Sequence Characters on Mitochondrial DNA of Swordfish in-situ Collected from Taiwanese Longliners

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Abstract

A survey on in-situ collection of swordfish (*Xiphias gladius*) meat samples from Taiwanese longliners has been carried out from 1999 to 2003. Sequence characters on D-loop segment of mitochondrial DNA were used as the basis to infer the stock structure of the swordfish resources. Total numbers of swordfish sampled were 175, within which 9 sampling units (2 units from the Pacific Ocean and 7 from the Indian Ocean) were identified.

Results obtained indicated that: (1) the length of D-loop segment of the swordfish mitochondiral DNA were 841 base pairs. (2) A total of 133 polymorphic sites with 142 unique haplotypes were identified among the 175 D-loop segments. (3) Samples drawn from the waters off northern Madagascar and the Bay of Bengal were two distinctive groups based on either fixation index or nearest-neighbor statistic analyses. (4) The comparison in molecular difference between the central and southern Indian group versus the group of western Pacific by using analyses of molecular variance appeared to be less significant than pairwise comparison between groups in the Indian Ocean.

Based on the findings of this study, it is suggested that the stock structure of swordfish in the Indo-Pacific region can be summarized as follow: (1) the area off northern Madagascar, (2) the Bay of Bengal and (3) the rest of Indian Ocean and the western Pacific three groups.

Introduction

Swordfish, *Xiphias gladius* (Linnaeus, 1758), is one of the most widely distributed species in oceanic fish biota, due mainly to its high temperature tolerance ranging from 6° to 26°C (Carey and Robison 1981). Swordfish are commonly found not only in tropic and temperate zones of the Indian, the Atlantic, and the Pacific Oceans, they are also quite abundant in the Mediterranean Sea, the Sea of Marmara, the Black Sea, and the Sea of Azov (FAO 1994).

It is evident that the swordfish resources had become one of the important economic elements for those fishers who were capable of accessing them. It was not until early 1990, when catch of swordfish in the Indian Ocean getting increased notably, the Taiwanese longliners became one of the major fleets that capable of utilization Indian swordfish resources.

Stock identification is one of the major tasks to be accomplished if any successful fisheries resource assessment is needed. As one of the major fleets fishing Indian swordfish, research efforts had been devoted for in-situ collection of swordfish meat samples on Taiwanese fleet in 1999-2003 to meet the aforementioned needs.

Base pair sequential character of mitochondria DNA (mtDNA) has recently becoming useful tool for genetic studies of fish populations as its distinctive characters of high evolution rate and maternal mode of transmission. There were few studies on swordfish population structure in world's Oceans by using DNA base pair sequential characters (Reeb et al. 2000). Although it is presumed that the swordfish population structure in the Indo-Pacific Oceans to be one breeding unit (Chow & Takeyama 2000), the area-time distribution of swordfish samples in the study is limited.

The main objective of this study was thus further investigating the swordfish stock structure in Indo-Pacific Oceans based on DNA sequential characters of meat samples in-situ collected by Taiwanese longliners.

Materials and methods

Sampling

From 1999 to 2003, a survey on in-situ collections of swordfish meat samples was carried out by Taiwanese fisheries research sector for collecting on-board swordfish meat samples from Taiwanese longliners fishing in the Indian and the Pacific Oceans. A total of 175 in-situ collected swordfish meat samples (Table 1), which belonged to 11 assigned fishing operations, have been successfully sampled and delivered to our Laboratory through various means, such as personal carrying, via air mail or transport vessels. All on-board swordfish meat samples were preserved immediately in 95% ethanol by fishing masters for further analysis.

These 175 swordfish meat samples were further categorized based on their area-time attributes, into 9 independent sampling units (Fig. 1). There were 7 and 2 sample units collected from the Indian Ocean and the Pacific Ocean, respectively. They are: Central Indian (ICen), Centro-south Indian (ICeS), Eastern Indian (IEast), Northern Indian (INor), Western Indian (IWest), off Eastern Madagascar (IMadE), off Northern Madagascar (IMadN), Central Pacific (PCen), Western Pacific (PWest).

Laboratory protocols

DNA extraction

Isolation of DNA was performed by standard phenol/chloroform procedure (Kocher et al. 1989). About 100mg of meat tissue was digested in 900 μl extraction buffer (10mg/ml DTT, 10mM Tris-HCl pH8.0, 2mM EDTA.), 50μl proteinase K (10 mg/ml) and 50 μl 20% SDS. The tissue was incubated overnight at 55°C, with gentle rotation inside a hybridization oven. It was then extracted once with an equal volume of saturated phenol, once with 1ml phenol/chloroform/isoamyl alcohol (25:24:1) and once with 1ml chloroform/isoamyl alcohol (24:1), followed by ethanol precipitation. The extracted DNA was then suspended in 50 to 100μl of sterile water.

PCR amplification and DNA sequencing

Two specific primers, modified from (1) the light-strand primer SP (5'-TAC CCC AAA CTC CCA AAG C-3') complementary to tRNApro (Alvarado Bremer., 1994) and (2) the heavy-strand primer S12S (5'-CAG AAG GCT AGG ACC AAA C-3') complementary to 12S rRNA (Kocher et al., 1989), were developed and used in this study.

Amplifications on control-region of mtDNA were performed in a final volume of 50μl. The following gradients will be sequentially added: 200μM dNTPs, 0.5 units of Super Taq DNA polymerase, 5μl of 10X buffer (50mM KCl, 10mM Tris-HCl (pH=8.3), 1.5mM MgCl2, 0.1% gelatin), 1μl of the extracted DNA, and 0.5μM of aforementioned primers. The PCR program were set as: (1) the denaturing for 5 min at 94°C; (2) followed by 35 cycles of the following settings: denaturing at 94°C for 1 min, annealing at 52°C for 70 sec, the extension at 72°C for 80 sec; (3) the final extension at 72°C for 10 min. The amplification procedure was performed in a Thermal Cycler System 9600 (Perkin Elmer). PCR product was purified using the QIA quick Gel Extraction Kit (Qiagen Inc.).

The sequencing reactions of the PCR product were then analyzed by using Applied Biosystems Prism 377 Automated Sequencer.

Data analysis

The DNA sequences were aligned with the sequence AH008720: Xiphias gladius (Reeb,C.A.,Arcangeli,L. and Block, B.A.2000) using GCG software package (Genetic Computer Group, Devereaux et al., 1984). Phylogenetic analyses of mtDNA control region sequence variation within swordfish was constructed by the UPGMA tree-building algorithm with Kimura's 2-parameter (Kimura, 1980) distance using MEGA (Molecular Evolutionary Genetic Analysis, Version 2.0, Kumar et al., 2001).

Genetic diversity analyses

The characters of Haplotype diversity Hd (Nei 1987), Nucleotide diversity π (Nei 1987), FST (Hudson et al. 1992b), and nearest-neighbor statistic Snn (Hudson 2000) were calculated for performing the genetic differentiation analysis (DnaSP version 4.0. Julio Rozas et al, 2003). Average sequence divergences between populations were computed and the resultant divergence matrix thus obtained will be used, based mainly on UPGMA algorithm, to create a clustering tree of populations. The probable geographic population units will then be determined by the results of molecular variance analyses.

Population genetic structure

AMOVA (analysis of molecular variance, Excoffier et al., 1992) is a hierarchical approach analogous to analysis of variance in which the correlation specified for molecular sequence

data. Estimates on genetic differentiation between populations were performed based on the results obtained by AMOVA. Various groupings on those populations suggested by (1) the analysis of DNA sequence, (2) population trees, and (3) geographic distribution were performed. The grouping that reveals maximal value of ΦCT and significantly differs from random organization of similar grouping is assumed to be the most probable geographic subdivisions (Stanley et al.1996).

Results

Molecular and genetic information of the control region

The target segment extracted by using SP and S12S primers contains about 969 base pair, which includes a portion of tRNApro , the control region or D-loop, a segment of tRNAphe, and a portion of 12S rRNA. The length of the control region, aligned from the 175 swordfish meat samples, is 841 base pair

Nucleotide composition in the D-loop of swordfish mtDNA showed that the ratio of Adenine (A): Thymine (T): Cytosine (C): Guanine (G) to be 33.2% : 30.1% : 21.8% : 14.9%, accordingly (Table 2). The combined composition of Adenine and Thymine (63.3%) is higher than that of Cytosine and Guanine in D-loop segment of swordfish mtDNA. It is obviously that the D-loop of swordfish mtDNA is also an A-T rich segment.

Information content in terms of nucleotide sequence is generally highly variable in the control region (Rosel and Block, 1996). A total of 133 polymorphic sites, including 33 singleton variable sites and 100 parsimony informative sites, were detected within the 841 base pair of swordfish mtDNA control region was obtained in this study. It is also noted that 68% of the total polymorphic sites appeared from 1 to 300 base pair sequence of the 5'-strand (Fig. 2).

Among 175 individuals of swordfish studied, there resolved 142 unique haplotypes. Notably, there were 29 unique haplotypes discovered among 39 individuals of swordfish collected from the Pacific Ocean and 118 unique haplotypes among 136 individuals from the Indian Ocean (Table 3).

Within location analyses on sequence character

Haplotype diversity index (h) were derived and used for interpretation the molecular genetic character of each sampling unit or location. The estimation of the h values for the seven sampling unit ranged from 0.914 (IMadN) to 1(ICen, IEast, ImadE, and INor). The pooled samples for (1) the Pacific Ocean (PWest and PCen), (2) the Indian Ocean and (3) the combined Pacific and Indian Oceans were also derived, as shown in Table 3. The lowest haplotype diversity value was 0.914, which was derived from the IMadN sample. In the IMadN sample, there were 8 unique haplotypes among 15 individuals, which indicated that more than 1 individual resolved the same haplotype.

The nucleotide diversity index (π) were also calculated based on the number of polymorphic sites observed in the sequence information of the control region. The nucleotide diversity index varied from 0.01126 (PWest) to 0.01861 (IMadE) among all sampling units. The π value of INor (0.01137), IMadN (0.01226), and PWest (0.01126) were smaller than other units. It is evident that the h and π index of sample IMadN appeared to have the lowest h value and a lower π value.

Between sampling units comparison on sequence information

The FST value and the Nm value, which were based mainly on number of polymorphic sites in sequence information of the control region, were computed for pairwise comparison between any two sampling units. The results of pairwise comparison indicated by FST estimator indicated that whenever a combination includes the IMadN and INor regions always showed higher FST value, which ranged from 0.04910 to 0.14376 (Table 4a). These results indicated that swordfish from IMadN and INor regions were quite different from the rest of locations.

The comparison between INor and ICen revealed the highest FST value (0.14376). Pairwise comparisons of the seven regions showed that the differentiation was mostly due to the IMadN and INor regions, which were significantly differentiated from the rest of regions. The lowest Nm value is 2.98 revealed between INor and ICen in the Indian Ocean (Table 4a).

The Snn value, which is also derived basically from the polymorphic sites yet provide statistic test by using permutation methodology, is also computed for further pairwise comparison. According to the value and significant test of Snn parameter, it is obvious that high value between pairwise comparisons indicated the distinguishing characteristic of the IMadN and INor locations. The values were ranged from 0.66667 to 0.88137 in IMadN and from 0.65833 to 0.90122 in INor (Table 4b). The degree of specification or genetic differentiation in these two regions (IMadN and INor), as reflected in terms of the high values of FST and Snn, appeared to be significant.

The FST value between PCen and PWest is 0.03940. The Snn value between the two populations in Pacific Ocean is 0.5982. These two values derived from the samples collected in the Pacific Ocean were comparatively rather small. It is thus indicated no significant difference, as reflected by FST and Snn values, between the PCen and the PWest these two sampling units collected from the Pacific Ocean.

Optimization on grouping of sampling units

Analysis of Molecular Variance (AMOVA) method was adopted in this study to develop and to choose the best grouping entity out of various grouping possibilities grouped from those sampling units.

The resultant UPGMA tree, followed by performing a clustering analysis using UPGMA criterion, was one of the main source of information for providing reasonable combinations of groupings for further analyses. One result obtained by using such analysis, based on the FST value, was shown in Fig. 3.

According the UPGMA tree, it implies that the IMadN and the INor regions may have already significantly differentiated away from other regions. It is also suggested, based on the results of further clustering analyses and the character of geographic locations, that six primary regional divisions out of original nine sampling units are appropriate to be grouped for detailed AMOVA analyses.

According to among-group variance component test results, five grouping schemes (Gp1, Gp2, Gp3, Gp4, and Gp5 of Table 5) out of all designated 6 grouping schemes are statistically significant.

The Gp1 grouping scheme, which comprised of three groups, consistently showed the highest value in among-group variance components was selected as the best grouping of all sampling units in present study. The selection of Gp1 grouping scheme to be the best

grouping scheme is based on the statistics of ΦCT value. The obtained value of ΦCT =0.04964 is the largest value appeared in all grouping schemes, as shown in Table 5.

Further between-ocean analyses by FST and Snn statistics

The results so far obtained indicated that characters of IMadN and INor appeared significantly different from the rest of sampling units. Further between-ocean analyses, which exclude IMadN and INor, by FST and Snn statistics were thus performed for better understanding the tendencies between the Pacific and the Indian Oceans. The results of these analyses were shown in Table 6. The minimum value of FST (0.03153) was observed when the pooled Pacific sample versus the pooled Indian sample, and the maximum value (0.17715) was appeared when the pooled Pacific versus INor. The minimum Nm (2.32) value was appeared when the pooled Pacific sample versus the INor, and the maximum Nm (15.36) was observed when the pooled Pacific sample versus the pooled Indian sample of excluding IMadN and INor (Table 6).

According the permutation test of Snn statistic, the IMadN sample was significantly different from all the remaining samples or groups. Notably, the pooled Indian sample (which excludes IMadN and INor) showed no significant different from the pooled Pacific sample by Snn statistic test (Table 6).

Summary and discussion

The results so far obtained can be summarized as follows:

(1) IMadN sampling unit showed different character from others in terms of haplotype diversity index (h);
(2) IMadN and INor sampling units are different from rest of samples from Indian Ocean according to FST and Snn statistics;
(3) the results of AMOVA indicate that the best scheme of grouping samples is to organize samples into three groups: the group of INor, the group of IMadN, and the group that includes rest of all sampling units;
(4) IMadN and INor these two sample units, which were drawn from the north and the northwestern parts of the Indian Ocean, always showed constant higher FST and lower Nm whenever any pairwise comparison was performed manifest itself the molecular genetic specialties of the two regions; and
(5) the extent of molecular genetic differentiation, based on Snn statistic, between the pooled Pacific sample versus the pooled Indian sample with exception of IMadN and INor appeared to be insignificant implies that a large genetic exchange between these two Oceans is very possible;

Phylogenetic analysis on D-loop sequences of swordfish obtained from the Atlantic Ocean and the Mediterranean Sea indicated that there are two clades, where clade Ⅱ was identified by only a single repeat of the 5'-TACA-3' sequence and clade I having two or three contiguous repeats of this sequence at the same position (Alvarado Bremer et al.1995; Kotoulas et al.1995). In this study, only 3 fish from Indian Ocean and none from the Pacific Ocean belong to clade Ⅱ, as shown in Table 7. All of the rest 172 swordfish specimens were clade I, including 28 individuals with three TACA repeats and 144 individuals with two TACA repeats (Table 7). The proportion of clade I was larger than clade Ⅱ in the Indian Ocean. Each of the 3 clade Ⅱ fish was from the ICen, the IWeS and

the IMadE sample units of Indian Ocean. Previous studies (Alvarado Bremer et al.1995, Rosel and Block 1996) also indicated that no clade Ⅱ fish was discovered In the Pacific Ocean and only very few swordfish of clade Ⅱ was also discovered off western Australia waters reported by Robert Ward et al (2001).

Hudson (2000) suggested that the Snn could be a suitable indicator for the degree of molecular genetic differentiation. It approaches unity when the populations at the two localities are highly differentiated, and is 0.5 when the populations are part of the same panmictic population. The two sample units of IMadN and INor drawn from northern Indian waters show very high level of Snn (larger than 0.85) when compared to the pooled Indian and the pooled Pacific samples. Although the permutation test of the Snn obtained between INor and the pooled Indian sample appeared no significant, the value of Snn (larger than 0.90) revealed to be still rather high. Less significant value of Snn was obtained between the pooled Indian and the pooled Pacific sample implies that gene exchange between the central and southern Indian Ocean swordfish and the Pacific ones may have existed. Pair-wise comparison in molecular genetic difference of between the pooled Pacific and the pooled Indian Ocean seemed less significant may have also stemmed from that there still is a through-flow (Meyers et al., 1995) connected the two. This through-flow must apparently more widely open in the ancient time when the India and Australian plate was further away from the eurasian plate. The constant existence of a through-flow between the current western Pacific and the current central and southern Indian Ocean explains, perhaps, the main causation for an insignificant molecular genetic difference between these two Oceans.

Nishikawa et al. (1974) indicated that a widely distributed swordfish larva was discovered in the western tropic Pacific, where archipelago islands are abundant, presumed there must exists a major spawning ground for swordfish. If this spawning at tropic archipelago region of an Ocean is one of the reproductive traits in swordfish, it is thus not irrational to comprehend the current results of isolating the off north Madagascar region and the Bay of Bengal to be different from rest of the Indian Ocean.

As abundant and widely distributed in all Oceans nowadays as swordfish, it must very likely also was very thriving in the ancient time when Madagascar and India were two large land masses surrounded by Oceans and were then located nearby the equator. Plate tectonic movements of these two lands resulted into the current Indian Ocean geography as well as its specific hydrographic characters. The water mass of eastward south equator current, which may have well included the through-flow from the western Pacific, is different from those originated from the Red Sea, which is high in salinity and nutrients and low in oxygen contents (Wyrtki, 1971; DiMarco et al., 2002). The circulation of water mass, in which the upper layer is less saline because that it receives a significant amount of fresh water from hinterland rivers and oceanic precipitation, in the Bay of Bengal seemed to be semi-enclosed and rather self-contained system driven by the seasonally reversing monsoons (Subramanian, 1993). As a rational inference, the off north Madagascar (IMadN) and the Bay of Bengal (INor) two areas appeared to be ecologically specific in the Indian Ocean. It is thus not irrational to recognize, based on D-loop sequence character analyses, the swordfish population in the Indian and the western Pacific region into the off north Madagascar (IMadN), the Bay of Bengal (INor), and the rest Indian and the Pacific three major groups.

Although much research effort had been endeavored for the collection of in-situ swordfish samples from the Taiwanese longline vessels, the number of sample collected in this study were still limited. Particularly, swordfish samples from the areas of the off north

Madagascar and the Bay of Bengal are still urgently needed if any further confirmation on the stock structures to be needed.

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Table 1 Detailed information of independent swordfish meat samples collected in this study.

Sample Code	Latitude	Longitude	Sample size	Sampling date
S1	0° S～11°S	63° E～84° E	20	Sep~Nov.2000
S2	30°S～32°S	82° E～89° E	15	Jul~Aug.2003
S3	8° S～12°S	112°E～122°E	23	May 2001
S4	30°S～32°S	65° E～68° E	5	Jun.2003
S5	17°S～24°S	51° E～68° E	11	Sep.1999
S6	7° S～16°S	43° E～57° E	15	Sep~Oct.1999
S7	11°N～12°N	86° E～88° E	5	Mar.2003
S8	2° N～2° S	58° E～66° E	25	Mar.2000
S9	3° N～5° S	57° E～67° E	17	Oct.2000
S10	4° N～12°S	133°W～153°W	21	Sep~Nov.2002
S11	18°N～20°N	127°E～130°E	18	Jun.2001
Total			175*	

*: These swordfish meat samples were kindly collected by Taiwanese longliners of Ming-Jie No.1, Tai-Yuan No.212, Yu-Sheng No.1,

Jin-Hung No.116, and Hai-Tsuen No.1.

Table 2 Nucleotide composition in the D-loop segment of swordfish mtDNA.

Sample unit	Sample size	Nucleotide composition (%)			
		T	C	A	G
ICen	20	30.2	21.7	33.1	15.0
ICeS	15	30.0	21.9	33.0	15.0
IEast	23	30.1	21.8	33.2	14.9
IMadE	16	30.2	21.7	33.1	15.0
IMadN	15	30.3	21.6	33.2	14.9
INor	5	30.0	22.0	33.1	15.0
IWest	42	30.2	21.7	33.2	15.0
PCen	21	30.2	21.8	33.2	14.9
PWest	18	30.1	21.8	33.3	14.8
	175	(Mean)30.1	21.8	33.2	14.9

Table 3 D-loop polymorphism characters in terms of haplotype diversity index(*h*) and nucleotide diversity index (π) for sampling units and Oceans.

Items	Sampling units									Oceans		
	ICen	**ICeS**	**IEast**	**IMadE**	**IMadN**	**INor**	**IWest**	**PCen**	**PWest**	**Pacific[b]**	**Indian[c]**	**Total[d]**
N(n)[a]	20(20)	15(14)	23(23)	16(16)	15(8)	5(5)	42(39)	21(15)	18(16)	39(29)	136(118)	175(142)
h	1	0.990	1	1	*0.914*	1	0.997	0.957	0.987	0.9800	0.9976	0.9967
S	65	64	68	72	33	20	90	55	40	71	129	133
π	0.01394	0.01779	0.01606	0.01861	0.01226	0.01137	0.01426	0.01498	*0.01126*	0.01331	0.01555	0.01476

a: N is the sample size of the unit and (n) is the number of haplotype of the unit;
b: pooled 2 sampling units of PCen and PWest from the Pacific Ocean;
c: pooled 7 sampling units of ICen, ICeS, IEast, IMadE, IMadN, INor and IWest from the Indian Ocean;
d: pooled total 9 sampling units of b and c;
S: the number of variable sites.

Table 4 Results of pairwise comparison in terms of F_{ST}, Nm and Snn between sampling units from the Indian Ocean.

(a) F_{ST} (above diagonal) and Nm (below diagonal) values obtained by pairwise comparison between sampling units

	ICen	**ICeS**	**IEast**	**IMadE**	**IMadN**	**INor**	**IWest**
ICen		0.00045	0.03354	0.02062	*0.08663*	*0.14376*	0.00815
ICeS	1117.10		0.01606	-0.00024	*0.07273*	*0.10643*	0.02584
IEast	14.41	30.64		-0.01783	*0.05772*	*0.05629*	0.01801
IMadE	23.74	-2097.00	-28.54		*0.04910*	*0.06292*	0.00428
IMadN	*5.27*	*6.37*	*8.16*	*9.68*		*0.12295*	0.08092
INor	*2.98*	*4.20*	8.38	7.45	*3.57*		0.14076
IWest	60.86	18.85	27.26	116.20	5.68	*3.05*	

(b) *Snn* (above diagonal) and P value of permutation test (below diagonal) obtained by pairwise comparison between sampling units

	ICen	**ICeS**	**IEast**	**IMadE**	**IMadN**	**INor**	**IWest**
ICen		0.48571	0.57752	0.61111	*0.79048*	*0.86000*	0.61774
ICeS	0.5366^{ns}		0.51316	0.37634	*0.66667*	*0.65833*	0.65100
IEast	0.1642^{ns}	0.4612^{ns}		0.45299	*0.82895*	*0.80952*	0.63883
IMadE	0.1016^{ns}	0.8474^{ns}	0.7034^{ns}		*0.81989*	*0.73016*	0.65661
IMadN	$*0.0012^{**}$	$*0.0296^{*}$	$*0.0000^{***}$	$*0.0000^{***}$		*0.85000*	0.88137
INor	$*0.0080^{**}$	$*0.3140^{ns}$	$*0.0586^{ns}$	$*0.1106^{ns}$	$*0.0096^{**}$		0.90122
IWest	0.1890^{ns}	0.2468^{ns}	0.0594^{ns}	0.1678^{ns}	$*0.0000^{***}$	$*0.0170^{*}$	

ns: not significant in permutation test (10,000 permutations)

* : significant in permutation test (10,000 permutations).

*:0.01<P<0.05, **:0.001<P<0.01, ***:P<0.001.

Table 5 AMOVA results for Indian and Pacific groups of swordfish.

Name	Grouping scheme	Variance component	% variance	Φ-Statistics
Gp1[a]	Group1{INor } Group2{IMadN } Group3{ICen; ICeS; IEast; IMadE IWest; PCen; PWest }	AG AP/WG WP	4.96 2.84 92.19	Φ_{CT}=0.04964* Φ_{SC}=0.02993* Φ_{ST}=0.07808*
Gp2	Group1{INor; IEast } Group2{IMadN } Group3{ICen; ICeS; IMadE IWest; PCen; PWest }	AG AP/WG WP	2.23 3.11 94.65	Φ_{CT}=0.02234* Φ_{SC}=0.03185* Φ_{ST}=0.05349*
Gp3	Group1{INor } Group2{IMadN } Group3{PCen; PWest } Group4{ICen; ICeS; IWest; IEast; IMadE }	AG AP/WG WP	3.69 1.98 94.33	Φ_{CT}=0.03694* Φ_{SC}=0.02055* Φ_{ST}=0.05673*
Gp4[a]	Group1{INor } Group2{IMadN } Group3{PWest } Group4{ICen; ICeS; IEast; IMadE IWest; PCen }	AG AP/WG WP	3.66 2.55 93.80	Φ_{CT}=0.03657* Φ_{SC}=0.02643* Φ_{ST}=0.06204*
Gp5[a]	Group1{INor } Group2{IMadN } Group3{PWest } Group4{ICen; ICeS; IMadE; IWest } Group5{IEast; PCen }	AG AP/WG WP	3.16 1.83 95.01	Φ_{CT}=0.03163* Φ_{SC}=0.01891* Φ_{ST}=0.04994*
Gp6	Group1{INor; IEast } Group2{IMadN } Group3{IMadE } Group4{ICen; ICeS; IWest; PCen; PWest }	AG AP/WG WP	1.25 3.42 95.33	Φ_{CT}=0.01250 Φ_{SC}=0.03466* Φ_{ST}=0.04672*

AG is the among-groups component of variance; AP/WG is the among-populations/within-group component of variance, WP is the within-population component of variance.
a: grouping scheme followed by the result of UPGMA tree, as shown in Fig.3;.
*: The significant level of the permutation test $P<0.05$

Table 6 Results of pairwise comparisons in terms of F_{ST}, Nm and Snn between groups of Indian and Pacific oceans

(a)F_{ST} (above diagonal) and Nm (below diagonal) values obtained by pairwise comparisons between groups.

	Pacific	Indian	IMadN	INor
Pacific		0.03153	0.10086	0.17715
Indian	15.36		0.06651	0.10432
IMadN	4.46	7.02		0.12295
INor	2.32	4.29	3.57	

(b)*Snn* (above diagonal) and P value of permutation test (below diagonal) obtained by pairwise comparison between groups

	Pacific	Indian	IMadN	INor
Pacific		0.67412	0.89691	0.94318
Indian	0.0708^{ns}		0.93957	0.90771
IMadN	0.0000^{***}	0.0000^{***}		0.85000
INor	0.0002^{***}	0.7662^{ns}	0.0096^{**}	

ns: not significant in permutation test (10,000 permutations)

* : significant in permutation test (10,000 permutations).

*:0.01<P<0.05, **:0.001<P<0.01, ***:P<0.001.

Table 7 The number of swordfish individuals by Calde I and Clade II by Oceans obtained in this study

	Indian	IMadN	INor	Pacific	Total
Clade I	113●	15●	5●	39●	172
Clade I^2	101●	9●	4●	30●	144
Clade I^3	12●	6●	1●	9●	28
CladeII	3■	0□	0□	0□	3
Total	116	15	5	39	175

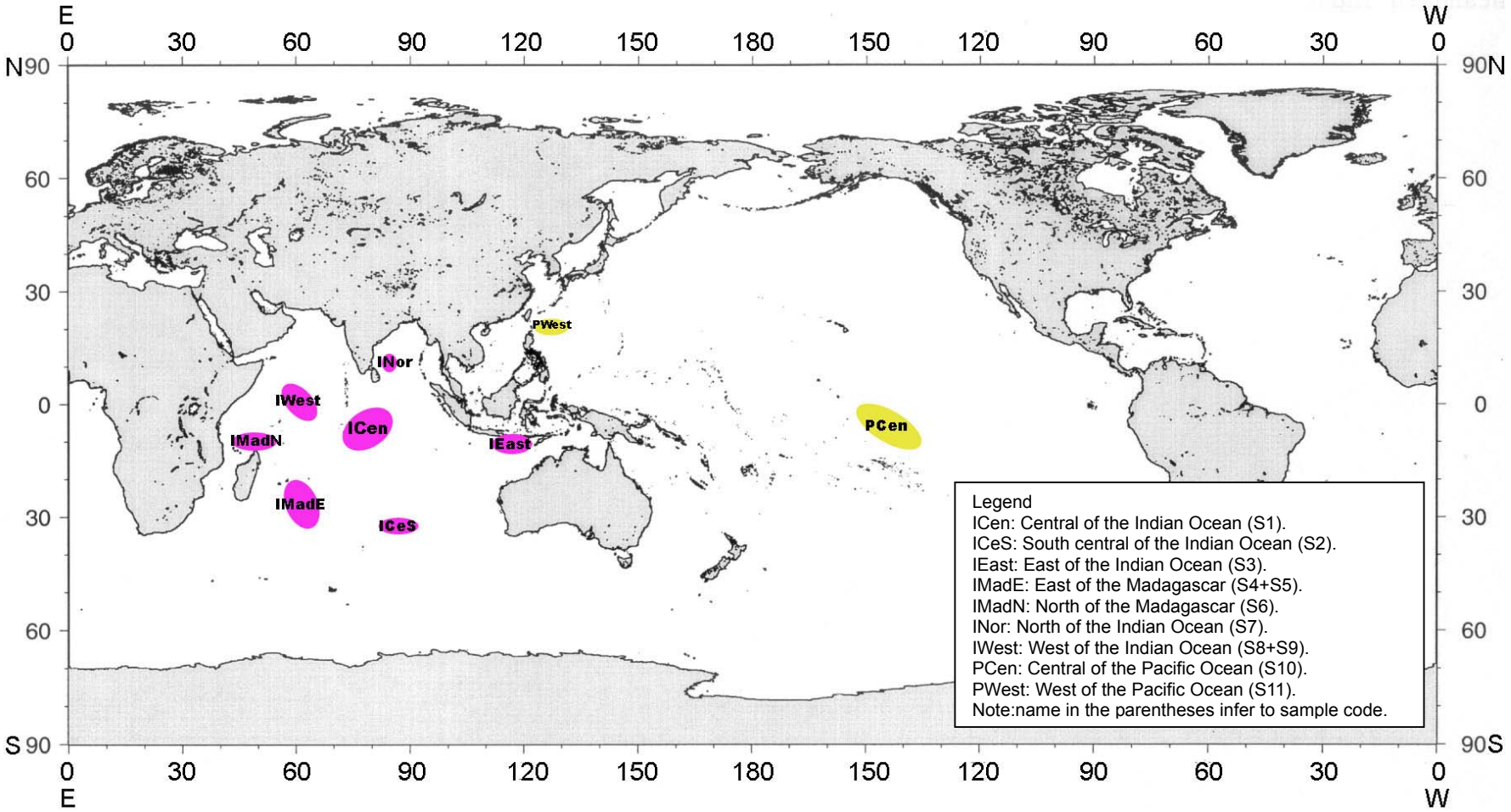

Fig. 1 Geographic distribution of sampling units (see Legend) of the swordfish collected in this study.

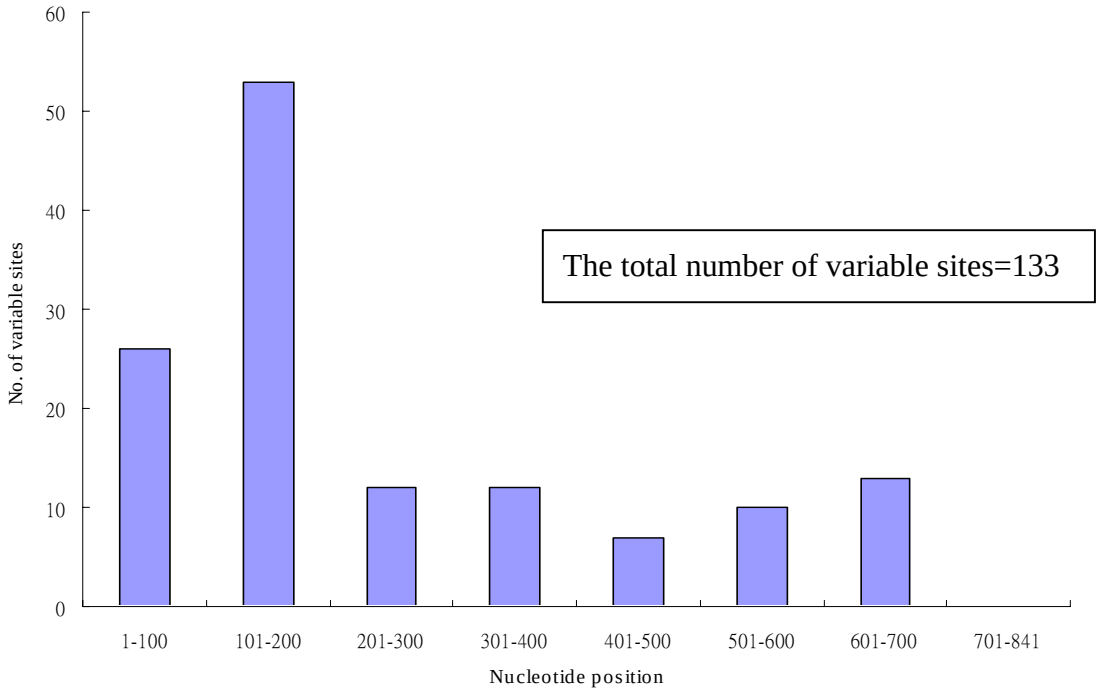

Fig. 2 The frequency distribution on the number of variable sites versus nucleotide position from D-loop in 175 swordfish individuals.

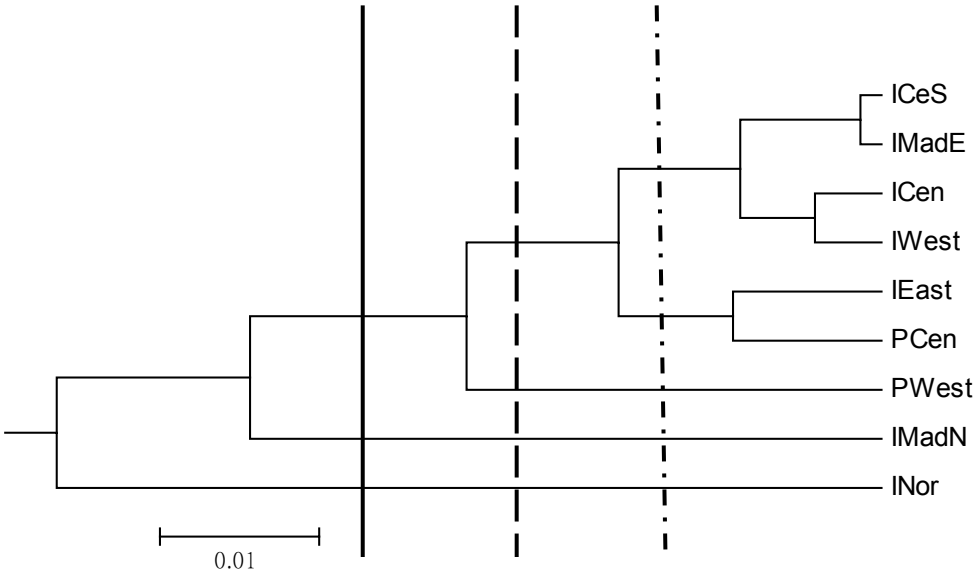

Fig. 3 Results of UPGMA tree derived from 9 sampling units (suggest Gp1,4,5 in Table 5).