST</sub>) using analysis of molecular variance (AMOVA; Excoffier et al., 1992) implemented in the program ARLEQUIN vers. 2.0 (Schneider et al., 1997). The historical population demography was evaluated using Tajima's *D* statistic (Tajima, 1989), Fu's *F*<sub>s</sub> (Fu, 1997) and mismatch distribution test (Rogers and Harpending, 1992). Tajima's *D* and Fu's *F*<sub>s</sub> were calculated and tested by 1000 replicated randomizations by ARLEQUIN. The mismatch distributions both observed and expected under the sudden expansion model were generated and the goodness-of-fit between them was tested, using 10000 bootstrap replicates as implemented in ARLEQUIN. Additionally the mismatch distribution expected under the constant model that population size would have been at equilibrium was generated using DnaSP ver. 4.0 (Rozas et al., 2003), compared with the former distributions.

Percent nucleotide difference (*p*-distance) including indels as a fifth substitution and Kimura's two parameter distance (K2P) with pairwise deletion option for indels were calculated to compare divergence between the Pacific and Atlantic saury species in three segments (control region, cytochrome *b* and ITS1) using MEGA 3.1.

## 3. Results

### 3.1. MtDNA control region sequence diversity of the Pacific saury population

Eighteen haplotypes were identified in the 141 fish, and 21 variable nucleotide sites were observed (Table 2) (DNA Data Bank of Japan: DDBJ accession numbersAB355938–AB355955). Haplotype 1 was most commonly observed over all samples, with a frequency of 62.1 to 87.1% per sample. Nucleotide sequence diversity (*π*) between all sequences obtained ranged from 0 to 2.49%, with a mean of 0.17%±0.05. Insertion of a di-nucleotide repeat (TA) was observed in one individual (haplotype 18) from Sea of Okhotsk (Table 2), and this single sequence was responsible for elevating the maximum value of sequence difference. Haplotype diversity (*h*) ranged from 0.243 (EC) to 0.599 (OH) with an overall estimate of 0.418 (Table 2).



**Fig. 1.** Pie graph representation for the frequencies of types 1 to 3 and the remaining haplotypes which were pooled due to their relatively low frequency into 'others'. See Table 1 for abbreviated local sample codes.

Frequencies of the 18 haplotypes are presented in Fig. 1, with types 4–18 pooled together due to their low frequency. Chi-square analysis of haplotype frequencies revealed no significant heterogeneity among the five local samples ( $P=0.5$ ). The global  $\Phi_{ST}$  was 0.004, with no significant proportion of genetic variation separating samples (0.38% of variance,  $P=0.293$ ). Both Tajima's  $D$  and Fu's  $F_s$  had significant negative values ( $D=-2.09$ ,  $P=0.002$  and  $F_s<-10^3$ ,  $P<0.001$ , respectively), indicating distinct departure from a null hypothesis with selective neutrality and population equilibrium. The mismatch distribution observed in the entire Pacific saury population showed better fitting with expectation under the sudden expansion model, being supported by test of the goodness-of-fit (sum of squared deviation:  $SSD=0.0006$ ,  $P=0.650$ ).

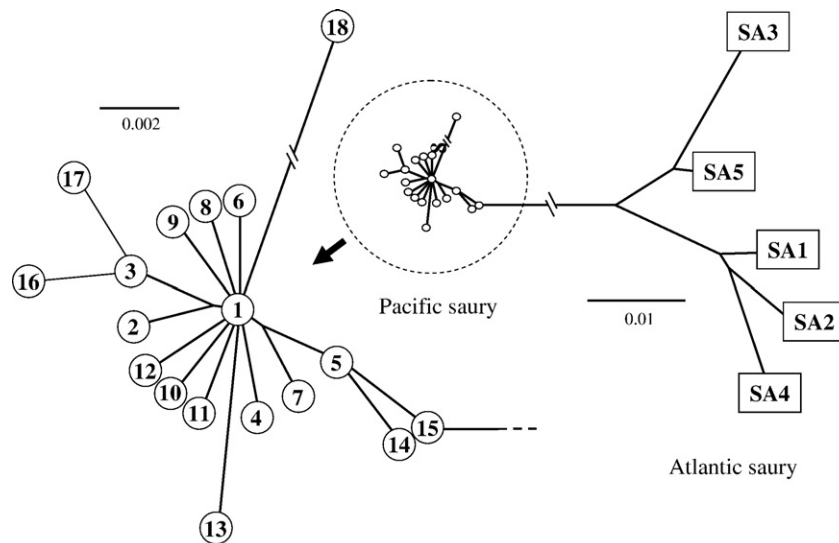
### 3.2. Nucleotide sequence difference in three DNA segments between the Pacific and Atlantic saury species

To determine whether the low sequence diversity in the Pacific saury was due to a slow mutation rate in the control region, nucleotide substitution between the Pacific and Atlantic sauries was compared in three DNA regions.

All five Atlantic saury had unique control region sequences (AB355956 to AB355960). Nucleotide sequence difference in the control region between these two species was  $7.47\pm 1.24$  ( $p$ -distance) and  $7.91\pm 1.40$  (K2P). The phylogenetic relationships among the 18 haplotypes of Pacific saury (Table 1) and the five of Atlantic saury are depicted in Fig. 2. Inclusion of Atlantic haplotypes splits the network into two clades and also shows a much lower diversity profile in the Pacific saury compared to the Atlantic saury. The Pacific saury is characterized by a star-like topology, where all haplotypes are distributed around haplotype 1 and connected to each other by a one to two nucleotide difference, except haplotype 18.

Partial sequences of cytochrome *b* gene (611 bp) were determined for an arbitrarily selected six Pacific saury and for all five individuals of Atlantic saury, in which two and three haplotypes (AB355961 to AB355965) were observed, respectively. Nucleotide sequence difference between species was  $4.64\pm 0.86$  ( $p$ -distance) and  $4.88\pm 1.13$  (K2P).

Size of entire ITS1 region sequenced for three individuals each of Pacific and Atlantic saury ranged from 397 to 399 bp and from 376 to 396 bp, respectively (AB355966, AB355967, AB447518 to AB447521). All sequences were unique, and nucleotide sequence difference



**Fig. 2.** Unrooted neighbor-joining network among 18 haplotypes of the Pacific saury (open circle) and five of the Atlantic saury (open rectangle) based on percent nucleotide difference ( $p$ -distance) without weighting between substitutions including indels treated as a fifth substitution. The numbers and codes in the circles and rectangles correspond to haplotype numbers shown in Table 2 and those deposited in DDBJ, respectively.

**Table 3**

Summary of percent nucleotide substitution (*p*-distance and K2P in the parenthesis) between congeneric fish species and between the Pacific and Atlantic sauries in three DNA segments

	cytochrome <i>b</i> *	control region*	ITS1 <sup>§</sup>
<i>Takifugu chinensis</i> / <i>T. rubripes</i>	-	-	0.18 (0.18) <sup>1</sup>
<i>Merluccius productus</i> / <i>M. gayi</i>	-	-	1.28 (0.76) <sup>2</sup>
<i>Salvelinus alpinus</i> / <i>S. malma</i>	0.78 (0.78)	3.19 (3.03)	1.34 (0.53) <sup>3</sup>
<i>Thunnus albacares</i> / <i>T. thynnus</i>	1.46 (1.48)	10.68 (11.27)	7.85 (4.62) <sup>4</sup>
<i>Thunnus albacares</i> / <i>T. alalunga</i>	2.43 (2.48)	14.84 (14.27)	8.69 (5.13) <sup>4</sup>
<i>Clupea harengus</i> / <i>C. palasii</i>	2.50 (2.55)	10.51 (11.52)	-
<i>Engraulis encrasicolus</i> / <i>E. japonicus</i>	2.50 (2.55)	4.99 (5.22)	-
<i>Trachurus japonicus</i> / <i>T. trachurus</i>	3.47 (3.57)	5.56 (5.80)	-
<i>Chaunax abei</i> / <i>C. tosaensis</i>	3.99 (4.15)	7.71 (7.47)	-
<i>Cololabis saira</i> / <i>Scomberesox saurus</i>	4.64 (4.88)	7.44 (7.91)	11.49 (5.23)
<i>Latimeria chalumnae</i> / <i>L. menadonesis</i>	4.67 (4.90)	5.33 (5.58)	-
<i>Alosa alosa</i> / <i>A. pseudoharengus</i>	5.16 (5.39)	31.40 (20.69)	-
<i>Salvelinus malma</i> / <i>S. fontinalis</i>	5.43 (5.69)	6.13 (5.65)	7.22 (5.22) <sup>3</sup>
<i>Auxis rochei</i> / <i>A. thazard</i>	6.53 (6.95)	10.49 (11.16)	-
<i>Salvelinus fontinalis</i> / <i>S. leucomaenis</i>	7.36 (7.85)	5.88 (5.65)	11.24 (8.20) <sup>3</sup>
<i>Beryx decadactylus</i> / <i>B. splendens</i>	7.44 (8.29)	12.44 (12.99)	-
<i>Contigaster coronatus</i> / <i>C. rivulatus</i>	7.49 (8.04)	15.46 (16.89)	-
<i>Anguilla australis</i> / <i>A. anguilla</i>	7.54 (7.54)	21.72 (22.03)	-
<i>Polymixia japonica</i> / <i>P. lowei</i>	8.54 (9.20)	11.31 (10.53)	-
<i>Labeo batesii</i> / <i>L. senegarensis</i>	9.82 (10.69)	19.07 (19.40)	-
<i>Oncorhynchus masou</i> / <i>O. mykiss</i>	9.83 (10.07)	8.07 (7.96)	6.85 (5.22) <sup>5</sup>
<i>Hemibarbus mylodon</i> / <i>H. labeo</i>	12.54 (13.98)	15.00 (14.30)	-
<i>Cobitis sinensis</i> / <i>C. striata</i>	14.02 (15.95)	11.73 (12.12)	-
<i>Hemibarbus mylodon</i> / <i>H. longirostris</i>	14.35 (16.80)	17.61 (16.85)	-
<i>Hemibarbus labeo</i> / <i>H. longirostris</i>	14.56 (16.94)	15.00 (12.92)	-
<i>Polypterus senegalus</i> / <i>P. ornatipinnis</i>	16.39 (18.83)	22.47 (24.92)	-
<i>Merluccius hubbsi</i> / <i>M. gayi</i>	-	-	13.32 (8.45) <sup>2</sup>
<i>Merluccius hubbsi</i> / <i>M. productus</i>	-	-	13.32 (8.25) <sup>2</sup>

\*Data of partial sequences of mitochondrial cytochrome *b* gene and control region were derived from the Mitochondrial Genome Database of Fish (<http://mitofish.ori.u-tokyo.ac.jp/>).

<sup>§</sup>ITS1 data were obtained from <sup>1</sup>Reza et al. (2008), <sup>2</sup>Perez et al. (2005), <sup>3</sup>Pleyte et al. (1992),

<sup>4</sup>Chow et al. (2006), <sup>5</sup>Domanico et al. (1997).

between species was  $11.49\% \pm 1.32$  (*p*-distance) and  $5.23\% \pm 1.06$  (K2P).

#### 4. Discussion

Our examination of mtDNA control region sequence variation detected no significant differences among five different geographic locations where Pacific saury were collected over a wide area of the North Pacific Ocean. The extremely low level of genetic variation found in the control region both within and among the samples provides a weak test for population differentiation, and consequently it is not surprising that we did detect very little genetic structuring (Fig. 2). Our study also contradicts results from previous allozyme analysis (Hara et al., 1982a,b; Kijima et al., 1984) that suggested saury populations in the north central to western Pacific were composed of several subpopulations. However, the statistical procedure used in those studies may have been flawed, because *t*-tests of allele frequencies were conducted on a large number of pairs (1035 to 3003) repeatedly sampled from 46 to 78 local samples without correcting the significance level for multiple comparisons. We re-examined their raw allozyme data in order to investigate heterogeneity among year or location of collection, and fish size groups within and between year samples, and found no significant heterogeneity in all combinations examined (results not shown). Thus, results of both the mtDNA analysis presented here and the previous allozyme analyses do not reject the null hypothesis of panmixia in the Pacific saury population.

The mitochondrial DNA control region is regarded as the fastest evolving part of the mitochondrial genome in many vertebrate species (Brown, 1985). However, the mtDNA control region sequence diversity of the Pacific saury population was an order of magnitude or more lower than the estimates obtained from many fish species which generally show 2 to 8% intra-specific nucleotide sequence diversity (Fujii and Nishida, 1997; Tabata and Taniguchi, 2000; Ely et al., 2005;

Nakadate et al., 2005). A considerably smaller diversity profile was observed in the Pacific saury compared to the Atlantic saury (Fig. 2). The haplotype diversity (0.418) was also considerably lower than the estimates of many marine fish species which are usually larger than 0.9 (see for example Ishikawa et al., 2001 and references therein). There may be several factors responsible for low genetic variability; 1) slow mutation rate, 2) evolutionarily recent origin, 3) small effective population size, 4) non-random lineage sorting, and 5) lineage extinction due to a severe bottleneck.

Nucleotide sequence divergences between the Pacific and Atlantic sauries in three DNA regions were compared with those between congeneric fish species. The nucleotide sequences of 45 species belonging to 20 genera were derived from the Mitochondrial Genome Database of Fish (<http://mitofish.ori.u-tokyo.ac.jp/>) and from previous publications (Table 3). The cytochrome *b* and control region sequences of a species derived from the database were of the same individual. Cytochrome *b* gene sequences derived from these species were homologous to those of Pacific saury. Since it was not possible to determine the homologous region in the control region between distantly-related taxa, 400 bp left domain sequences were adopted. No large departure was observed between *p*-distance and K2P in cytochrome *b* and in control region sequences. It is a little surprising that the *p*-distance among congeneric species was consistently and sometimes considerably larger than K2P in ITS1, which was apparently due to the number of indels present between species. Nucleotide sequence divergence largely varied even between the congeneric (supposed to be closely related) species, where the *p*-distance ranged from 0.78 to 16.39% in the cytochrome *b* gene, from 3.19 to 31.4% in the control region and from 0.18 to 13.32% in ITS1. Nucleotide sequence divergences between the Pacific and Atlantic sauries in three DNA regions are well in the range of those between congeneric fish species, indicating a moderate mutation rate in the control region sequence of Pacific saury. It is unlikely that random extinction of mtDNA lineages caused by population dynamics may be responsible for selecting lineages sharing similar sequences.

The historical demographic indices, the mismatch distributions and the star-like tree topology (Fig. 2) shown in the Pacific saury have been assumed to be the signature of a drastic bottleneck followed by explosive population expansion (Magoulas et al., 1996). Sequence diversity of the Pacific saury population was ten fold lower than that found in a recently collapsed and recovered stock of Atlantic sardine (*Sardina pilchardus*) showing nucleotide sequence diversity ranging from 1.4 to 2.3% (Atarhouch et al., 2006). Thus, the Pacific saury may be grouped into the category of marine fish having small values of both haplotype and nucleotide diversities (Grant and Bowen, 1998), which may be due to a recent population bottleneck or founder event by single or a few mtDNA lineages.

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