

Genetic Diversity and Population Structure of Chum Salmon in the North Pacific

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Abstract: Analysis of population structure and stock identification has been carried out by a number of different methods. In this study, we used SNPs in 730bp long sequences of the mtDNA COIII-ND3-ND4L region in order to examine genetic diversity and population structure of chum salmon in the North Pacific. In a total of 201 individuals analyzed, 48 from Korea, 44 from Japan, 45 from Alaska, 29 from Canada, 20 from Washington (USA) and 15 from east Sakhalin Island (Russia), as many as 55 variable sites comprising 51 different haplotypes were identified. Some of the haplotypes were population-specific so that they can be used for stock identification. Canadian and one of the Korean populations showed relatively high levels of haplotype diversity (h , $0.70 \pm 0.11 \sim 0.92 \pm 0.06$) while Russian and one of the Korean populations showed low variability (h , $0.37 \pm 0.15 \sim 0.42 \pm 0.16$). Pairwise FST and AMOVA analyses of the populations revealed that Korean and Japanese chum salmon are genetically indistinguishable and so are Russian, Alaskan, and Canadian salmon ($F_{ST} < 0.1$). Therefore, we suppose that the chum salmon populations in the North Pacific can be grouped, in general, into three genetic population units: a Korea-Japan unit, a Russia (east Sakhalin)-Alaska-Canada unit, and a Washington (USA) unit.

Keywords: chum salmon, genetic diversity, population structure, *Oncorhynchus keta*, ND3

INTRODUCTION

Stock identification and population structure analysis establish a basis for management of fisheries resources. Since molecular markers were introduced in stock assessment and conservation of salmon populations (Ferguson et al. 1995), microsatellite DNA (Beacham 1996; Beacham et al. 2003) and single nucleotide polymorphisms (SNPs; Sato et al. 2001, 2004) have recently been in use for such studies. SNP methods that use differences in DNA sequences are becoming more popular because of their easy standardization and application to high throughput assay systems (Brumfield et al. 2003; Melton 2003).

For chum salmon (*Oncorhynchus keta*) populations distributed widely in the North Pacific, Sato et al. (2004) examined SNPs in about 500bp sequences at the 5' end of the mitochondrial (mt) DNA control region. They analyzed as many as more than 2,100 individuals and found 20 variable sites defining 30 haplotypes. The variability appeared rather small (haplotype diversity, 0.63 ± 0.01) taking into account the relatively large number of individuals analyzed. Previ-

ously, Park et al. (1993) reported a low level of chum salmon intraspecific variation in the control region.

The present study investigated SNPs in the mtDNA COIII-(tRNA-gly)-ND3-(tRNA-Arg)-ND4L region, about 730bp long sequences. This region had been used for phylogenetic analysis of Pacific salmon (*Oncorhynchus*) (Domanico and Phillips 1995; McKay et al. 1995). Because it was known to have a high level of variability compared to other regions of the mtDNA (Thomas and Beckenbach 1989; Domanico and Phillips 1995), we also used this region in analyzing genetic diversity and population structure of chum salmon in the North Pacific.

MATERIALS AND METHODS

Samples

A total of 201 individual chum salmon were obtained from 11 populations on both sides of the North Pacific and in the Bering Sea (two Korean, two Japanese, one Russian (east Sakhalin), two Canadian, one Washingtonian (USA) and

three Alaskan populations) (Table 1; Fig. 1.). The sample size for each population ranged from 13 to 35 individuals. These numbers appear to be small, but in fact they are large enough for analysis of population diversity using nucleotide sequence data. Studies on optimal sequencing strategies for surveying molecular genetic diversity revealed that the realistic values for optimum sample size are relatively small, three to 10 (Pluzhnikov and Donnelly 1996), or 8 or fewer individuals (Felsenstein 2006). In addition, populations with the lower variability require a smaller sample size than more heterogeneous populations (Israel 1992).

The individual samples were collected when fish returned to their natal rivers. Liver or muscle tissues were taken from each individual and stored in ethanol until DNA extraction.

DNA Extraction and PCR Amplification

DNA was extracted from the stored specimens using a blood and cell culture DNA midi kit or a DNeasy tissue kit (Qiagen, Germany) following the manufacturer's protocol. The target DNA, COIII-ND3-ND4L region of the mitochondrial DNA, was amplified by PCR with the primer pair of COIII forward (5'-TTACAATCGCTGACGGCG-3') and ND4L reverse primers (5'-GGTGCGGTG AAACGC-GAGTC-3'). The reaction mixture consisted of Hotstar Taq polymerase (2.5unit, Qiagen), 10X PCR buffer (5 µl), 10mM each dNTPs (2.5 µl), 25 pmoles of each primer and 0.5–1.0 µg DNA. PCR procedures were as follows: preheating at 95°C for 15 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 1 min, and completion with final extension at 72°C for 10 min. PCR products were examined by 1% agarose-gel electrophoresis and purified by a PCR purification kit (Takara, Japan). The amplified DNA was either cloned into the vector pCR2.1-TOPO with the TOPO-TA cloning system (Invitrogen, USA) or directly sequenced. The insert DNA

or the purified DNA was sequenced using Automated DNA sequencer 377 or 3100 (Applied Biosystems, USA).

Population Genetic Data Analysis

The sequence data were aligned by ClustalW (Thompson et al. 1994). Haplotype diversity (h) and nucleotide diversity (π) were calculated using the ARLEQUIN program (Schneider et al. 2000). The extent of population subdivision was evaluated by analysis of molecular variance (AMOVA, Excoffier et al. 1992) and by estimation of pairwise F_{ST} values (Slatkin 1995). The Tamura and Nei model (1993) with a gamma distribution parameter alpha 0.3441 was applied in the AMOVA analysis.

RESULTS

Genetic Diversity

Nucleotide sequences of the mitochondrial DNA COIII-ND3-ND4L region were obtained in 201 individuals from two Korean (KS1 and KS2), two Japanese (JS1 and JS2), one Russian (east Sakhalin, RSC), three Alaskan (ALS1, ALS2 and ALS3), two Canadian (CS1 and CS2) and one Washingtonian (USA, AS) chum salmon populations (Table 1; Fig. 1). Comparison of the 730bp sequences revealed a total of 55 single nucleotide polymorphism (SNP) sites. These SNPs discriminate 51 different haplotypes (A1-A17, B1-B21, C1-C4, D1-D9; Table 2). Haplotype A1 is the most common sequence in Korean and Japanese populations but it is not observed in any other populations except a single individual in the Russian population. Haplotypes A2 through A17 that are differentiated from A1 by one or two nucleotides also occur only in Korean and Japanese populations. On the other hand, haplotype B1 is the most common sequence in Russian, Alaskan and Canadian populations. This haplotype is also observed in a few individuals in Washingtonian, Ko-

Table 1. Sampling locations with the latitudes and the longitudes, year of collection (Y) and the number of chum salmon samples (N).

Sample name	Population	Y	Sampling location	Latitude / Longitude	N
KS	KS1	1999/2000/2001	Namdae River, KangwonDo	38.44'20"N, 128.37'17"E	6/14/15
	KS2	2000	Wangpi River, KyoungsangbukDo	36.59'23"N, 129.24'15"E	13
JS	JS1	1998/2000	Shokanbetsu River, Hokkaido	43.46'59"N, 141.31'59"E	2/22
	JS2	2000	Hakodate, Hokkaido	41.46'59"N, 140.45'00"E	20
AS	AS	1999/2000	Quilcene River, Washington	47.49'21"N, 122.52'28"W	6/14
CS	CS1	2000	Nitnat River, Vancouver Island	48.49'12"N, 125.06'00"W	14
	CS2	2001	Fraser River, British Columbia	49.06'00"N, 123.09'58"W	15
ALS	ALS1	1991	Noatak River, Alaska	66.58'53"N, 162.30'23"W	15
	ALS2	1994	Gisasa River, Alaska	65.15'44"N, 157.40'45"W	15
	ALS3	1995	American River, Alaska	65.25'30"N, 165.46'57"W	15
RSC	RSC	2003	Taranai River, Sakhalin	46'37'30"N, 142'26'00"E	15
Total					201

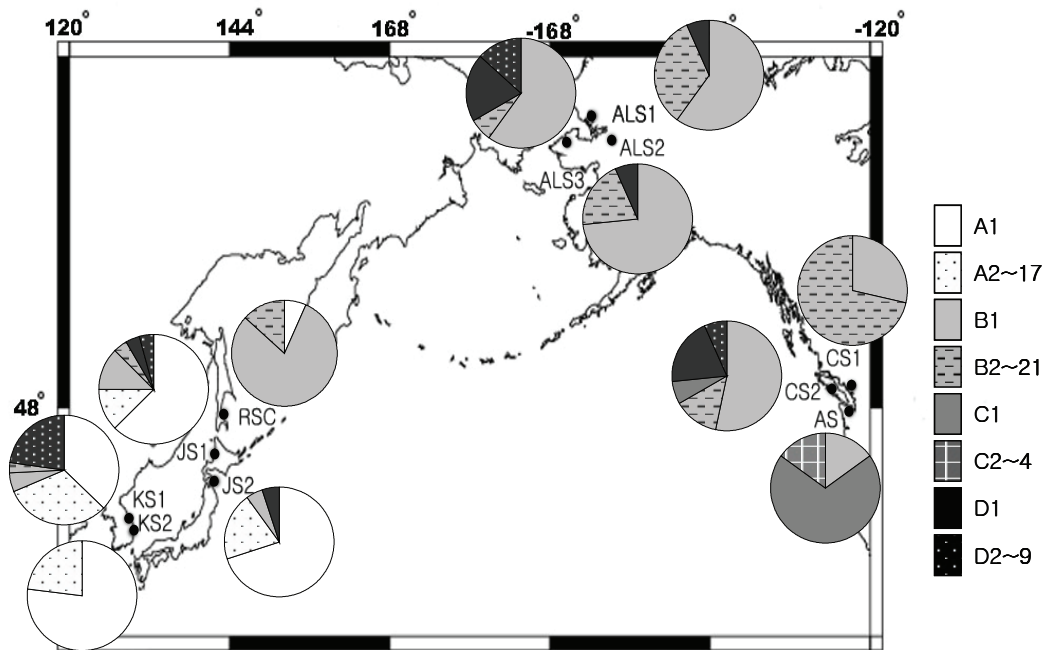


Fig. 1. Pie chart diagrams showing the haplotype frequency in each population of chum salmon.

rean and Japanese populations, showing its wide distribution around the North Pacific. B1 related haplotypes, B2 through B21, occur mostly in Canadian and Alaskan populations. Haplotype C1 and its derivatives, C2 to C4, occur almost exclusively in the Washingtonian population. Haplotype D1 is another common sequence observed in both eastern and western Pacific populations such as Japanese, Canadian and Alaskan populations. D1 derived haplotypes, D2 to D5, however, occur only in Korean populations. Haplotypes C1 and D1 are different from B1 by a single nucleotide.

Haplotype frequency distribution among the populations shows population-specific patterns of haplotype composition (Fig. 1). In Korean and Japanese populations, haplotype A1 and its related haplotypes, A2-A17, constitute more than 65% of the individuals, while in Russian (east Sakhalin), Alaskan and Canadian populations, haplotypes B1 and its related haplotypes, B2-B21, occur in more than 65% of the individuals. These haplotypes are found in Korean and Japanese populations at zero to 17%. On the other hand, the Washingtonian population consists of haplotype C1 and its differentiated haplotypes, C2-C4, in up to 85% of individuals (Fig. 1).

Haplotype diversity in each population ranges from 0.37 ± 0.15 to 0.92 ± 0.06 (Table 3). Relatively high levels of diversity were observed in Canadian (CS1, 0.92 ± 0.06 ; CS2, 0.70 ± 0.11) and one of the Korean populations (KS1, 0.84 ± 0.05). The other Korean population (KS2) and the Russian population (RSC) showed relatively low levels of diversity, 0.42 ± 0.16 and 0.37 ± 0.15 , respectively. Japanese (JS1 and JS2), Alaskan (ALS1 to ALS3) and Washingtonian (AS) populations showed intermediate levels of diversity (0.47 to

0.64). The overall haplotype diversity among the populations turns out to be relatively high, 0.83 ± 0.02 (Table 3). Nucleotide diversities, however, are low in almost all chum salmon populations (0.00071 to 0.0053) as is the overall diversity (0.0036 ± 0.0013).

Population Differentiation

Pairwise F_{ST} values (Slatkin 1995) among the populations were significantly greater than zero ($p < 0.01$) in comparisons between any two of the populations except the comparisons between Korean and Japanese populations and between the Russian, Alaskan, and Canadian populations (Table 4). In these comparisons, the values were < 0.1 , not significantly different from zero, even though the examined DNA was mitochondrial. These results suggest that Korean and Japanese populations are genetically indistinguishable as are the Russian, Alaskan, and Canadian populations. When the extent of population subdivision was evaluated by analysis of molecular variance (AMOVA, Excoffier et al. 1992), grouping the populations into three geographical groups, KS-JS/RSC-ALS-CS/AS, resulted in significantly strong levels of genetic structuring among the three groups (Table 5, $\Phi_{ct} = 0.37$, $p < 0.05$). Other population groupings such as KS-JS/RSC-ALS/CS-AS and KS-JS/RSC-ALS-CS-AS did not show significant population structure among groups.

DISCUSSION

Single nucleotide polymorphisms observed in the mtDNA COIII-ND3-ND4L region in the present study were large

Table 2. Fifty-one different haplotypes and their distribution among 11 populations.

Haplotype	Locality											Total
	KS1	KS2	JS1	JS2	AS	CS1	CS2	ALS1	ALS2	ALS3	RCS	
A1	13	10	15	14	0	0	0	0	0	0	1	53
A2	1	0	0	0	0	0	0	0	0	0	0	1
A3	2	1	0	0	0	0	0	0	0	0	0	3
A4	1	0	0	0	0	0	0	0	0	0	0	1
A5	3	1	0	0	0	0	0	0	0	0	0	4
A6	1	0	0	0	0	0	0	0	0	0	0	1
A7	0	0	0	1	0	0	0	0	0	0	0	1
A8	1	0	0	0	0	0	0	0	0	0	0	1
A9	1	0	0	0	0	0	0	0	0	0	0	1
A10	0	0	0	1	0	0	0	0	0	0	0	1
A11	1	0	0	0	0	0	0	0	0	0	0	1
A12	0	0	0	1	0	0	0	0	0	0	0	1
A13	0	0	1	0	0	0	0	0	0	0	0	1
A14	0	0	0	1	0	0	0	0	0	0	0	1
A15	0	1	0	0	0	0	0	0	0	0	0	1
A16	0	0	1	0	0	0	0	0	0	0	0	1
A17	0	0	1	0	0	0	0	0	0	0	0	1
B1	2	0	3	1	3	4	8	9	11	9	12	62
B2	0	0	0	0	0	0	1	0	0	0	0	1
B3	0	0	0	0	0	0	0	2	0	0	0	2
B4	0	0	0	0	0	0	0	2	2	0	0	4
B5	0	0	0	0	0	0	0	1	0	0	0	1
B6	0	0	0	0	0	0	0	0	1	0	0	1
B7	0	0	0	0	0	2	0	0	0	0	0	2
B8	0	0	1	0	0	0	0	0	0	0	0	1
B9	0	0	0	0	0	0	0	0	0	1	0	1
B10	0	0	0	0	0	0	1	0	0	0	0	1
B11	0	0	0	0	0	0	0	0	0	0	1	1
B12	0	0	0	0	0	0	0	0	0	0	1	1
B13	0	0	0	0	0	1	0	0	0	0	0	1
B14	0	0	0	0	0	1	0	0	0	0	0	1
B15	1	0	0	0	0	0	0	0	0	0	0	1
B16	0	0	0	0	0	1	0	0	0	0	0	1
B17	0	0	0	0	0	1	0	0	0	0	0	1
B18	0	0	0	0	0	1	0	0	0	0	0	1
B19	0	0	0	0	0	1	0	0	0	0	0	1
B20	0	0	0	0	0	1	0	0	0	0	0	1
B21	0	0	0	0	0	1	0	0	0	0	0	1
C1	0	0	0	0	14	0	1	0	0	0	0	15
C2	0	0	0	0	1	0	0	0	0	0	0	1
C3	0	0	0	0	1	0	0	0	0	0	0	1
C4	0	0	0	0	1	0	0	0	0	0	0	1
D1	0	0	1	1	0	0	3	1	1	3	0	10
D2	5	0	0	0	0	0	0	0	0	0	0	5
D3	1	0	0	0	0	0	0	0	0	0	0	1
D4	1	0	0	0	0	0	0	0	0	0	0	1
D5	1	0	0	0	0	0	0	0	0	0	0	1
D6	0	0	1	0	0	0	0	0	0	0	0	1
D7	0	0	0	0	0	0	1	0	0	0	0	1
D8	0	0	0	0	0	0	0	0	0	1	0	1
D9	0	0	0	0	0	0	0	0	0	1	0	1
Total	35	13	24	20	20	14	15	15	15	15	15	201

Table 3. Haplotype diversity ($h \pm SD$) and nucleotide diversity (π) in each population. N = number of samples.

Population	N	$h (\pi)$	Population	N	$h (\pi)$
KS1	35	0.8437 \pm 0.0528 (0.003615)	CS2	15	0.7048 \pm 0.1139 (0.001327)
KS2	13	0.4231 \pm 0.1645 (0.000861)	ALS1	15	0.6381 \pm 0.1288 (0.001054)
JS1	24	0.6087 \pm 0.1118 (0.002216)	ALS2	15	0.4667 \pm 0.1478 (0.000710)
JS2	20	0.5211 \pm 0.1346 (0.001503)	ALS3	15	0.6286 \pm 0.1253 (0.001402)
AS	20	0.5053 \pm 0.1256 (0.000784)	RSC	15	0.3714 \pm 0.1532 (0.000930)
CS1	14	0.9231 \pm 0.0604 (0.005316)	Total	201	0.8286 \pm 0.0188 (0.0036)

Table 4. Pairwise F_{ST} values among the chum salmon populations.

	KS	JS	AS	CS	ALS
JS	0.02850				
AS	0.34341*	0.45266*			
CS	0.19546*	0.28319*	0.25986*		
ALS	0.31521*	0.39329*	0.39681*	0.03347	
RSC	0.35041*	0.44361*	0.49551*	0.08399	0.01469

* $p < 0.01$ **Table 5.** Hierarchical analyses of molecular variance for chum salmon.

Source of variation	Variance component	Percentage of variation	F-statistics (Φ)
Among three groups (KS-JS/RSC-CS-ALS/AS)	0.1738	36.62	0.3662*
Among populations within groups	0.0093	1.97	0.0311*
Within populations	0.2915	61.42	0.3858*

* $p < 0.05$

enough to analyze genetic diversity and population structure of chum salmon. The level of sequence variability, 55 variable sites in about 730bp sequences (Table 2), turns out to be higher than those reported in the control region. Park et al. (1993) identified only four nucleotide variations in the entire D-loop region (approximately 1kb long) from an analysis of 29 individuals. Sato et al. (2004) found only 20 variable sites in about 500bp sequences at the 5' end of the control region from an analysis of more than 2,100 individuals. Haplotype diversity reflects a difference in variability. The overall haplotype diversities were 0.83 (\pm 0.02) in the present study (Table 3) and 0.63 (\pm 0.01) in Sato et al. (2004). Levels of haplotype diversities among populations show a different pattern between the two studies. In the present study, relatively high levels of haplotype diversities were observed in Canadian (CS1, 0.92 \pm 0.06; CS2, 0.70 \pm 0.11) and one of the Korean populations (KS1, 0.84 \pm 0.05), while in Sato et al. (2004) Japanese populations showed higher levels of diversity (average, 0.63 \pm 0.01) than any other populations. Diversities in Korean and North American populations were as low as 0.37 \pm 0.08 and 0.34 \pm 0.02, respectively (Sato et al. 2004). These contradictory results may result from different sampling strategies as well as the use of different molecular markers. Sato et al (2004) used a special strategy for sampling Japanese chum salmon in order to minimize human

influences on diversity such as transplantation and hatchery operations by avoiding sampling hatchery-released stocks. Such a strategy was not applied to other populations, e.g. for Korean chum salmon. Moreover, the two Canadian populations (CS1 and CS2) that showed high levels of diversity in the present study were not included in Sato et al (2004).

Differences in haplotype frequency distribution among populations and among some population-specific haplotypes are useful for identification of source populations in mixed-stock fisheries. For example, a high percentage of haplotype A1 in a catch shows the presence of either Korean or Japanese populations; the presence of haplotype D2 further distinguishes Korean populations from Japanese populations (Table 2; Fig. 1). A high percentage of haplotype C1 distinguishes Washingtonian populations from others. The present results were obtained from analysis of 11 chum salmon populations around the North Pacific. Further extensive studies on the COIII-ND3-ND4L sequences for more populations will increase the power of resolution for population discrimination in chum salmon.

Pairwise F_{ST} values (Slatkin 1995) among the populations suggest that Korean and Japanese chum salmon are genetically indistinguishable as are Canadian, Alaskan, and Russian salmon ($F_{ST} < 0.1$, Table 4). Results of analysis of molecular variance (AMOVA, Excoffier et al. 1992) further

support such a grouping of chum salmon populations: grouping the populations into three geographical groups, KS-JS/RSC-ALS-CS/AS, resulted in significantly strong level of genetic difference among the groups (Table 5, $\Phi_{ct} = 0.37$, $p < 0.05$) as expected from the F_{ST} estimation. Therefore, the genetic structure of the chum salmon populations appears as follows: a Korea-Japan population unit, a Russia-Alaska-Canada population unit, and a Washington population unit. This population structure is reflected in the haplotype frequency distribution (Fig. 1). Korean and Japanese populations share haplotype A1 at no less than 37% of their individuals, and Canadian, Alaskan, and Russian populations share haplotype B1 at about 30%. The Washingtonian population has almost exclusively haplotype C1 and its derived haplotypes C2 to C4 (Table 2).

This population structure is partly supported by the results of Sato et al. (2004). Although they grouped populations based on geography and nation (i.e. Japan, Russia, and North America), the haplotype frequency distribution is somewhat consistent with the grouping of populations in the present study. In Sato et al. (2004), Korean and Japanese populations were characterized by a high frequency of the mtDNA control region with haplotypes A1, B3, and C1 (Table 3 in Sato et al. 2004). Russian and North American populations also have common features in that they share haplotype B3 with highest frequency but do not contain haplotype A1, which distinguishes them from the Korean-Japanese populations. One population in Russia, Primorye, which is located in the southernmost part of eastern Russia in the East/Japan Sea, is an exception -- this population contains haplotypes A1, B3, and C1 with a high frequency which is similar to the Korean and Japanese populations. Therefore, the Primorye population should be classified as a part of the Korea-Japan population unit. Washingtonian populations have features both common with and different from other North American populations in the mtDNA control region sequences (Sato et al. 2004). Although the Washingtonian populations possess haplotypes B3 and B13 common with the North American populations, they also have unique haplotypes B10, B14, and B16 (Table 3 in Sato et al. 2004).

The proposed population genetic structure in the present study is also consistent with the results of other genetic studies with minisatellite DNA (Taylor et al. 1994), restriction fragments and allozymes (Seeb and Crane 1999a,b). Minisatellite DNA variation among Japanese, Russian, northwest Alaskan (Yukon river), southeast Alaskan and British Columbian populations showed that Japanese populations are distinctively separated from the others ($D_2 > 30$, Fig. 5 in Taylor et al. 1994). Restriction enzyme site polymorphism in the ND5-ND6 region revealed that there are two major chum salmon groups in the North Pacific: Japanese populations and others including the Russian and Washingtonian populations (Table 6 in Seeb and Crane 1999b). On the other hand, an allozyme study by Seeb and Crane (1999a) appears to show a different relationship in that an UPGMA tree showed a cluster

of Russian and northwest Alaskan populations together with Japanese populations rather than with southeast Alaskan and British Columbian populations. However, the UPGMA tree topology was not statistically tested so that close affinities of Russian and northwest Alaskan populations to Asian populations cannot be corroborated. Multidimensional scaling analyses of the same data set in the same study and also in a successive study (Seeb and Crane 1999a,b), in fact, showed that there is clear distinction of Japanese populations from all the other populations. For the Washingtonian populations, separation from Alaskan and British Columbian populations was also shown in the allozyme studies (Seeb and Crane 1999a, 1999b). Although this separation was not as distinct as the one between Japanese and Russian-northwest Alaskan populations, the Washingtonian populations were a separate cluster in the multidimensional scaling plots and formed a separate clade by themselves in the UPGMA tree.

Differentiation of the three genetic population units may have occurred recently. The number of nucleotide differences between the haplotypes A1, B1 and C1, the representative haplotypes of each population unit, is limited to only one to three in about 730bp long sequences of the COIII-ND3-ND4L region. The overall nucleotide diversity among the populations is also as low as 0.0036 ± 0.0013 , which is similar to the value observed in the mtDNA control region ($\pi = 0.0037$, Sato et al. 2004).

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