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Population Genetic Structure of Pantropical Spotted Dolphin, *Stenella attenuata*, in Waters of Taiwan and South China Sea Based on Mitochondrial DNA Control Region Sequences

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ABSTRACT: A total of 447 base pairs of the mitochondrial DNA control region from 34 pantropical spotted dolphins, *Stenella attenuata*, in the waters of Taiwan and South China Sea were sequenced and analyzed. In this study, we tested the null hypothesis of non-differentiation genetic structure of the spotted dolphin of Taiwanese and adjacent waters. There were three putative populations in this study ─ Taiwan Strait, eastern Taiwan and South China Sea. The genetic structure and population differentiation of the putative populations were estimated to clarify the population status. There were 14 variable sites within the sequences and 13 haplotypes defined. Among the haplotypes, nine were unique and four were shared. The South China Sea population had three haplotypes, all of these were unique. The haplotype diversity estimates of the three populations range from 0.8883 in South China Sea, followed by 0.8182 in Taiwan Strait and 0.7778 in eastern Taiwan. Nucleotide diversity estimates range from 0.0096 in eastern Taiwan, 0.0078 in South China Sea and 0.0049 in Taiwan Strait. The Minimum-spanning network does not describe a clear pattern of haplotype and geographical locale relationship. Neighbor-joining and Maximum Likelihood phylogenetic analyses with *Stenella longiroatris* as outgroup also could not divide the haplotypes into clades representing the three putative populations. The Analysis of Molecular Variance (AMOVA) results show two patterns of population differentiation. The analysis using haplotype frequency, Fst, indicates significant population subdivision between South China Sea and Taiwan Strait (Fst = 0.1761, p = 0.0156), and between South China Sea and eastern Taiwan populations (Fst = 0.2029, p = 0.0059). However, the analysis using genetic distance and frequency information, Φst, does not reveal significant population differentiation between any pair of comparisons. Additionally, the relatively lower genetic diversity estimates of pantropical spotted dolphins in Taiwanese waters suggest that future monitoring is needed. A more systematic collecting method and expanding sampling area should be carried out for future investigations.

KEY WORDS: Mitochondrial DNA, Taiwan, South China Sea, *Stenella attenuata*, Population structure.

INTRODUTION

Dispersal and vicariance are two important factors in shaping the geographical distribution of genetic traits of animals (Avise, 2000). Most cetaceans have a very high mobility in marine environment where geographical barriers are not usually detectable. It is not surprising that little genetic differentiation among populations of cosmopolitan marine mammals such as sperm whale, *Physeter macrocephalus* (Lyrholm *et al*., 1996; Lyrholm and Gyllensten, 1998; Lyrholm *et al.*, 1999), and killer whale, *Orcinus orca* (Hoelzel *et al*., 1998; Hoelzel *et al*., 2002) had been reported. However, studies on several cetaceans have revealed local divergence between intraspecific populations. For example, small toothed whales such as

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striped dolphin, *Stenella coeruleoalba* (See Garcia-Martinez, *et al.*, 1999), finless porpoise, *Neophocaena phocaenoides* (See Yang *et al.*, 2002) and Dall's porpoise, *Phocoenoides dalli* (See Hayano *et al.*, 2003) have significant genetic differentiation among local populations. Bérubé *et al.* (1998) also suggested that fin whale (*Balaenoptera physalus*) populations in Atlantic, Mediterranean and Cortez Seas possibly have some limited gene flows between adjacent waters. In a review article, Hoezel (1994) suggests that feeding ecology, habitat usage and demographic history could be the causes of genetic differentiation of local populations in cetaceans.

Pantropical spotted dolphin, *Stenella attenuata,* is distributed in the tropical and subtropical waters worldwide (Evans, 1987). In eastern tropical Pacific (ETP), Perrin (1975a; 1975b) first suggested that geographical variation between inshore and offshore populations existed based on morphological differences. Further studies also supported distinct inshore and offshore populations in ETP based on skull morphology (Douglas *et al.*, 1984; Schnell *et al.*, 1986; Perrin *et al.*, 1994). Schnell *et al*. (1986) revealed that the population differences related well with several oceanographic parameters such as sea surface temperature, solar insulation, and depth of thermocline. Moreover, significant genetic differences between inshore and offshore populations was also proved based on the analysis of mitochondrial and nuclear DNA (Escorsa-Trevino *et al.*, 1999). In the western Pacific, pantropical spotted dolphins are found from southern Japan (Miyazaki *et al.*, 1974; Zhang, 2001) through Taiwan to Australia. According to stranding and sighting records (Chu, 1996; Yeh, 2000; Chen, 2001), they have been seen on the eastern/western sides of Taiwan, although the oceanic environment on each side is greatly different. Taiwan Strait is located in the continental shelf and the average depth of it is around 60 meters. In contrast, the sea floors deepen sharply more than 1000 meters within knots off eastern Taiwan coast (Fig. 1). In addition, fish fauna between Taiwan Strait and eastern Taiwan are relatively different (Dai, 2003). In such diverse oceanographic habitats, population status of pantropical spotted dolphin in waters around Taiwan is still unknown.

Pantropical spotted dolphin is one of the most fishery-interacted dolphins among cetaceans in Taiwanese waters. According to the field records in fishing ports of Taiwan (LS Chou, unpublished data), the pantropical spotted dolphin is the species with the highest frequency of unnatural deaths. In Taiwan, all cetaceans have been protected by Wild Animal Conservation Law since 1990. For effective managements on dolphins and whales, it is important to clarify the population status and genetic structure of the dolphin that is known to have relatively higher non-natural mortality. In this study, we tested the null hypothesis of non-differentiation genetic structure of pantropical spotted dolphin of Taiwanese and adjacent waters. The genetic structure and population differentiation of putative populations were estimated to clarify the population status of pantropical spotted dolphin around Taiwan.

MATERIALS AND METHODS

Sample collection

Muscle or skin samples collected from 34 pantropical spotted dolphins from 1995-2001 were used in this research. These samples were obtained by three methods. A: Stranded animals were collected from localities in Taiwan Strait and eastern coastal Taiwan (n = 14, see Fig. 1). B: Dolphins killed incidentally by fishery were collected from Shiti Port, Taiwan (n =16) and Beihai, Qanxi, China (n = 1). C: Three samples from the Philippines waters, two

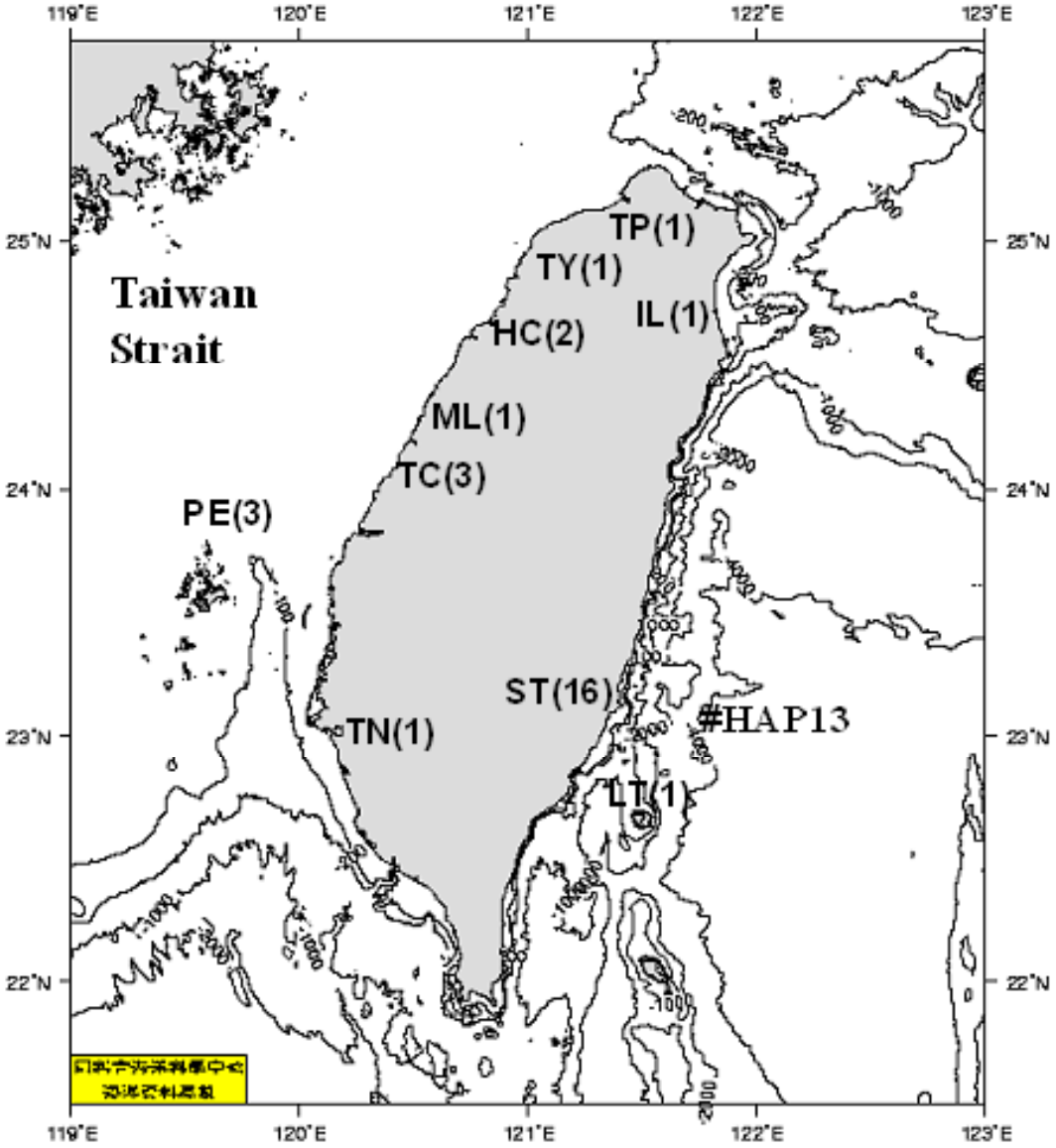

Fig. 1. Map of regions where samples of pantropical spotted dolphins were collected in the Taiwanese waters. Numbers in parentheses are sample sizes. HC-Hsinchu, IL-Ilan, LT-Green Island, ML-Miaoli, PE-Penghu, ST-Shiti, TC-Taichung, TN-Tainan, TP-Taipei, TY-Taoyuan. #HAP13 indicates the locality where the samples of haplotype HAP13 were obtained by fishery. This map is modified from a water depth file of Ocean Data Bank, National Center for Ocean Research, Taiwan.

from Cagayan de Tawi-Tawi and one from the Philippines, were courtesy from Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, USA (Loan No. 19). The tissue was preserved either in 20% dimethyl sulfoxide saturated with NaCl, or frozen at –20℃ until DNA extraction.

DNA amplification, sequencing, and analysis

DNA was extracted following the standard proteinase K digestion and phenol-chloroform extraction, as described in Rosel and Block (1996). A total of 447 base pairs (bp) of mitochondrial DNA (mtDNA) control region fragment was amplified using primers

L15824 5'-CCTCACTCCTCCCTAAGACT-3', and H16265 5'-GCCCGGTGCGAGAAGA GG-3' (Rosel *et al*., 1999). The polymerase chain reaction (PCR) was performed on a PTC200 (MJ Research) thermocycler systems in 50 μl volumes containing 10-100 ng of extracted DNA templates, 20 mM of Tris-HCl (pH 8.4), 1.5 mM MgCl, 0.15 mM of each dNTP, 1 unit of Taq DNA polymerase (QIAGEN™, Gmbh, Germany), and 0.3 μM of each primer. The cycling profile consisted of initial denaturation at 94℃ for 3 minutes, followed by 35 cycles of denaturation at 94℃, annealing at 55℃, and extension at 72℃ for 30 seconds each. After amplification, the PCR products were purified by QIAquick PCR Purification kit (QIAGEN™, Gmbh, Germany). Then the PCR products were sequenced in both directions using the Big Dye™ Terminator Cycler Sequencing Ready Reaction Kit. Sequencing was performed on an ABI Prism 310 automated sequencer.

The resultant sequences were aligned using Genetic Computer Group (GCG, Wisconsin Package Version 10.3-Unix) program. Then, the multiple sequence file was checked and corrected by eye to determine the variable sites and unique haplotypes. The haplotype diversity (h; Nei, 1987), nucleotide diversity (π; Nei, 1987) within each population were calculated using the program Arlequin version 2.0 (Schneider *et al.*, 2000). Relationships among unique haplotypes were described by computing a minimum spanning network using the algorithm MINSPNET (Excoffier and Smouse, 1994) in program Arlequin 2.0. In addition, phylogenetic trees including all haplotypes and one haplotype of same species from eastern Pacific Ocean (GenBank Accession No. U09710; Rosel *et al*., 1995) were reconstructed using the neighbor-joining (NJ) method implemented in program MEGA version 2.0 (Kumar *et al*., 2001) and maximum likelihood (ML) method implemented in program PAUP* version 4.0b10 (Swofford, 2002) with homologous sequences of spinner dolphin (*S. longirostris*) as the outgroup, to address relationships among haplotypes and among populations. The confidence of each branch was generated through bootstrap resampling for 1000 permutations. Then, population differentiation was tested using the analysis of molecular variance (AMOVA) to compute F-statistic (Fst) (Wright, 1978) using haplotype frequencies, and the F-statistic analogs, Φ-statistics (Φst), using the Tamura-Nei distance algorithm (Tamura and Nei, 1993) and a gamma parameter with $\alpha = 0.5$ (Rosel *et al*., 1999) by program Arlequin 2.0. The pairwise distances among all haplotypes were estimated by MEGA 2.0. The AMOVA model assumes that individuals are arranged into populations and populations nested into groups. In the present case, we stratified only by population, not by populations into groups. Thus, the Φst and Fst are the correlation of a random pair of haplotypes drawn from within a population on a random pair drawn from the whole individuals. Further, the significance of both Fst and Φst was tested against a null distribution constructed by 1000 permutations of the data (Schneider *et al.*, 2000). In the present analysis samples were arranged into different populations according to their collection localities. Samples collected from west coast of Taiwan and Penghu Island were assigned to Taiwan Strait population (n = 12). Dolphins collected from eastern coast of Taiwan and Green Island were assigned to eastern Taiwan population (n = 18). Samples form the Philippines and Beihai were assigned to South China Sea population (n = 4).

RESULTS

There were 14 variable sites within the 447 bp of mtDNA control region sequences from 34 pantropical spotted dolphins, accounting for 3.13 % of the total sequence. All sequence differences were transition substitutions. Two insertion/deletion were detected, one individual

Table 1. Variable nucleotide sites defining each unique mtDNA control region haplotype from 34 pantropical spotted dolphins. Sequences identity to reference sequence of the first haplotype (HAP1) indicated by dot.

Haplotype	Variable sites in the DNA fregment													
	77	102	157	209	302	305	326	339	340	347	351	360	386	427
HAP1	C	T	C	G	T	A	C	T	T	A	T	G	C	C
HAP2	.	.	.	.	.	.	.	.	C	.	.	.	.	.
HAP3	.	.	.	.	.	.	T	.	.	.	.	.	.	.
HAP4	.	.	.	.	.	.	.	.	.	.	.	A	.	.
HAP5	.	.	.	.	.	.	.	C	.	.	.	.	.	.
HAP6	.	.	.	.	.	.	.	.	C	.	.	A	.	.
HAP7	.	.	.	.	.	.	.	.	C	.	.	.	T	.
HAP8	.	.	.	.	.	.	.	C	.	.	.	A	.	.
HAP9	.	.	.	A	.	.	.	.	.	.	.	.	.	T
HAP10	T	.	.	.	.	.	.	.	C	.	.	.	T	.
HAP11	.	.	.	.	C	.	.	C	.	.	C	A	.	.
HAP12	.	C	.	A	.	.	T	.	C	.	.	.	.	T
HAP13	.	C	T	A	.	G	T	C	C	G	.	.	.	T

from eastern Taiwan population and one sample from South China Sea population, respectively. A total of 13 haplotypes was defined (Table 1). Nine of the 13 haplotypes were unique and four were shared between putative populations. Of these, six haplotypes were identified from 12 individuals of the Taiwan Strait population, and two of the six haplotypes were specific. Eight haplotypes, including four specific ones, were found in the 18 individuals of the eastern Taiwan population. The four individuals of the South China Sea population had three haplotypes, all of these were unique. Haplotype HAP1 was the most frequent type, occurring in 13 individuals, including five individuals and eight individuals in the putative Taiwan Strait and eastern Taiwan populations, respectively. HAP13 was the second frequent haplotype, occurring in four individuals in the eastern Taiwan population. The two most divergent haplotypes were HAP11 and HAP13, differed by 11 transitions, or 2.46 % of the total sequence. These two haplotypes were unique in South China Sea and eastern Taiwan populations, respectively (Table 2).

The Minimum-spanning network describes relationships among individual haplotypes (Fig. 2). A clear pattern of haplotype and geographical locale is not detected. The most common shared haplotype, HAP1, is located at the central position in the network. Both unique haplotypes of Taiwan Strait were derived from HAP1. Two unique haplotypes, HAP5 and HAP9, of South China Sea were also derived from HAP1. The unique haplotypes of eastern Taiwan, HAP8, HAP6, HAP10 and HAP13, were derived from the four shared haplotypes, HAP1, HAP2, HAP7, and HAP12, respectively. Differentiation among most haplotypes was low with the majority of neighboring haplotypes being separated by a single substitution, whereas HAP13, HAP12, HAP9, and HAP11 by two to four substitution steps. Extensive homoplasy in the data is evident in two possible connections in the network.

Both NJ and ML phylogenetic analysis with *Stenella longiroatris* as outgroup could not divide the fourteen haplotypes into clades representing the three putative populations. The topology of the ML tree (not showed here) is similar to NJ tree with a slight difference in bootstrapping values of same clades (Fig. 3). Most of the shared haplotypes of Taiwan Strait and eastern Taiwan populations, i.e., HAP1, HAP2, HAP7, one specific haplotype of eastern Taiwan, HAP6, and two unique haplotypes of South China Sea , HAP5 and HAP9, are at the

Table 2. Haplotype frequency of the three putative populations of the pantropical spotted dolphin in the western Pacific Ocean.

Haplotype	Putative Population			Total
	Taiwan Strait	Eastern Taiwan	South China Sea	
HAP1	5	8		13
HAP2	1	1		2
HAP3	2			2
HAP4	1			1
HAP5			1	1
HAP6		1		1
HAP7	1	1		2
HAP8		1		1
HAP9			2	2
HAP10		1		1
HAP11			1	1
HAP12	2	1		3
HAP13		4		4
Total	12	18	4	34

basal positions of the phylogenetic reconstruction tree relative to the specific haplotypes of each population, i.e., HAP 10 and HAP13 of eastern Taiwan, and HAP11 of South China Sea, and the haplotype of ETP.

The haplotype diversity estimates of the three populations range from 0.8883 in South China Sea, followed by 0.8182 in Taiwan Strait and 0.7778 in eastern Taiwan. Nucleotide diversity estimates range from 0.0096 in Eastern Taiwan, 0.0078 in South China Sea and 0.0049 in Taiwan Strait. We compared the genetic diversity of the pantropical spotted dolphin, Atlantic spotted dolphin, harbor porpoise and dusky dolphin. Of these, pantropical spotted dolphin has the smallest values of the diversity estimates (Table 3).

The AMOVA results show two patterns of population differentiation. The analysis using haplotype frequency (Fst) indicates significant population subdivision between South China Sea and Taiwan Strait (Fst = 0.1761, p = 0.0156), and between South China Sea and eastern Taiwan populations (Fst = 0.2029, p = 0.0059). However, the analysis using genetic distance and frequency information, Φst, does not reveal significant population differentiation between any pair of comparisons (Table 4). From the two analyses, it is indicated that there is no genetic differentiation between pantropical spotted dolphins of Taiwan Strait and eastern Taiwan populations.

DISCUSSION

There are 13 haplotypes of mtDNA control region sequences defined in 34 pantropical spotted dolphins in this study. Among them, all haplotypes from South China Sea are unique. Shared haplotypes occurred only in Taiwan Strait and eastern Taiwan waters indicating strong genetic connection or gene flow between two populations. The South China Sea population shows the highest haplotype diversity (h) among the three putative populations although the sample size of it is very small. It is not surprising that a higher diversity appears in this marine biodiversity hot spot (Roberts *et al*., 2002).

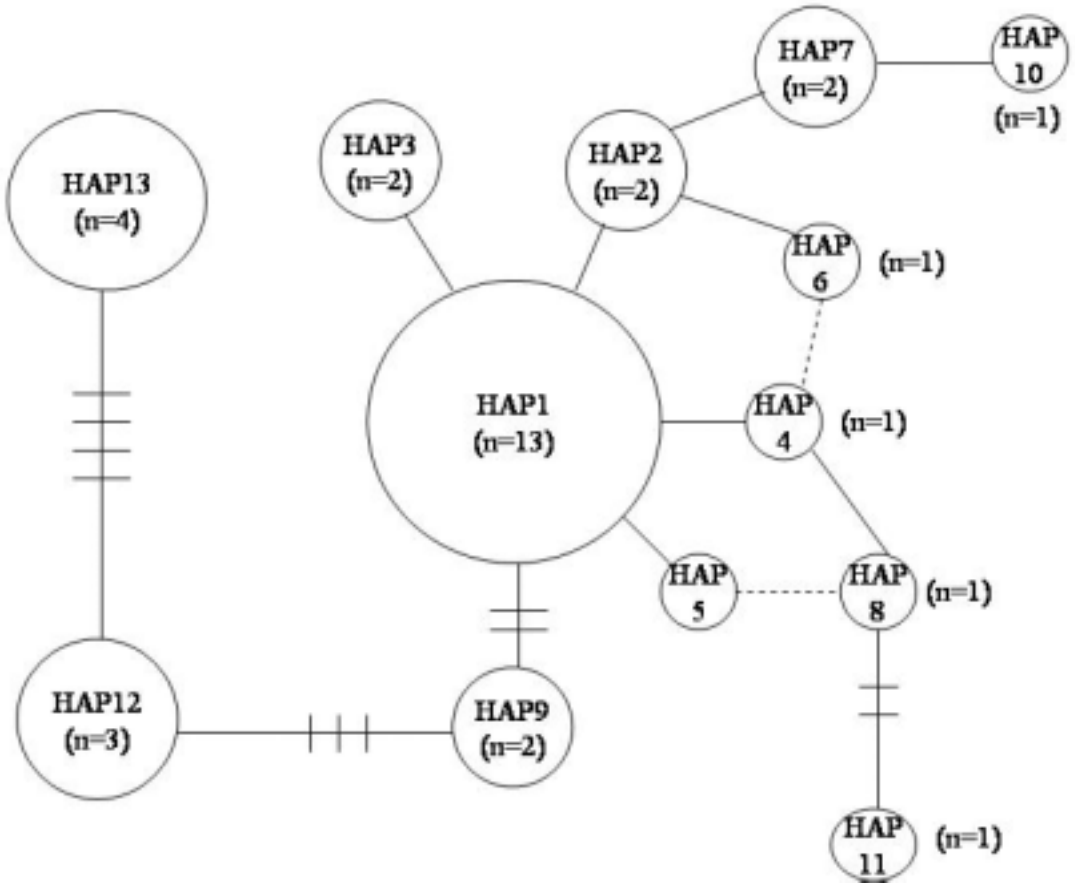

Fig. 2. Haplotype networks showing the relationships among thirteen pantropical spotted dolphin mtDNA control region haplotypes of three putative populations. Circles with shading are the haplotypes appearing in each population. Haplotype numbers correspond with the number in Table1. All haplotypes are separated by at least one substitution. Multiple substitutions between haplotypes are indicated by hash marks. Alternative connections between haplotypes (dotted lines) indicate homoplasy in in the DNA sequence data.

The eastern Taiwan population has the highest nucleotide diversity (π) whereas the h of it is the lowest. The lowest h of it could be caused by the high frequencies of two most common haplotypes, HAP1 and HAP13 (8/18, 44.4% and 4/18, 22.2%, respectively), in eastern Taiwan. However, the divergent haplotypes of HAP10, HAP12, and HAP13, occur in this locality leading to high nucleotide differences between pairwise individuals. Thus, relative divergent haplotypes appear in this locality constitutes the highest π in eastern Taiwan. The low π (0.00493) of Taiwan Strait is much less than that of eastern Taiwan (0.0096). Although the Taiwan Strait population has a higher *h* than the eastern Taiwan population, the nucleotide differences between pairwise haplotypes are low, mostly just one substitution. This situation has caused low nucleotide diversity in Taiwan Strait. In the last glacial period, the sea level had been lower than 140 meters in the Eastern China Sea. About 15000 years ago, at least, the Taiwan Strait did not even exist (Emery *et al.*, 1971). Thus, the pantropical spotted population in the Taiwan Strait may be relatively young, or newly established, compared to the other two populations. Thus, the nucleotide changes in the dolphins in the Taiwan Strait could be few due to a shorter evolutionary history. However, sampling bias could be a possible reason of the lower genetic diversity in Taiwan Strait.

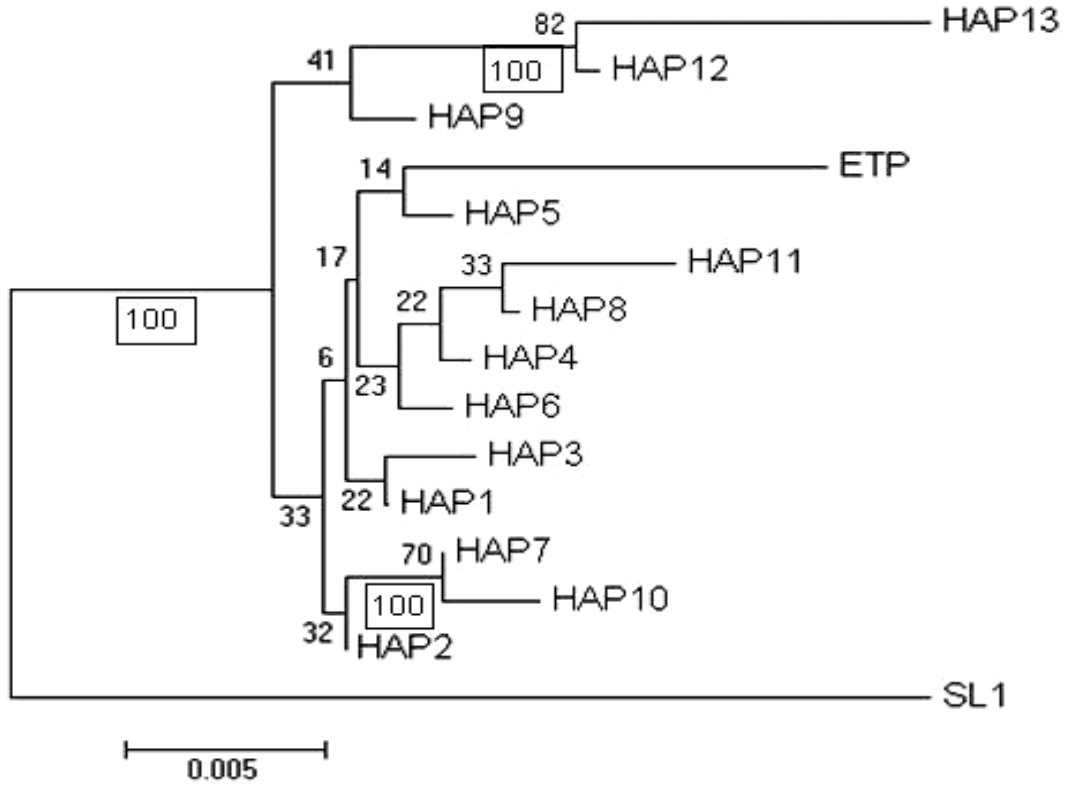

Fig. 3. Phylogenetic relationship of fourteen pantropical spotted dolphin mtDNA control region haplotypes reconstructed by using Neighbor-joining (NJ) algorithm based on the Tajima-Nei model with *Stenella longirostris* (SL1) as outgroup. Bootstrap values from 1000 iterations indicated above or below branches. Topology of Maximum Likelihood (ML) phylogenetic reconstruction based on 70% majority consensus rule is similar to NJ algorithm with slight differences on bootstrap values. Number in box below the branch indicated the confidence value of ML.

Both Fst and Φst estimates could not reject the null hypothesis of non-differentiation of the genetic structure of the spotted dolphin between the Taiwan Strait and the eastern Taiwan waters. Hoelzel (1994; 1998) had suggested that resource specialization, i. e. different habitat usage and diverse prey ecology, may be an important mechanism for the generation of genetic differentiation among some sympatric and parapatric populations of cetacean species rather than the geographical distance. However, for the pantropical spotted dolphins of the Taiwanese waters, diverse habitat, i.e. shallow continental shelf vs. deep continental slope, did not lead to significant population differentiation between the two areas.

The measurement of Wright's Fst estimated by haplotype frequency in AMOVA revealed significant population differentiation between the South China Sea and the other two populations in Taiwanese waters. However, Φst, the analysis using genetic distance and frequency information, indicated that there was no significant population differentiation between any pair of comparisons. O'Corry-Crowe *et al*. (1997) had suggested that the analysis using haplotype frequency may be a better estimate of population differentiation in situations where many very closely related haplotypes exist and little phylogeographical structure is observed in the data. In the present study, the unique haplotypes of the South China Sea are similar to most haplotypes of the Taiwanese waters with only one or two nucleotide substitutions. Phylogentic reconstruction of the haplotypes also did not show

Table 3. Genetic diversity estimates based on mtDNA control region sequences for the pantropical spotted dolphin populations and other small toothed whale populations.

Species and locality	N	No.of h[b]	Haplotype diversity	Nucleotide diversity	Population Status or Fishery impact	Note
Pantropical Spotted dolphin						
Taiwanese Waters[a]	30	10	0.7931 +/- 0.0667	0.0078 +/- 0.0046	Unknown	This study
Taiwan Strait	12	6	0.8182 +/- 0.0957	0.0049 +/- 0.0033	Unknown	This study
Eastern Taiwan	18	8	0.7778 +/- 0.0861	0.0096 +/- 0.0056	Unknown	This study
Southern China Sea	4	3	0.8333 +/- 0.2224	0.0078 +/- 0.0060	Unknown	This study
Atlantic Spotted dolphin						
Western Atlantic	122	28	0.8428 +/- 0.0276	0.0136 +/- 0.0075	Suggested as an management unit	Bero, 2001
Gulf of Mexico	77	17	0.8975 +/- 0.0163	0.0106 +/- 0.0060	Suggested as an management unit	Bero, 2001
Harbor porpoise						
Gulf of Maine	80	35	0.839 +/- 0.039	0.009 +/- 0.005	High level of nonnatural mortality	Rosel *et al*., 1999
Gulf of St. Lawrence	40	24	0.967 +/- 0.014	0.011 +/- 0.006	High level of nonnatural mortality	Rosel *et al*., 1999
Newfoundland	42	24	0.872 +/- 0.049	0.012 +/- 0.007	High level of nonnatural mortality	Rosel *et al*., 1999
West Greenland	50	26	0.967 +/- 0.010	0.013 +/- 0.007	High level of nonnatural mortality	Rosel *et al*., 1999
Mid-Atlantics	41	28	0.950 +/- 0.023	0.012 +/- 0.007	High level of nonnatural mortality	Rosel *et al*., 1999
Dusky dolphin						
New Zealand	169	76	0.972	0.022 +/- 0.011	1 or 2 historical population expansions	Harlin *et al*., 2003

a: Taiwan Strait plus Eastern Taiwan
b: haplotype

strong phylogeographical subdivision among putative populations. Thus, Fst could significantly separate the South China Sea population from the populations of the Taiwanese waters whereas Φst could not. However, the small sample size from the South China Sea implies that further research should be conducted to clarify the relationships among dolphins in those localities.

The analysis of phylogentic reconstruction shows that HAP1 and HAP2 are at the relative basal positions of the phylogenetic tree than the others. These two shared haplotypes of the Taiwan Strait and the eastern Taiwan waters revealed that relative ancient haplotypes exist in Taiwanese waters. In addition, the more derived haplotype, HAP13, also occurs in eastern Taiwan where nucleotide diversity is the highest among the three localities. These characteristics suggest that eastern Taiwan is an important locality with diverse genetic potential. Moreover, the collection site, a locality with a depth of 4000 m, constituting HAP 13 specimens was more offshore than the other samples of Taiwan (Fig. 1). Similarly, the

Table 4. Population pairwise Fst/Φst and significant values for putative pantropical spotted dolphin populations in waters around Taiwan estimated by AMOVA using mitochondrial DNA haplotype frequency and genetic distance information.

	Putative population		
	Taiwan Strait	Eastern Taiwan	South China Sea
Fst			
Taiwan Strait	-	0.3740	0.0059
Eastern Taiwan	-0.0014	-	0.0156
South China Sea	0.1761	0.2029	-
Φst			
Taiwan Strait	-	0.3333	0.0811
Eastern Taiwan	0.0132	-	0.3604
South China Sea	0.1696	0.0463	-

Fst or Φst are below diagonal, P-value above.

offshore ETP sample was also a relatively derived haplotype. The phenomenon of more derived genetic characteristics exists in offshore individuals should be tested and clarified in the future studies.

As the Taiwan Strait and the eastern Taiwan populations were suggested with no subdivision by AMOVA, pantropical spotted dolphins around the Taiwanese waters should be managed as an identical management unit. Compared to other small toothed whales in several studies, the genetic diversity estimates of pantropical spotted dolphins in the present study are relatively lower than those of Atlantic spotted dolphin (Bero, 2001), harbor porpoise (Rosel *et al.*, 1999), and dusky dolphin (Harlin *et al.*, 2003). All genetic diversity estimates of the above studies were based on the same genetic marker, hyper variable domain 3 of mtDNA control region, and the same diversity indices (Nei, 1987). Among them, harbor porpoise experiences high levels of non-natural mortality owing to interactions with commercial fisheries throughout its distribution range (Escorsa and Dizon, 2000; Rosel *et al.*, 1999; Reeves and Leatherwood, 1994). Nevertheless, the genetic diversity estimates of harbor porpoise are higher than those of pantropical spotted dolphins in Taiwan. The comparisons revealed that continuous monitoring on pantropical spotted dolphin in Taiwanese waters is necessary in the future. However, the small sample size and the relatively limited collection area of our study could also bias the estimates. Thus, under the regulation of Wild Animal Protection Law, a more systematic collection method and expanding the sampling area, i.e. biopsy sampling in inshore and offshore localities, are needed for future investigations. Moreover, accurate bycatch rate estimation will be necessary for cetaceans around Taiwanese waters to clarify the impacts from fisheries.

In addition, significant skull morphological differentiation was revealed between the pantropical spotted dolphins of Taiwanese and Japanese waters (Yao *et al.*, in revision). It is not yet clearly known if the geographic variation reflects the existence of only environmental variation, or the existence of more than one breeding population in the western Pacific Ocean. Further research on the distribution, estimation of population size, and population genetics needs to be carried out to clarify these questions.

Appendix

Samples used in this study

Number	Locality	Sex	Year	Date	Body length (cm)	Body weight (kg)
ST9501	Eastern Taiwan	M	1995			
BH9501	South China Sea	F	1995	20-Dec	134	26.1
PE9509	Taiwan Strait	F	1995	17-Nov	190.5	
PE9510	Taiwan Strait	M	1995	17-Nov	149.5	
TC9601	Taiwan Strait	M	1996	20-Jun	200	81.8
TC9602	Taiwan Strait	M	1996	20-Jun	197	79.8
TC9603	Taiwan Strait	F	1996	20-Jun	195	75
TN9605	Taiwan Strait	F	1996	3-Apr	195	82
PE9711	Taiwan Strait	M	1997	15-Mar	123.5	19.5
IL9801	Eastern Taiwan	M	1998	2-May	205	71.6
LT9901	Eastern Taiwan	F	1999	18-Mar	201	
ST9903	Eastern Taiwan	M	1999	20-Jul	206	
ST9911	Eastern Taiwan	F	1999	11-Nov	173	55
ML9902	Taiwan Strait	M	1999	12-Aug	197	
TP9902	Taiwan Strait	M	1999	13-Sep	170.5	
ST0010	Eastern Taiwan	M	2000	27-Jul	189.5	
ST0018	Eastern Taiwan	F	2000	9-Oct	173	
ST0023	Eastern Taiwan	F	2000		127.5	46
ST0028	Eastern Taiwan	F	2000	17-Oct	190	
ST0029	Eastern Taiwan	M	2000	17-Oct	195	
ST0036	Eastern Taiwan	M	2000	29-Oct	181	62
ST0037	Eastern Taiwan	M	2000	29-Oct	215	93
ST0038	Eastern Taiwan	M	2000	29-Oct	216	98
ST0039	Eastern Taiwan	M	2000	29-Oct	201	94
ST0041	Eastern Taiwan	F	2000	21-Nov	178	
ST0042	Eastern Taiwan	F	2000	21-Nov	195	
ST0043	Eastern Taiwan	M	2000	21-Nov	175	
HC0002	Taiwan Strait	F	2000	19-Jun	188	
HC0003	Taiwan Strait	M	2000	19-Jun		
TY0008	Taiwan Strait	M	2000	29-May	168	
ST0103	Eastern Taiwan	F	2001	3-Feb	129	
Z5551	South China Sea	U	1996	15-May		
Z5552	South China Sea	U	1996	15-May		
Z5748	South China Sea	U	1996	13-Jun		

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以粒線體 DNA 控制區序列探討台灣及南中國海海域泛熱帶斑海豚 (*Stenella attenuata*) 的族群結構

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摘　　要

本研究是利用粒線體 DNA 控制區序列之 447 個鹼基探討台灣及南中國海海域泛熱帶斑海豚 (*Stenella attenuata*) 的族群結構與遺傳多樣性。我們將這些海域的海豚，區分為三個預設族群，包括台灣海峽、台灣東部海域與南中國海域，並且檢測此三海域的海豚族群並無族群分化之假說。在 34 個體的 DNA 序列中，共有 14 變異位點，可以分為 13 個單套型。在這些單套型中，有 9 個是地區特有型，包括台灣海峽 2 個、台灣東部海域 4 個與南中國海域 3 個。而南中國海域並無地區共有之單套型。遺傳多樣性估計值包含單套型多樣值(haplotype diversity, h) 與核甘酸多樣值 (nucleotide diversity, π) 兩種，三個族群的數值範圍分別是 h 介於 0.7778-0.8883，以南中國海域最高; π 介於 0.0049-0.0096，以台灣東部海域最高。與數種小型齒鯨同段 DNA 的遺傳多樣性估計值相比，本研究的估計值均偏低。由最小幅網路分析與親緣關係建構法分析三個族群之 DNA 單套型，均無明顯的區域性分化趨勢。分子變異分析結果發現，由單套型頻度資料所估算出的 Wright's fixation index, Fst，顯示南中國海族群分別與台灣海峽 (Fst = 0.1761, p = 0.0156) 以及台灣東部海域 (Fst = 0.2029, p = 0.0059) 兩個族群有顯著的分化。然而，由遺傳距離資料所估算的分化指數，Φst，任何族群間均無顯著分化。這是由於南中國族群之 DNA 單套型雖然具地區獨特性，但是其與台灣兩個族群大多數之單套型的相異度並不大所致。雖然有極多的族群研究顯示，棲地的差異是造成共域或鄰域之鯨豚族群分化的重要因素之一，然而由我們的研究結果顯示，位於亞洲大陸棚的台灣海峽淺水域與大陸斜坡陡降的台灣東部深水域的泛熱帶斑海豚族群，彼此之間並無顯著分化。未來的研究中，增加南中國海樣本數以及更系統性的在台灣海域蒐集樣本，將有助於釐清南中國海與台灣海域族群分化的問題，並且也可持續監測這些海域之海豚的遺傳多樣性，作為保育管理單位的建議。

關鍵詞：粒線體 DNA、台灣、南中國海、泛熱帶斑海豚、族群結構。

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