Thesis for the Degree of Master of Engineering

Taxonomic status of common dolphin in the East Sea, Korea

by

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Taxonomic status of common dolphin in the East Sea, Korea

동해에 서식하는 참돌고래의 분류학적 위치

Advisor: Prof. Yong Ki Hong

by
Ji Hye Kim

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Abstracts

참돌고래는 동해에 약 30,000 마리가 서식하고 있는 돌고래이다. 참돌고래 속(Genus)dms 긴부리참돌고래와 짧은부리참돌고래 두 종을 포함하고 있다. 동해의 참돌고래는 형태적 분류를 통해 긴부리참돌고래로 알려져 있다. 정확한 동 판별을 위한 유전학적 종 분류 등과 집단 분석은 아직 이루어지지 않았다. 본 연구에서는 두개골 측정과 미토콘드리아 DNA 분석을 통하여 분류학적 위치를 명확히 정립하고, 미토콘드리아 DNA 분석을 통해 집단 구조를 알아보고자 하였다. 유전 분석을 위한 표본은 2011년부터 2013년까지 혼획된 참돌고래의 시료를, 두개골 측정은 기존에 제작된 두개골 표본을 사용하였다. 본 연구 이전에 이루어진 긴부리 및 짧은부리참돌고래 분류에 대한 연구와 비교한 결과 두개골 측정 값은 긴부리참돌고래의 특징을 나타내었고, 미트콘드리아 DNA 과변이부위의 염기서열 분석 결과 짧은부리참돌고래로 밝혀졌다. 주요 유전자형은 연도별, 월별 비교 결과 집단간 차이를 거의 보이지 않았으며, 유전자형 빈도 분포에서도 참돌고래의 하부 계군 구조를 추정하기 어려웠다. 매년 새로운 유전자형이 발견되었지만 이는 표본의 수의 증가와 관련 있는 것으로 추정되며 유전자형의 빈도분포에 변화를 주는 요인으로 판단하기는 어려웠다.

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I. Introduction

Common dolphins, genus *Delphinus* are one of the most widespread cetacean species in the world. They are inhabited mainly in tropical and warm-temperate waters (Evans 1994; Perrin 2002). As common dolphins show great morphological variability throughout their wide geographical distribution, these species have classified more than 20 different species since its first description (Evans 1994; Hershkovitz, 1966).

Previously, most of taxonomic studies on common dolphins focused on morphological characters. Morphological studies to understand species identification, sexual dimorphism, and population structure between or within species are particularly based on rostrum length to zygomatic width ratio including skull characters such as beak length, total body length, and tooth counts (Bell et al., 2002; Heyning et al., 1994; Murphy et al., 2006; Perrin 2003; Westate 2007).

In spite of the controversy of the taxonomic classification within *Delphinus* spp, two species are generally accepted: the short-beaked common dolphin, *Delphinus delphis* Linnaeus, 1758 and the long-beaked common dolphin, *D. capensis* Gray, 1828. Heyning and Perrin (1994) documented the separation between sympatric two morphotypes of genus *Delphinus* inhabiting off Californian coast based upon external morphological characters, such as the pigmentation patterns, overall body size, and skull variation.

Heyning et al. (1994) claimed *D. delphis* is smaller than *D. capensis*. However, additional morphological studies for the common dolphins have observed high variability

compared with body size, beak length, and rostrum length to zygomatic width ratio for common dolphins off the Californian waters (Bell et al., 2002; Jefferson et al., 2002; Jordan 2012; Murphy et al., 2006; Westgate 2007). Moreover, Bell et al. (2002) founded skull differentiation of common dolphins according to different water depths off southern Australia.

Recently, molecular analysis has been increased for many cetacean species (LeDuc et al. 1999; Pichler et al., 1998; Rose et al., 1994; Stockin et al., 2013; Wang 1994). Mitochondrial DNA, which is only maternally inherited, has been used for population structure studies (Wilson et al., 1985). Mitochondrial DNA (mtDNA) has several advantages for phylogenetic analysis; mitochondrial gene has high variability, and evolutionary rate is estimated about ten times faster than nuclear protein-coding gene (Ballard and Whitlock 2004; Hoelzel et al., 1991; Lin and Danforth, 2004). For these reasons, mtDNA gene as molecular marker is a powerful tool for species identification, population structure, recent demographic history, taxonomic classification within or among closely related species (Hoelzel et al., 1991; Lin and Danforth, 2004; Sergio et al., 2005; Wang 1994). The study of genetic analysis using mitochondrial DNA supported genetic differentiation between two species, whose genetic divergence was 1.11% (Rosel et al., 1994). The author referred long- and short-beaked common dolphins were reciprocal monophyly. However, the long- and short-beaked common dolphin for phylogenetic study of family Delphinidae based upon mtDNA cytochrome b gene showed that they are not reciprocal monophyly and genetic study using the amplified

fragment length polymorphism (AFLP) markers indicated that the two species are recently diverged (Amaral et al. 2007, Kingston & Rosel 2004, LeDuc et al. 1999).

Although the studies on two species were restricted to the area off the Californian coast, the result of both morphological and molecular data was very evident to separate the two species. Despite the sympatry, long-beaked common dolphin appears to be restricted within 180 km off the coast and short-beaked common dolphin occurs farther to thousands of kilometers from the coastline (Heyning et al., 1994; Rosel et al., 1994).

Common dolphin is the most abundant cetacean species in the East Sea, and the population size is estimated at about 30,000 individuals (Unpublished data, Cetacean Research Institute). The by-catch of this species is reported more than 250 individuals annually (Ahn et al., 2014), and the number of the common dolphin by-catch has increased recently. The increasing by-catch of the dolphins has raised concerns about the conservation of this species in Korean waters.

The common dolphins in the Korean peninsula have been identified as long-beaked common dolphins, *D. capensis,* which are only defined by external morphological characteristics such as pigmentation patterns, rostrum length, and tooth count. However, morphological study has not yet been conducted in detail.

It is very important to identify the species and understand the population structure of the common dolphins which is the top predators in their habitat in order to manage and conserve the species. However, there has been no genetic study on the identification of the species and the stock structure of the population in Korean waters.

The aims of this study are (1) to investigate molecular analysis using mtDNA control region combined with skull measurement in order to identify the taxonomy the common dolphins in Korean waters, and (2) to confirm whether common dolphins inhabiting the East Sea are belonging to a single stock.

II. Materials and methods

1. Sample collection and DNA extraction

For osteological measurements, 16 skulls collected in the East Sea from 2004 to 2013 were used (Fig. 1).

Muscle and skin were collected from 784 individuals of common dolphins that were caught incidentally by several fishing gears in the same region from 2011 to 2013. Of these, 658 samples with good conditions were used for this study.

Total genomic DNA was extracted from muscles or skins was preserved frozen (-20℃) using the DNeasy Blood & Tissue Kit (Qiagen. USA) following manufacturer's instructions. The DNA quality was examined via electrophoresis on 1.5% agarose gels in 0.5 x TAE buffer.

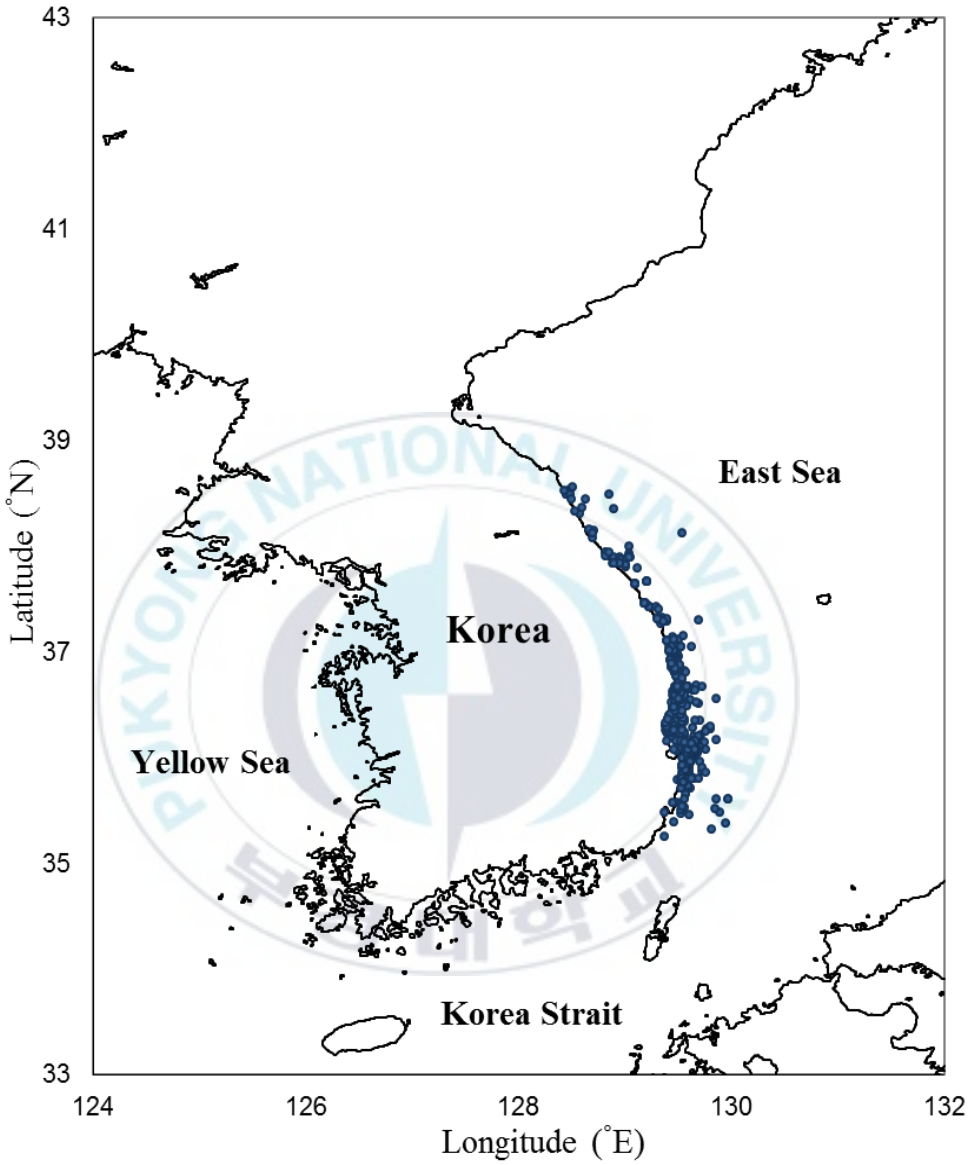

Fig. 1. By-catch locations of common dolphins analyzed in this study (blue solid circles) from 2011 to 2013

2. Skull measurement

For osteological measurement, 16 skulls were measured as described in Perrin (1975); Benke (1993) and Heyning et al (1994). Measurements of each skull were taken to an accuracy of 0.01 mm using digital vernier calipers for measurement of ≤ 310 mm or 0.1 mm using tape measure for measurement of > 310 mm without information on body length and age (Table 1). Tooth was counted on both upper and lower jaws of each side. Simply, skull variation of common dolphins in the eastern coast of Korea is compared with published morphotypic data for condylobasal length, rostrum length, zygomatic width, rostrum length to zygomatic width ratio, and tooth count from other areas. Skull morphometric data were analyzed using Excel. Statistical features of skull measurement for the common dolphins in Korean waters by sex are presented in Appendix 1. To compare with values generated in this region, published osteological data are added from other geographic populations within the genus (Table 2).

3. Mitochondrial DNA amplification

For the mtDNA data, a 469 base pair (bp) fragment of control region from 658 individuals was amplified by PCR using MTF-F; 5'-CCTCCCTAAGACTCAAGGAAG-3' primer (Arnason et al., 1993) and Dlp-5R; 5'-CCATCGATGTCTTATTTAAGGGGAAC-3' premer (Baker et al., 1996; Dalebout et al., 1998). The PCR reaction mixtures were performed in 10 μL volumes as following: 1 μL of genomic DNA, 0.8 μL dNTP mixture (2.5 mM each), 0.4 μL each primer (10 Pm), 1 μL 10 x PCR buffer, and 0.1 μL of Taq polymerase (5 units/μ) (TAKARA). The amplification protocol consisted of an initial denaturation for 10 minutes at 95℃, followed by 30 cycles of 30 seconds at 94℃, 30 seconds at 50℃, 1 minutes at 72℃, with a final extension step of 5 minutes at 72℃. The PCR products were purified with a PCR purification Kit (Qiagen, Hilden, Germany) and the purified products were stored at -20℃ until required. The purified DNA subsequently were used in direct cycle sequencing reactions with the Big-Dye Terminator v3.1 cycle Sequencing Kit (Applied Biosystems Foster City, CA, USA) according to manufacturer's instructions. The sequencing reaction was performed in volumes of 10 μL and with the following temperature profile: 25 cycles of 96℃ for 10 seconds, 5 seconds at 50℃, 4 minutes at 60℃. The sequences were determined using an ABI 3130xl automated DNA sequencer (Applied Biosystems). Sequence data were edited by eyes using BioEdit v.7.2.0 and were aligned using multiple alignments Clustal X.

In order to define the classification of common dolphin inhabiting Korean waters, additional published sequences of long- and short-beaked common dolphins were obtained from Genbank (Accession numbers: HE680096-HE680141 of short-beaked common dolphins, HE680142-HE680202 of long-beaked common dolphins). Sequences obtained from Genbank were truncated in order to have the same length determined in this study.

4. Statistical analyses

For phylogenetic tree, the distance matrix for the neighbor-joining analysis was performed by the Tamura-Nei distance method using Mega v. 6.06 with $\alpha = 0.5$ and consensus trees were constructed from 1000 bootstrap replications. A 50% criterion for the retention of nodes was applied. Only haplotype frequency

Genetic diversity within Korean waters was evaluated as the number of haplotypes, estimate nucleotide diversity (π), gene diversity (H), and Genetic distances F_{ST} (using only haplotype frequency), which were calculated using ARLEQUIN v3.5 (Excoffier and Schneider 2005). The selective neutrality of Tajima's D and Fu's *F*s, and mismatch distribution analyses were used to explore demographic history and were calculated using 1,000 simulations using ARLEQUIN v3.5 (Schneider et al., 2000). To investigate the demographic history of Korean common dolphin, the time of expansion (t) was calculated using the equation $\tau=2ut$, where τ is the mode of the mismatch distribution (in units of evolutionary time) and u is the mutation rate for the whole sequence used. The value u was calculated using the formula $u=\mu k$, where μ is the mutation rate per nucleotide and k is the number of nucleotides assayed (Yang et al., 2008). the mutation rates (μ) was used by 7×10^{-8} (s.s.yr^{-1}) (Harlin et al., 2003).

Table 1. Description of skull measurements for the common dolphins used in this study.

Morphometric characters	Abbreviation	No.
Condylobasal length	CBL	1
Rostrum length	RL	2
Distance from tip of rostrum to external nares	DREN	3
Width of rostrum at base	WRB	4
Width of rostrum at 60mm from base	WR60	5
Width of rostrum at midlength	WRM	6
Width of premaxillaries at midlength of rostrum	WPRM	7
Width of rostrum at ¾ length	WR¾	8
Greatest preorbital width	GPRW	9
Greatest postorbital width	GPOW	10
Least supraorbital width	LSW	11
Greatest width of external nares	WEN	12
Greatest width of premaxillaries	WPR	13
Least width braincase parietal borders	BCW	14
Length top perimeter skull	LTPS	15
Zygomatic width	ZW	16
Greatest width basioccipital	BOW	17
Greatest width of internal nares	GWIN	18
Greatest length of left pterygoid	GLP	19
Distance from tip of rostrum to internal nares	DRIN	20

Table 1. (continued)

Length of upper left tooth row	LUTR	21
Length orbit(left side)	OL	22
Length antorbital	AOL	23
Greatest length temporal fossa (left side)	LTF	24
Greatest whdth temporal fossa (left side)	WTF	25
Length mandible (left side)	ML	26
Length mandibular fossa (left side)	LMF	27
Length mandible left tooth row to tip	LMTR-T	28

III. Results

1. Taxonomic identification of common dolphin in Korea

A. Comparing skull

Taxonomic identification of common dolphins for osteological study was mostly determined by condylobasal length (CBL), rostrum length (RL), zygomatic width (ZW), rostrum length to zygomatic width ratio (RL/ZW), and tooth count (TL).

In Korean waters, male common dolphins had CBL range from 465 to 524 mm (n=9), RL from 290 to 344 mm, ZW from 181 to 203 mm, RL/ZW ratio from 1.50 to 1.78. For female dolphins, CBL ranged from 452 to 488 mm (n=6), RL from 288 to 304 mm, ZW from 180 to 196 mm, RL/ZW ratio from 1.58 to 1.72. The numbers of upper and lower tooth for male were 48-57 and 49-56, respectively. For female common dolphins, the ranges of tooth count were 51-60 and 53-56, respectively. Values for CBL, RL, ZW, and RL/ZW of common dolphin with unknown gender, were 471.5 mm, 304.4 mm, 186.6 mm, and 1.63, respectively. The numbers of upper and lower tooth count were 56 and 53, respectively (Table 2).

Table 2. Cranial parameters for taxonomy of *Delphinus* sp. Mean values and range (in parentheses) are shown. Morphotypic variability was observed according to geographic areas. Note: Cal = California; wNA = western North Aatlantic; eNA = eastern North Aatlantic; sAU = southern Australia; NZ = New Zealand; IndO = Indian Ocean; Kor = Korea in this study; TC = tooth count, M = males, F = females, P = sexes pooled.

Species	Sex	CBL (mm)	RL (mm)	ZW (mm)	RL/ZW ratio	Upper left TC	Lower left TC	Region
D. delphis	M	421.5 (392 - 445)	254.4 (227 - 275)	184.9 (173 - 195)	1.37 (1.21 - 1.46)	49 (42 - 54)	47 (41 - 53)	Cal [a]
	F	406.3 (382 - 442)	244 (218 - 264)	179.6 (170 - 190)	1.36 (1.23 - 1.47)			
	M	444.7 (412 - 481)	271.7 (248 - 298)		1.42 (1.31 - 1.54)			wNA [b]
	F	434.9 (411 - 456)	265.8 (250 - 281)		1.44 (1.34 - 1.54)			
	M	446.8 (411 - 479)	273.6 (237 - 295)		1.43 (1.31 - 1.57)	41 - 53	42 - 53	eNA [b, c]
	F	430.6 (397 - 452)	263.3 (234 - 285)		1.43 (1.31 - 1.54)			
	P		271.4 (225 - 311)	178.2 (152 - 202)	1.52 (1.36 - 1.73)	47.9 (43 - 54)		sAU [d]
	M	463.6 (446 - 495)	291.6 (276 - 322)	192.2 (181 - 207)	1.50 (1.39 - 1.59)	51 (45 - 55)	49 (45 - 53)	NZ [e]
	F	446.4 (423 - 469)	279 (261 - 298)	185.3 (175 - 200)	1.49 (1.40 - 1.61)	50 (47 - 55)	50 (43 - 55)	
D. capensis	M	473.6 (446 - 498)	302 (286 - 321)	189.1 (181 - 204)	1.60 (1.52 - 1.67)	53 (48 - 59)	51 (47 - 55)	Cal [a]
	F	465.5 (445 - 486)	296.2 (281 - 314)	180.8 (173 - 191)	1.64 (1.55 - 1.77)			
	P	486 (449 - 543)	312.4 (278 - 348)	191.1 (170 - 212)	1.64 (1.46 - 1.77)	54	52	IndO [f]
D. c. tropicalis	P	502.9 (456 - 575)	336.2 (298 - 398)	179.8 (160 - 206)	1.85 (1.60 - 206)	60	57	
Delphinus sp.	M	491.7 (465 - 524)	317.2 (290 -344)	190.5 (181 - 203)	1.67 (1.50 - 1.78)	54 (48 - 57)	52 (49 - 56)	Kor
	F	470.4 (452 - 488)	303.6 (288 - 304)	184 (180 - 196)	1.65 (1.58 - 1.72)	55 (51 - 60)	54 (53 - 56)	
	P	471.5	304.4	186.6	1.63	56	53	

[a] Heyning & Perrin (1994)
[b] Westgate (2007)
[c] Murphy et al. (2006)
[d] Bell (2001)
[e] Jordan (2012)
[f] Jefferson &Van Waerebeek (2002)

B. Comparing Mitochondrial DNA control region sequence

The 469 bp of mtDNA control region sequence for *Delphinus* including published sequence obtained from Genbank were identified 125 haplotypes showing 82 polymorphic sites; 75 were transition, 5 were both transition and transversion, and 2 were insertion/deletion (Table 3).

Haplotypes of common dolphin in the Korean waters correspond with haplotypes of *D. delphis* lineage. No shared haplotypes were observed between common dolphins in the Korean waters and added long- or short-beaked common dolphin off the California.

The six nucleotide differences (site 110, 156, 248, 287, 289 and 292) indicate distinction between haplotypes of *D. capensis* and *D. delphis* lineages (Rosel et al., 1994). Previous genetic studies suggested one fixed and five frequency differences observed between the two species of *Delphinus* (Rosel et al., 1994; Segura, 2001). However, in this study six sites were frequency differences between the two forms of *Delphinus*; all sites were transition in this study (Table 3). There is no fixed difference although transition in 156 bp was only one haplotype KH16.

Table 3. Polymorphic sites of common dolphin mtDNA control region haplotypes including published sequence of long-beaked (Dc1-Dc36) and short-beaked common dolphin (Dd1-Dd37). Dots indicate identity to top sequence. Dashes represent insertion/deletion (indel) events. Asterisks indicate fixed or frequency differences between long- and short-beaked common dolphins (sites: 110, 156, 248, 287, 289 and 292).

Haplotypes	1111111 1111111111 1122222222 2222222222 2233333333 3333333333 3334444444 44 244478889 9990000001 1123555567 8901144666 7777888889 9900001122 2456678899 9990015555 55 7034783791 7891345690 3816012681 3351858259 3589123792 3401243535 8665927901 2357800124 56 * * * ***
Dc1	-ACGATGACT CACTCACATG TA-GATCATG TATTCCGTAC CATCTCCCGT CCTCGTTAAC CGTTTTAATC TCTATATTTA CT
Dc2	-..... ....-..... ....A..... ....C..... ....
Dc3	-..... ....-..... ....C..... ....
Dc4	-..... ....-..... ....G.. .....C. ....
Dc5	-..... ....-..... ....T .....C. ....
Dc6	-..... ....-..... ....A....T .....C. ....
Dc7	-..... ....-..... ....T ....
Dc8	-..... ....-...T... ....C .T.....T ....C..... ....
Dc9	-..... .G..... ..-...T... ....T ....
Dc10	-..... .G..... ..-...T... ..... .G..... .....T ....
Dc11	-..... ....-...T... ..... .G..... .....T ....
Dc12	-..... ....-...T... ..... .G..... ..C.....T ....
Dc13	-..... ....T...T... ....T ....
Dc14	-..... ....T...T... ....T ....G..... ..
Dc15	-..... ....-...T... ....T ..C..... ....
Dc16	-G..... ....-...T... ....T ....
Dc17	-..... ....-...T... ....T ....
Dc18	-..... ....-...T... ....T .....G... ....
Dc19	-..... ....-...T... ..C..... ....C.....T ....
Dc20	-..... .....C. ..-...T... ....C.....T ....C.. ..
Dc21	-..... ....-...T... ....T .....C. .....C.. ..
Dc22	-..... .....T... ..-...T... .....C.. ..... .T.....T ....C.. ..
Dc23	-..... ....-...T... ....T ....C.. ..
Dc24	-.....G.. ....-...T... ....G.... T.....T ....
Dc25	-..... ....-...T... .... T.....T ....
Dc26	-..... .....G.... ..-...T... C..... .....TA. .....T ....
Dc27	-..... .....G...A ..-...T... ....TA. T.....T ..... .T..... ..

Table 3. (continued)

Dc28	-..... .....G.... ..-...T... ....TA. T.....T .....G.. ....
Dc29	-...G..... .....G.... ..-...T... ....TA. T.....T ....
Dc30	-..... .....G.... ..-...T... ....TA. T.....T T..... ....
Dc31	-..... .....G.... ..-...T... ....TA. T.....T ....
Dc32	-..... .....G.... ..-...T... ....TA. T.....T ....C. ..
Dc33	-..... .....G.... ..-...T... ....T.TA. T.....T ....
Dc34	-..... .....G.... ..-...T... ....T.TA. .....T ....
Dc35	-..... ....-...T... ....T.TA. T.....T ....
Dc36	-..... .....G.... ..-...T... ....T.. T.....T ....
Dd1	A.T..... .....A ..-...AG.. ....T..... .....TTAC .....GT ..C..... ....
Dd2	-..... .....A ..-...TG.. .....G. .....TTAC .....T ....
Dd3	-..... .....A ..-...TG. .....G. ...T..TTAC .....T ....
Dd4	-..... .....G...A ..-...TG.. .....G. .....TTAC T.....T .....C..C. ....
Dd5	-..... .....A C.-...TG.. ..C...A... .....T.TAC .....T ...C..... ....C..... T.
Dd6	-..... .....A ..-...TG.. .....T .....T.AC .....T ....
Dd7	-..... .....A ..-...TG.. ...C....G. .....TTTAC .....T ....
Dd8	-..... .....A ..-...TG.. .....TA... .....TTTAC .....T C
Dd9	-..... ....-...TG.. ....TTTAC .....T ..C..... C..... ..
Dd10	-.T..... .....A ..-...TG.. ....TTA. .....T ....C... ..
Dd11	-.T..... .....A ..-...TG.. ....TTA. .....T ..... C.....C... ..
Dd12	A.....C ....T....A ..-...AG.. ....T.A... T.C.C.TTA. T.....GT .....C. ....
Dd13	-.....T. .....A ..-...TG.. ..... T...C.TTAC T.....T ....
Dd14	-..... .....A ..-..CTG.A .G..... .....TTA. .....T ....C....A .....G..CG ..
Dd15	-..... .....A ..-...TG.. .....A... .....TAC T.....C..T .....A ....
Dd16	-..... .....A ..-...TG.. .....A... .....T.TAC T.....C..T .....A ....
Dd17	-..... .....A ..-...TG.. .....A... .....TAC T.....T .A.....CA ....
Dd18	-..... .....A ..-...TG.. .....A... .....TAC ....A....T .....A ....
Dd19	-..... .....A ..-...TG.. .....A... ....C..TAC ....T....T ....
Dd20	-..... .....A ..-...TGC. .....A... ....C..TAC ....T....T ....
Dd21	-..... .....A ..-...TG.. ..C...A... .....T.TAC .....T ....
Dd22	-..... .....G.A ..-...TG.. ..C...A... .....TAC .....T .....A ....
Dd23	-..... .....A ..-.G.TG.. .....A... ....CT.TAC T...A....T ..... C..... ..
Dd24	-.....A... .....A ..-...TG.. .....A... .....T.TAC .....T ....
Dd25	-..... .....A ..-...TG.. .....A... ....CT.T.C T..T.....T .....CA ....
Dd26	-..... .....A ..-...TG.. ....TAC .....T ..C..... ...G.G.... ..
Dd27	-..... .....A ..-...TG.. .....A... .....TTTAC .....T ..C..... ....
Dd28	-..... ...C.....A ..-...TG.. ....T.TAC .....C...T ..C.....C. ...G..... ..

Table 3. (continued)

Dd29	-.T..... ..TC.....A ..-...TG.. ....TATA. .....T ....C..... .T..... ..
Dd30	-.T..... ..TC.....A ..-...TG.. ....TATA. ....A....T ....C..... .T..... ..
Dd31	-.T..... ..T.....A ..-...TG.. ....TATAC .....C.G.T ..C..... ....
Dd32	-..... .....A ..-.G.TG.. ..C...A... .....TAC T.....T ..C..... ....
Dd33	-..... .....A ..-....G.. ..C...A... .....TAC T.....T ....
Dd34	-..... T.....A ..-...TG.. ....TAC T.....T ....
Dd35	-..... .....A ..-....G.. .....T.... .....TAC T.....T ....
Dd36	-..... ....-...TG.. ....TAC .....T ..C..... ....
Dd37	-.T..... ....-...TG.. ....A.AC .....T ....
KH1	-..... .....A ..T...TG.. .....A... .....TAC .....C..T ....
KH2	-..... .....A ..-A..TG.. .....A... .....TTAC ....A....T ....
KH3	-..... .....A ..T...TG.. .....A... .....TAC .....T ....
KH4	A..... .....A ..-...TG.. .....A.G. .....TAC T...A....T .....C. ....
KH5	-..... .....A ..-A..TG.. .....A... .....TTA. ....A....T ....
KH6	-..... .....A ..T...TG.. .....A... .....TAC T.....T ....
KH7	A..... .....A ..T...TG.. .....A... .....TAC .....T ....
KH8	-..... .....A ..-A..TG.. .....A... .....TTAC T...A..... ....
KH9	-..... .....A ..-...TG.. .....G. .....TAC ....A....T .....C. ....
KH10	-....C.... .....A ..-A..TG.. .....A... .....TTAC ....A....T ....C..... ....
KH11	A..... .....A ..-...TG.. .....A.G. .....TAC TT..A....T .....C. ....
KH12	-..... .....A ..-A..TG.. .....A... .....TTAC ....A....T .....C. ....
KH13	A..... .....A ..-...TG.. .....G. .....TAC ....A....T ....C...C. ....
KH14	A..... .....A ..-...TG.. .....G. .....TAC ....A....T .....C. ....
KH15	A..... .....A ..-...TG.. ....TAC T...A....T .....C. ....
KH16	-..... .....A .G-...T... .....A... ...T.TT.AC T.....T ....
KH17	-..... .....A ..-...TG.. .....GT .....TAC ....A....T .....C. ....
KH18	A..... ....T....A ..-...TG.. .....G. .....TAC ....A....T .....C. ....
KH19	-.T..... .....G.A ..-...TG.. .....G. .....ATAC .....T ....
KH20	-..... .....A ..-...TGA. .....TA... .....TA. .....T ....
KH21	-..... T.....A ..-...TG.. .....G. .....TAC ....A....T .....C. ....
KH22	-..... .....A ..-A..TG.. .....A... .....TAC T...A..... ....
KH23	-..... .....A ..-A.CTG.. .....A... .....TTAC ....A....T ....
KH24	-..... .....A ..T...TG.. .....A.G. .....TAC .....C..T ....
KH25	-..... .....A ..-A..TG.. .....A.G. .....TTAC ....A....T ....
KH26	-..... .....A ..T...TG.. ..C...A... .....TAC .....T ....
KH27	A..... .....A ..-...TG.. .....G. .....TA. ....A....T .....C. ....
KH28	-..... .....A ..-A..TG.. .....A.G. .....TT.C ....A....T ....

Table 3. (continued)

KH29	-..... .....A ..T...TG.. .....A... .....TA. .....T ....
KH30	-..... .....A ..T...TG.. ..C...A... .....TAC .T.....T ....
KH31	A..... .....A ..T...TG.. .....G. .....TAC ....A....T .....C. ....
KH32	-..... .....A ..T...TG.. .....A... .....TAC ....A....T ....
KH33	A..... .....A ..-...TG.. ....TAC ....A....T .....C. ....
KH34	-..... ....-...TG.. .....A... .....TA. T.....T ....
KH35	A..... .....A ..-...TG.. .....G. .....TAC ....A....T ....
KH36	-..... .....A ..-A..TG.. .....G. .....TTAC ....A....T ....
KH37	-..... .....A ..-A..TG.. .....A... .....TTAC ....A....T .....G.. ....
KH38	-..... .....A ..-...TG.. .....A... .....TA. T.....T ....
KH39	-.T..... .....G.A ..-...TG.. .....G. ....C.ATAC .....T ....
KH40	A..... .....A ..-...TG.. .....G. ....C..TAC ....A....T .....C. ....
KH41	-..... .....A ..-A..TG.. .....A... .....TTAC .....T .....C. ....
KH42	-..... .....A ..T...TG.. .G....A... .....TA. .....T ....
KH43	A..... .....A ..-...TG.. .....A.G. .....TAC ....A....T .....C. ....
KH44	-..... .....A ..T...TG.. .....A... .....T.TAC .....T ....
KH45	-..... .....A ..-...TG.. .....G. .....TAC .T..A....T .....C. ....
KH46	-.T..... .....G.A ..-...TG.. ....ATAC .....T ....
KH47	-..... .....A .G-A..TG.. .....A... .....TTAC ....A....T ....
KH48	A..A..... .....A ..-...TG.. .....G. .....TAC ....A....T .....C. ....
KH49	-..... .....A ..-A..TG.. .....A... .....TTAC T...A....T ....
KH50	-..... .....A ..-...TG.. .....G. ....C.TTAC .....T ....
KH51	-..... .....A ..-A..TG.. .....A... ....C.TTAC ....A....T .....C. ....
KH52	A..... .....A ..-...TG.. .....A.G. .....TAC T...A....T ....C...C. ....

C. Phylogenetic analysis

In order to confirm the phylogenetic relationship within common dolphin population in the East Sea, Korea, phylogenetic tree were reconstructed using neighbor-joining (NJ) method. The NJ unrooted tree showed divergence between *D. capensis* and *D. delphis* haplotypes. Most common dolphin haplotypes in Korean waters clustered with *D. delphis* inhabiting off the California. However, common dolphins in the Korean waters were observed in segregated clade, which may differ from *D. delphis* inhabiting Californian waters, although some haplotypes showed closely related with published *D. delphis* off the Californian waters.

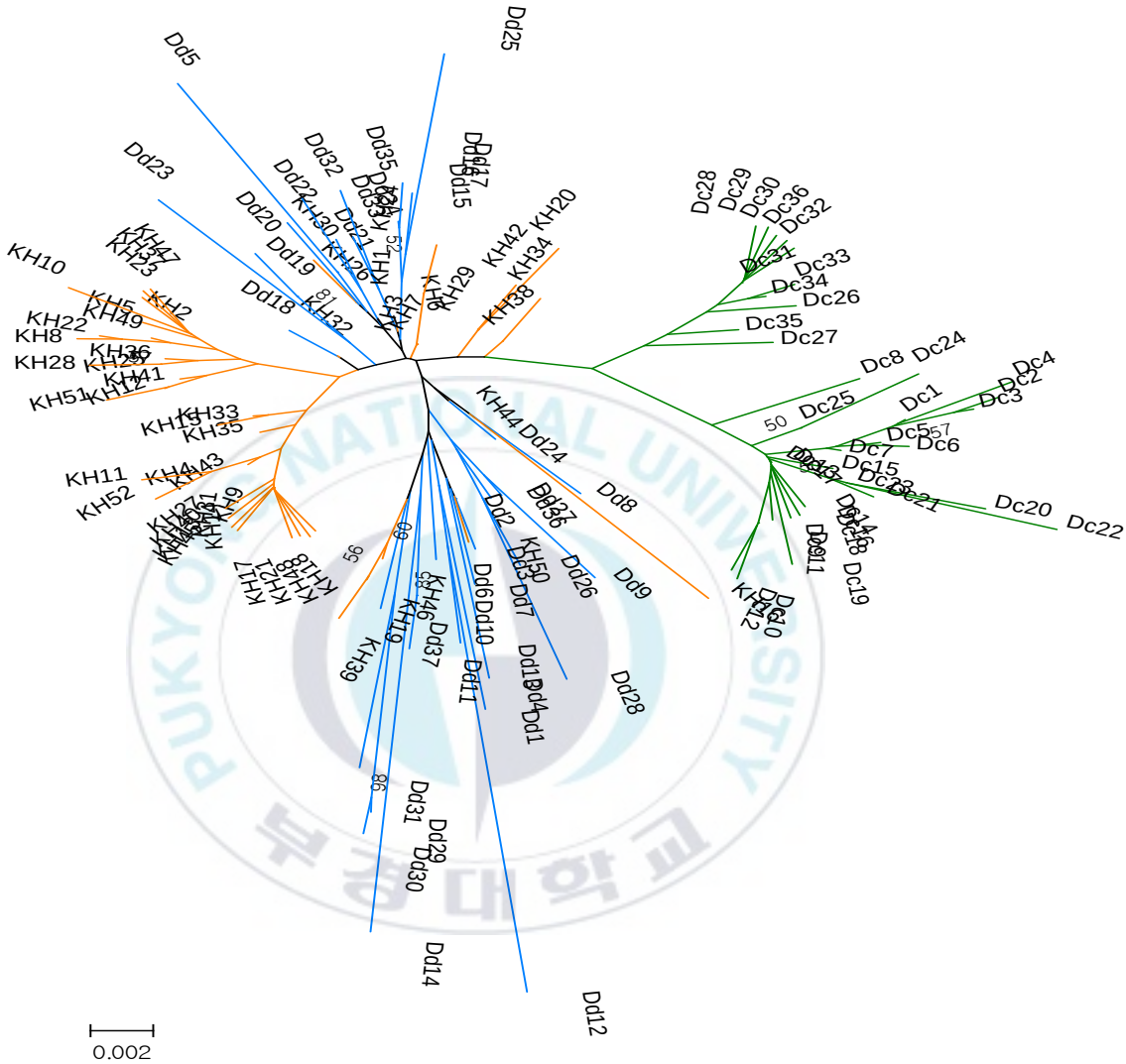

Figure 2. Unrooted neighbor-joining tree based on 469bp of the mtDNA control region including 125 haplotypes of short- and long-beaked common dolphins as determined by Neighbor-joining analysis. Colors represent the geographical morphotype: green – Californian long-beaked common dolphins, blue – Californian short-beaked common dolphins, orange – Korean common dolphins

2. Population genetic analyses of Korean common dolphin

A. Genetic diversity and differentiation

52 haplotypes of common dolphin in Korean waters were identified showing 36 polymorphic sites, at which 32 were transition, 1 was transversion, and 1 was both transition and transversion and 2 were insertion/deletion (Table 3). The results of the frequency for the common dolphin inhabiting the East Sea indicated that haplotype 14, which was occurred each year, was considered a major haplotype, with an average frequency of 18.2%. Next, the average values for haplotype 3, 2, and 4 were 14.3%, 7.6%, and 7.4%, respectively. These four haplotypes represented approximately 50% of the total frequency.

Overall, gene and nucleotide diversity (average over loci) for common in the Korean waters was 0.924 (SD±0.005) and 0.011 (SD±0.006), respectively. Haplotype diversity was highest in 2011 at 0.994, and lowest in 2013 at 0.896. Nucleotide diversity was highest in 2011 at 0.00944, and lowest in 2013 at 0.00885.

Pairwise F_{ST} values were estimated 0.00178 between 2011 and 2012, 0.00628 between 2011 and 2013, 0.00331 between 2012 and 2013, respectively (overall F_{ST} = 0.1217). The 2013 population was significant different from both 2011 and 2012 population (P<0.05).

Table 4. Number of mtDNA haplotypes of common dolphins in the East Sea from each year

	Year			Total
	2011	2012	2013	
1	2	4		6
2	12	24	14	50
3	23	29	42	94
4	17	17	15	49
5	4	3	1	8
6	4	7	2	13
7	5	5	4	14
8	4	9	7	20
9	3	5	1	9
10	3	4		7
11	3	4	1	8
12	11	12	16	39
13	4	8	1	13
14	19	52	49	120
15	5	3	4	12
16	5	5	5	15
17	3		6	9
18	1			1
19	2	5	2	9
20	4	11	10	25
21	4	12	5	21
22	4	7	8	19
23	2	1		3
24	1			1
25	6	8	2	16
26	1	2		3
27	2		1	3
28	2	1		3
29	1	3	2	6

Table 4. (continued)

30	1	1		2
31	1			1
32	1	1	1	3
33	1	2	2	5
34	1	1	1	3
35	1	3	4	8
36	1			1
37	1	2		3
38	1	3		4
39	1	2	4	7
40	1		1	2
41		1		1
42		1	1	2
43		4	4	8
44		1		1
45		1		1
46		1		1
47		1		1
48		1		1
49		2	2	4
50		1		1
51			1	1
52			1	1
Total	168	270	220	658

B. Demographic history

The selective neutrality and mismatch distribution analysis are useful to test for population expansion.

Tajima's D and Fu's *F*s values were estimated -0.37619 (P = 0.39500) and -17.14388 (P ≦ 0.00400), respectively. Although Tajima's D values were not significant, Fu's *F*s values were negative and significant values, suggesting possible population expansion. The mismatch distribution for common dolphin in Korean waters was weakly bimodal distribution but did not differ significantly from the simulated mismatch distribution based on the sudden expansion model [sum of squared differences (SSD) = 0.020, *P*=0.007] (Fig. 3).). using estimated value of τ=6.27, and the mutation rate $\mu = 7 \times 10^{-8}$, the expansion time were estimated at approximately 95,000 ybp (years before present).

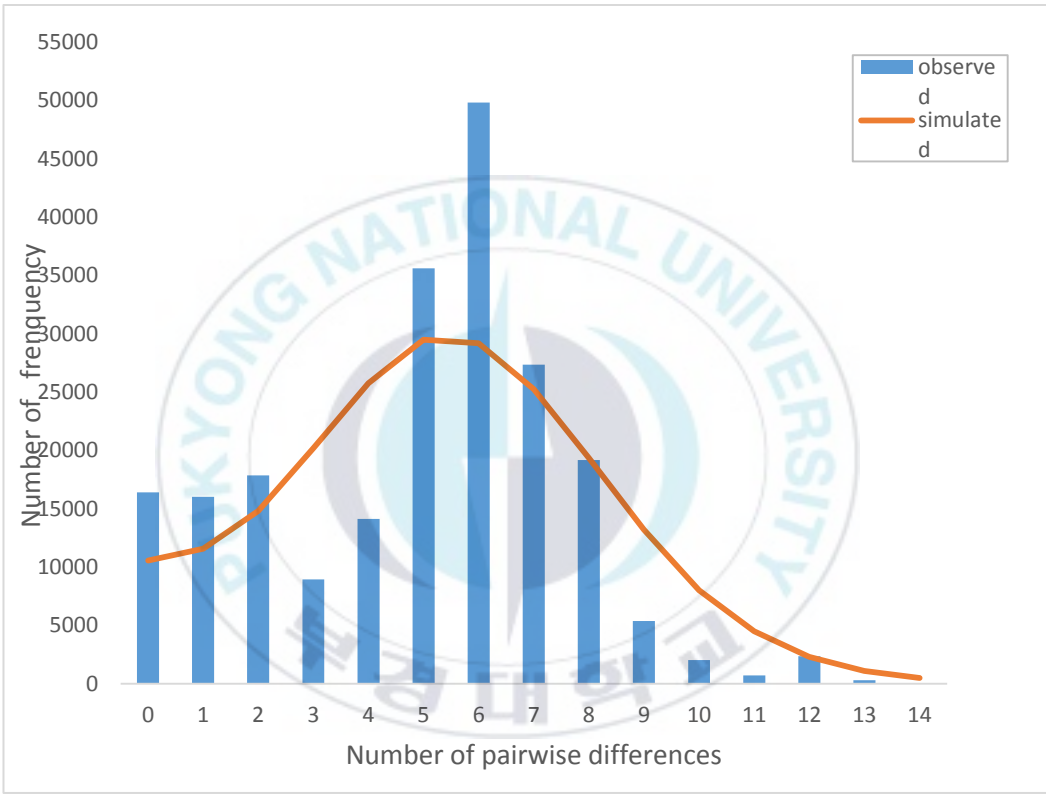

Figure 3. Mismatch distribution for common dolphin in Korean waters under a model of sudden expansion.

IV. Discussion

To compare with long- and short-beaked common dolphin from other areas, common dolphins in Korean waters were measured for CBL, RL, ZW, RL/ZW ratio, and TL. Distinction between long- and short-beaked common dolphins in osteological studies was particularly determined from the range of RL/ZW ration (Heyning et al., 1994; Murphy et al., 2006). Common dolphins used in this study appear more similar in osteological data to values published *D. capensis* than *D. delphis*, although *Delphinus* spp. are overlapped with some values published for both *D. delphins* and *D. capensis*. Furthermore, Common dolphins in Korean waters are large form on all osteological values han *D. capensis* in Californian waters (Heyning et al., 1994) (Table 2).

Numerous morphological studies on population of common dolphin have reported high levels of variability in total body length and skull size *D. delphis* according to areas; North Atlantic, southern Australia and New Zealand compared with *D. delphis* and *D. delphis* off the California (Bell 2001; Murphy et al., 2006; Amaral et al., 2007; Westagate 2007; Jorden 2012;). These author agued large forms when compared with *D. delphis* in waters off the Californian and the values of skull size varied from each region (Table 2). Moreover, minimum and maximum the values for total body length of mature *D. delphis* were recorded 164 – 201 cm and the values for *D. capensis* were 193 – 235 cm from Californian waters (Heyning et al., 1994). Jorden (2012) demonstrate TBL for common dolphin inhabiting the New Zealand are measured 187 – 241 cm. when compared with

Heyning and Perring' data (1994), these values were regarded as a large form of *D. delphis*. Similarly, Murphy et al (2006) found TBL for *D. delphis* off the NE Atlantic were also a larger form not only *D. delphis* but *D. capensis* off the California (Murphy et al., 2006). Previously, Amaha (1994) agued common dolphins off southern Australia and New Zealand have an intermediate form compared with two species from Californian waters.

Bell (2001) also claimed short-beaked common dolphin inhabiting deep water have a tendency to larger skull and longer rostra than common dolphin inhabiting shallow water in southern Australia. Furthermore, teeth and rostral differences between inshore and offshore waters seemed to be adaptive features for feeding owing to foraging type (Evans, 1982; Perrin, 1975). Common dolphin has highly mobile and they are considered to be moved along their prey items (Bilgmann et al., 2008; Burgess, 2006; Perrin et al., 2001; Pinela et al., 2011). The distribution of common dolphins has been correlated with the distribution of favorite prey species (Young and Cockcroft, 1994).

Taxonomy of genus *Delphinus* is still considered complex and unresolved due to incongruence between morphological and genetic data (Amaral et al., 2007; Natoli et al., 2006). Genetic divergence of *Delphinus* spp. was relatively low than closely related taxa within family Delphinidae (Amaral et al., 2012; LeDuc et al. 1999; Natoli et al., 2006). For instance, in sympatric two forms of genus *Tursiops* off the Chinese waters, *T. truncates* and *T. aduncus* was nucleotide divergence of 4.4% and clearly reciprocal monophyly. The congruence between morphological and genetic data for genus *Tursiops*

in Chinese waters is strong evidence that two species are distinct (Wang et al., 1994). *Stenella attenata* and *S. longirostris* was genetic diversity of 4% (Dizon et al., 1991).

The long- and short-beaked common dolphin for phylogenetic study of family Delphinidae based upon mtDNA cytochrome b gene are not reciprocal monophyly (Amaral et al. 2007, LeDuc et al. 1999). Kingston and Rosel (2004) revealed long- and short-beaked common dolphins are recently diverged using amplified fragment length polymorphism (AFLP). Furthermore, a phylogeographic study of the common dolphin identified by mitochondrial DNA and microsatellite loci suggested the long-beaked common dolphins has evolved independently in different regions and convergence on the same morphotype (Natoli et al. 2006).

While common dolphins based on osteological data are closely related to values of long-beaked common dolphin, status of common dolphin inhabiting Korean waters revealed as *D. delphis* using mtDNA sequence. NJ tree for the phylogenetic relationships showed common dolphin in Korean waters was more closely related to *D. delphis* than *D. capensis*. However, clade of the Korean haplotypes seems to be segregate from the Californian short-beaked common dolphin haplotypes. Similarly, in California, although morphological data has shown large size based on RL/ZW ratio (1.65 – 2.2) as well as the long-beaked common dolphin, the short-beaked common dolphin were identified by genetic data (Segura 2011).

Estimated genetic diversity for common dolphin in East Sea was high values in this region, based on mtDNA. Diversity of mtDNA haplotype and number of singletons

are indicative of a large effective population size in this study. Although new haplotype appeared in every year, this was caused by an increase in the number of total samples and did not represent a change in the haplotype distribution. The distributions of major haplotypes 2, 3, 4, and 14 seem to be all year round in Korean waters (Table 2). Pairwise F_{ST} values suggest that there is no significant differentiation among stock by each year. The mtDNA data suggested that there is no common dolphin subpopulation in Korean waters. These were consisted of a single stock.

However, nucleotide diversity has relatively low value than haplotype diversity in this study. Similarly, high values of haplotype diversity and low of nucleotide diversity were reported for many cetacean species (Abrahams 2014; Cassens et al. 2003; Harlin et al. 2003; Natoli et al. 2006; Pichler and Baker 2000). Low level of nucleotide diversity but high level of haplotype diversity is regarded as recent population expansion. Also, these patterns of genetic diversity, high haplotype and low nucleotide diversity are generally large population size that has been consistent with population expansion (Rogers and Harpending 1992). mtDNA data provide evidence to suggest that the Korean population has undergone expansion. The potential possibility for a population expansion was supported by the Tajima's D test, the Fu's test, and the mismatch distribution analysis. Negative values of Tajima's D ($\theta > \pi$) are indicative of either a selective sweep or a recent population bottleneck while positive values ($\theta < \pi$) is indicating either balancing selection or admixture of two genetically different populations (Pichler 2002; Rand 1996).

The mismatch distribution of the population in Korean waters was similar to the expected unimodal distributions for a population that has experienced past demographic expansion. Sudden demographic expansion usually represents unimodal distribution in the distributions of pairwise differences, whereas bimodal distribution is stable over long periods of time (Rogers and Harpending 1992; Excoffier 2004).

Incongruence between morphological and genetic study for the long- and short-beaked common dolphins was suggested as incomplete lineage sorting or hybridization (Amaral et al., 2007&2012; Segura 2011). Amaral et al (2007) elucidated a group of highly divergent individuals, 'Clade X', genetic divergence between Clade X and *D. capensis* was 1.76% and between Clade X and *D. delphis* were 1. 59%. Consequently, it suggested existence of hybridization between two species. As stated above, even though some osteological data for common dolphin are similar to values for *D. capensis*, genetic data are revealed as *D. delphis*. For the reason, some common dolphin off the Californian waters was regarded as hybridization (Segura 2011).

Recently, taxonomy of *Delphinus* identified by morphological characters such as beaked length, coloration, overall body length is not valid by genetic data. As mentioned previously, morphological variability on skull character such as beak length between and within *Delphinus* SP. might relate more to the local adaption than phylogenetic lineage (Amaral et al., 2007; Esteves and Oviedo, 2007; Natoli et al., 2006; Murphy et al., 2006; Pinela et al., 2011; Segura 2011).

In conclusion, the taxonomic status of common dolphins in Korean waters is

regarded as a larger form of *D. delphis*. However, skulls and genetic data are non-congruence. Future investigation should attempt to clarify in more detail population structure of common dolphins in East Sea using nuclear microsatellite loci.

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Appendix. 1. Mean ± SD, range and sample size for skull measures in Korean common dolphins.

Character	Male (n = 9)		Female (n = 6)		Unknown (n =1)
	Mean ± SD (mm)	Range (mm)	Mean ± SD (mm)	Range (mm)	Mean
CBL	491.7 ± 23.01	465 - 524.3	470.4 ± 11.77	451.5 - 487.8	471.5
RL	317.2 ± 19.83	290.2 - 343.5	303.6 ± 8.28	288.4 - 311.1	304.41
DREN	365.5 ± 19.82	338.5 - 394.5	349.8 ± 10.22	333.5 - 364.5	350.5
WRB	90.9 ± 3.14	87.1 - 97.3	87.4 ± 3.03	83.1 - 91.5	85.29
WR60	61.6 ± 4.14	57.9 - 68.4	59.7 ± 2.92	54.4 - 63	59.88
WRM	51.1 ± 3.3	47.6 - 57	48 ± 1.08	46.7 - 49.1	50.12
WPRM	24.5 ± 2.32	21.5 - 28.3	23.9 ± 1.11	22.5 - 25.2	22
WR¾	37.6 ± 3.68	33.5 - 46.2	34.6 ± 2.68	32.1 - 38.9	39.51
GPRW	171.9 ± 8.58	156.4 - 184.4	165.5 ± 5.39	160.6 - 175.5	164.7
GPOW	192.7 ± 7.82	180.6 - 204.1	186.7 ± 5.47	181.1 - 197.1	187.86
LSW	169 ± 6.48	157.4 - 179.5	163 ± 3.12	158.9 - 166.8	165.39
WEN	48.1 ± 1.99	44.4 - 50.8	46.4 ± 3.05	41.5 - 49.8	47.47
WPR	77 ± 3.33	72.1 - 82.1	74.5 ± 1.32	72.8 - 76.6	74.24
BCW	149.2 ± 8.15	142.4 - 168.8	145.9 ± 14.4	129.8 - 172	140.71
LTPS	136 ± 4.9	129.7 - 144	131 ± 3.1	125.9 - 133.8	131.91
GZW	190.5 ± 7.9	180.8 - 203.3	183.9 ± 6.21	180.1 - 196.3	186.56
BOW	90.4 ± 2.82	85.3 - 94	86.5 ± 4.57	81.4 - 93.9	86.98
GWIN	51.4 ± 4.93	46.9 - 60.4	48.2 ± 3.14	45.7 - 53.9	50.63
GLP	78.3 ± 5.91	70.9 - 89.6	72.2 ± 4.23	67.8 - 79.4	75.15
DRIN	347.2 ± 23.15	314 - 384.9	335.1 ± 8.69	327.5 - 351.4	332.5
LUTR	270.7 ± 19.36	236.4 - 293.1	261.5 ± 7.84	248.8 - 273.3	261.89
OL	50.8 ± 1.33	48.8 - 52.6	49.9 ± 2.56	47.2 - 53.7	49.02
AOL	45.1 ± 4.86	40.1 - 52.9	40.6 ± 2.81	37.5 - 43.9	44.23
LTF	75.2 ± 5.51	68.7 - 83.8	70 ± 4.26	62 - 74	69.95
WTF	59 ± 4.41	52.2 - 67.1	56.5 ± 4.04	49.7 - 60.8	55.27
ML	425.2 ± 21.69	397.5 - 458	412.6 ± 6.14	405.5 - 424	401.5
LMF	133 ± 36.67	115.9 - 229.9	119.2 ± 7.83	112.1 - 133.3	108.15
LMTR-T	264.2 ± 16.51	237 - 287	258 ± 4.73	250.2 - 262.8	254.73

Appendix. 2. Number of mtDNA haplotypes of common dolphins in the East Sea from each month

Haplotypes	Month												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
KH1	2	1	1				1			1			6
KH2	4	7	8	7	4	1	3	3	4	5	1	3	50
KH3	5	5	14	28	10	1	5	2	5	12		7	94
KH4	4	3	5	10	6		2	4	2	5	2	6	49
KH5	2			2					1		1	2	8
KH6	3		1	3		1	1	1	1		1	1	13
KH7		3		5			1		2	3			14
KH8		3	2	4	5		2	1	1	1		1	20
KH9		4	1	3					1				9
KH10		2	1	1	1		2						7
KH11		1	3						1	1		2	8
KH12	3	3	6	4	3		4	4	4	2	1	5	39
KH13		1	3		1		3	1	1	1	1	1	13
KH14	6	10	14	25	18	4	4	13	7	7	6	6	120
KH15	1		2	5	1		1		1		1		12
KH16	1		1	7	1		2	1	1	1			15
KH17	1		2	2	3							1	9
KH18			1										1
KH19		1	3	1				1		3			9
KH20			3	7	3	2	2		2	3	1	2	25
KH21	3	1	6	3	1	1				1	3	2	21
KH22	2		1	7	2			3	2		1	1	19
KH23				3									3
KH24				1									1
KH25			4	4	4				1	2	1		16
KH26				2				1					3
KH27				1	1			1					3
KH28	1			2									3

Appendix. 2. (continued)

KH29		2		1	1		1			1			6
KH30			1		1								2
KH31					1								1
KH32				1	1					1			3
KH33			1	2			1	1					5
KH34	1			1				1					3
KH35	2				3		1		1			1	8
KH36										1			1
KH37		1						1		1			3
KH38	1	1	1									1	4
KH39		2	2		1			1				1	7
KH40				1								1	2
KH41		1											1
KH42		1		1									2
KH43		1	3	3								1	8
KH44			1										1
KH45			1										1
KH46			1										1
KH47				1									1
KH48				1									1
KH49			2	1			1						4
KH50							1						1
KH51			1										1
KH52				1									1
Total	42	54	96	151	72	10	38	40	38	52	20	45	658