RAFFLES BULLETIN OF ZOOLOGY **62**: 188–199
Date of publication: 2 May 2014
http://zoobank.org/urn:lsid:zoobank.org:pub:65BDC7AF-4087-44CF-B3A7-454C76B64737

The "Southern form" of short-finned pilot whale (*Globicephala macrorhynchus*) in tropical west Pacific Ocean off Taiwan

Ing Chen[1,2], Hsin-Yi Yu[1], Wei-Cheng Yang[3], Shin Nishida[4], Tomohiko Isobe[5], Shinsuke Tanabe[5], Alastair Watson[6] and Lien-Siang Chou[1*]

Abstract. The short-finned pilot whale (*Globicephala macrorhynchus*) is a mid-sized toothed whale, widely distributed throughout warm-temperate to tropical regions of the Atlantic, Pacific, and Indian oceans. There is suggestive evidence that the taxonomy within the species is unresolved: a "Southern form" in southern Japanese waters is considered distinctive in its morphological, ecological, and genetic characteristics to a "Northern form" found in northern Japanese waters, and each might represent different subspecies or species. Short-finned pilot whales in the tropical west Pacific are poorly studied and their taxonomic status remains uncertain. The aim of this study was to determine the phenotype(s) and genotype(s) of pilot whales sighted or sampled from Taiwanese waters and compare them with whales found in adjacent Northeast and Southeast Asian waters. We examined 892 photographs taken from 14 groups of whales at sea around Taiwan, as well as the body length and photographic records of 19 whales stranded on the Taiwanese coast including genetic data (derived from a 689-bp mitochondrial DNA control region fragment) from eight of these. We also examined a group of short-finned pilot whales in a video clip filmed in the South China Sea, as well as a 593-bp mitochondrial DNA control region fragment sampled from two whales in Philippine waters. Our results revealed morphological and genetic similarities to available data from the more northerly located "Southern form" short-finned pilot whales from Japan, and indicate that the Southern form whales are indeed distributed more southward into the tropical west Pacific: viz, in the waters around Taiwan, in the South China Sea, and in the Philippines. The extent of the southern boundary for the range of the Southern form of short-finned pilot whales in the tropical west Pacific Ocean could be better defined with further observational and genetic studies.

Key words. Short-finned pilot whale, *Globicephala macrorhynchus*, Northwest Pacific, Taiwan, southern form, mitochondrial DNA genotypes

INTRODUCTION

The short-finned pilot whale (*Globicephala macrorhynchus*) is a mid-sized odontocete ranging widely throughout warm-temperate to tropical pelagic waters of the Atlantic, Pacific, and Indian oceans (Rice, 1998). In cetacean phylogeny, it is closely related to its congener, the long-finned pilot whale (*G. melas*) (See LeDuc et al., 1999; Oremus et al., 2009; Vilstrup et al., 2011), which has an anti-tropical distribution in the north Atlantic Ocean and cold-temperate Southern Hemisphere waters (Davies, 1963). Studies on short-finned pilot whales from Japanese waters have shown that there are two "forms" – a "Northern form" and a "Southern form", based on differences in their morphology (Yonekura et al., 1980; Miyazaki & Amano, 1994), oceanographic distribution and habitat preferences (Kasuya et al., 1988), and their genetics (Wada, 1988; Kage, 1999). The Northern and Southern forms are geographically segregated by the Kuroshio Current front off the east coast of Japan (Kasuya et al., 1988; Kage, 1999; Kasuya, 2011). In Japanese waters, the Northern form is distributed north in the Oyashio Current between latitudes 35–43°N (surface water temperature 12–24°C), whereas the Southern form is distributed south of the front and occur between latitudes 25–39°N (surface water temperature >18°C) (Kasuya et al., 1988; Miyashita, 1993)—the southernmost extent is around the Ryukyu Islands, which lie about 600 km north from the northern tip of Taiwan. Taylor et al. (2008) reported that the taxonomic status of these two "forms" is unresolved, and that they may represent different subspecies or species. A recent assessment of the worldwide phylogeny of genus *Globicephala* concluded that the Southern form of short-finned pilot whales is likely a distinct subspecies, or at least a distinct evolutionarily significant unit within the pilot whale species (Oremus et al., 2009).

[1]Institute of Ecology and Evolutionary Biology, National Taiwan University, No.1, Sec.4, Roosevelt Road, Taipei 10617, Taiwan; Email: chouls@ntu.edu.tw (*Corresponding author LSC)

[2]School of Biological and Biomedical Sciences, University of Durham, South Road, Durham DH1 3LE, UK; Email: ingechen@ntu.edu.tw (IC)

[3]Department of Veterinary Medicine, National Chiayi University, No.580, Xinmin Road, Chiayi City 60054, Taiwan

[4]Science Education, Faculty of Education and Culture, University of Miyazaki, 1-1 Gakuen-Kibanadai-Nishi, Miyazaki, Miyazaki 889-2192, Japan

[5]Center for Marine Environmental Studies, Ehime University, 2-5 Bunkyo-Cho, Matsuyama, Ehime 790-857, Japan

[6]Department of Physiological Sciences, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, Oklahoma 74078, USA

ISSN 2345-7600 (electronic) | ISSN 0217-2445 (print)

Short-finned pilot whales occur in the more tropical waters of several Southeast Asian countries south of Japan, including Taiwan (Chen, 2001; Yeh, 2001), the Philippines (Dolar et al., 1997), and Indonesia (Rudolph et al., 1997; Kreb & Budiono, 2005). However, this species is little studied in this tropical west Pacific region. An earlier worldwide genetic analysis of pilot whale phylogeny (Oremus et al., 2009) lacked samples from the tropical west Pacific, and thus left the pilot whales from these waters with an uncertain phylogenetic status. Pilot whales occasionally strand in this region (Rudolph et al., 1997; Aragones et al., 2010), and at least one case was suspected of being associated with military activities that took place in Taiwanese waters in 2005 (Yang et al., 2008). Incidental catches from fisheries as well as legal or illegal local small-scale whaling may also have affected the population(s) of pilot whales in this region (Perrin et al., 2005; Robards & Reeves, 2011). The identification of specific forms and regional populations of pilot whales in the tropical west Pacific becomes a key component to establish appropriate local conservation management plans.

The objective of this study was to determine phenotype(s) and genotype(s) of short-fined pilot whales from Taiwanese and adjacent southward waters. This was achieved by analysing data from photographs, strandings, and genetics and by comparing this with known forms of pilot whales elsewhere. We hypothesized that the short-finned pilot whales in Taiwanese waters are of the Southern form, based on the oceanographic similarities between Taiwan and southern Japan, the consistent productive influence of the north-flowing Kuroshio Current connecting these two broad regions (Wyrtki, 1975). It is possible though, that the short-finned pilot whales in the tropical west Pacific are a separate form of pilot whale, comparable to the different forms of spinner dolphins (*Stenella longirostris*) in shallow waters of inner Southeast Asia versus those from deeper waters of the northwest Pacific (Perrin et al., 1999), or as in Fraser's dolphins (*Lagenodelphis hosei*) between the Philippine and Japanese waters (Perrin et al., 2003).

MATERIAL AND METHODS

In this study, the Southern form of short-finned pilot whale was diagnosed by external appearance and/or genetic characteristics as previously defined in comparative studies of the Northern and Southern forms of short-finned pilot whales in Japanese waters and genetic variation in worldwide pilot whale specimens (Kasuya et al., 1988; Kasuya & Tai 1993; Oremus et al., 2009). The defining external characters for the Southern form are: 1) a relatively shorter adult body length (4–5 m for males, 3–4 m for females; compared to 5–6 m and 4–5 m for Northern form males and females respectively); 2) poorly defined or no saddle patch over the dorsum of the body just caudal to the fin; and 3) a square-shaped profile to the forehead in dorsoventral view in mature males. Reference photographs for identifying Southern and Northern forms can be found in Kasuya et al. (1988: Fig. 1), Kasuya & Tai (1993: Fig. 1), and Kasuya (2011: Fig. 12.3). The genetic characteristics, as in the mitochondrial DNA (mtDNA) haplotypes for the Southern form short-finned pilot whales, were compared with those from Oremus et al. (2009).

Photographic data. The photographs examined in this study were taken by trained operators opportunistically during onboard research surveys or whale watching tours off the east coast of Taiwan (1998–2010, Table 1), primarily during April–November within the region enclosed by 23–25°N, 121.5–122.5°E (Fig. 1). Relevant sighting information recorded includes date, location, species, and estimated group size. The significant sexual dimorphism in pilot whales allowed for sex identification of some photographed individuals without much difficulty: those of a relatively larger body size, and with a larger, bulbous head and larger fin were identified as adult males (Jefferson et al., 2008), whereas those being closely accompanied by another whale less than half of its body size (likely calf) were identified as adult females. All photographs taken at sea or of strandings (2002–2012; Table 2) that showed a clear view of the dorsum of the body (to evaluate presence of saddle patch) or of a male forehead were selected for examination.

Necropsy measurement and sex data. There were 27 short-finned pilot whales stranded on Taiwanese coasts in 19 events (2002–2012; Table 2; Fig. 1). This included one mass stranding of nine whales on the east coast (S6 in Table 2; Fig.1). Photographs were available for 14 whales and external morphological measurements and/or necropsy records were available for 19 whales. Straight-line total body length was measured from the most rostral end of the upper jaw to the notch between the flukes (Norris, 1961), and sex

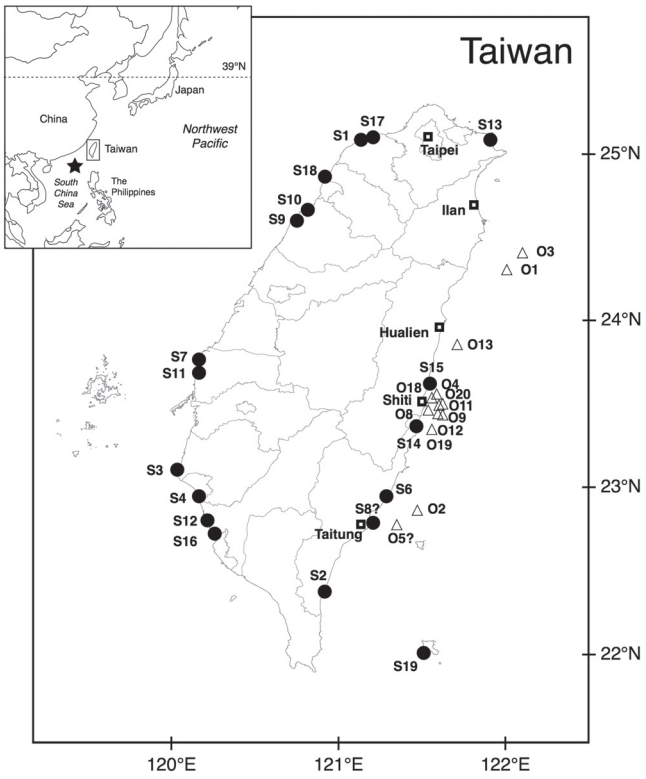

Fig. 1. Sighting observation (open triangles) and stranding (solid circles) locations of short-finned pilot whales around the coasts of Taiwan (1998–2012). See Tables 1 and 2 for event codes; sighting or stranding events without a detail location record (i.e., GPS position) were not indicated in the map. The star locates a group sighting near the Dongsha plateau recorded on video-clip (see text; Supplement A).

Table 1. Locations, dates and numbers of photographs of short-finned pilot whales sighted off the east coast of Taiwan from independent research surveys or whale watching tours (between April and October, 1998–2010). Group size was estimated by experienced onboard observers and number of identifiable whales for each group was confirmed by HYY via photo-identification technique. Short dash (–) indicates no data available.

Event	Date	Reference Terrestrial Landmark	Estimated Group Size	n of Photos Taken	n of Photos of the Body Part of Interest		n of Identifiable Whales
					Caudal Dorsum	Head of Adult Males	
O1	30 April 1998	Ilan	45	15	4	1	4
O2	11 June 1999	Taitung	2	–	–	–	–
O3	6 September 1999	Ilan	25	26	7	3	8
O4	1 September 2002	Shiti	200	15	3	1	3
O5	19 April 2003	Taitung	100	3	1	–	3
O6	22 September 2003	Hualien	–	6	5	–	4
O7	13 July 2004	Shiti	40	–	–	–	–
O8	18 September 2004	Shiti	30	86	53	6	–
O9	24 August 2006	Shiti	80	7	1	–	–
O10	3 September 2006	Hualien	100	43	8	2	7
O11	3 September 2006	Shiti	100	4	–	–	2
O12	4 September 2006	Shiti	–	4	4	–	4
O13	30 September 2006	Hualien	50	–	–	–	–
O14	28 July 2007	Hualien	–	14	7	–	4
O15	1 August 2007	Hualien	–	8	3	–	6
O16	9 September 2008	Hualien	–	27	6	–	8
O17	10 October 2008	Hualien	60	–	–	–	–
O18	11 October 2008	Shiti	4	–	–	–	–
O19	16 July 2010	Shiti	90	631	254	28	–
O20	17 July 2010	Shiti	40	–	–	–	–

was determined by observation of the genital and mammary slits. Sexual maturity was determined in nine whales by examination of their reproductive organs at necropsy by the attending veterinarian. Females were classified as adult if either milk was found in a mammary gland, a corpus luteum or corpus albicans was present in an ovary, or was pregnant (Perrin & Reilly, 1984); otherwise a sub-adult. Males were classified as adult if either a testis weighed more than 400 g or having a body length over 400 cm (Kasuya & Marsh, 1984).

Genetic analysis. Two different genetic protocols were applied in this study because our Taiwanese and Philippine samples were processed in separate laboratories. For Taiwanese samples, muscle and skin samples collected from eight stranded whales were processed for genetic analysis (Table 2). DNA was extracted and purified by AxyPrep™ Multisource Genomic DNA Miniprep Kit (Axygen Biosciences) and a 689 base pair (bp) fragment from the mtDNA control region was targeted and amplified by consensus primers M13-Dlp1.5-L (Ross et al., 2003) and Dlp-8G (Oremus et al., 2009) with established polymerase chain reaction (PCR) procedures. The PCR was performed in a Bioer thermocycler (model GenePro) in a volume of 25 µL. In each PCR reaction, 5–50 ng of genomic DNA was added in TAQXpedite™ PCR system (Epicentre). The cycling profile consisted of an initial denaturation at 98°C for 30 s, followed by 35 cycles of 10 s at 95°C, 10 s at 55°C, and 30 s at 68°C. Amplified mtDNA fragments were purified and subjected to cycle-sequencing reaction (ABI PRISM™ Cycle Sequencing Kit, Applied Biosystems), then sequenced in both directions by an ABI 3730 automated DNA sequencer.

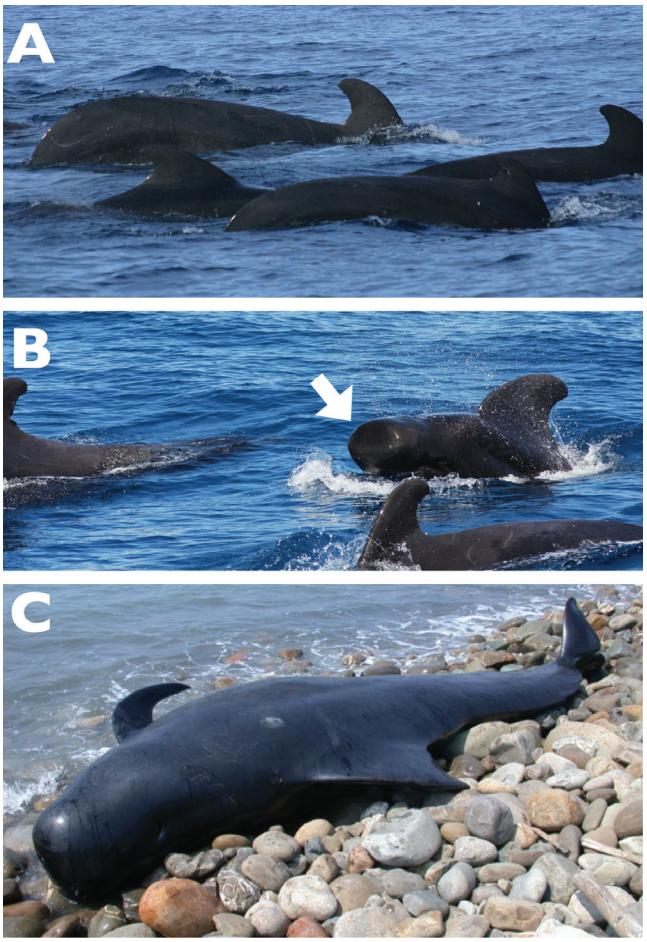

Fig. 2. Short-finned pilot whales sighted around Taiwan—offshore and beached on the coasts of Taiwan—showing two external morphological characteristics that define the Southern form (Kasuya et al., 1988). A, the post-fin dorsum was uniformly black in all whales observed, four shown here from group of about 30 whales (O8, Table 1), a lighter coloured saddle patch was not seen; B, a male with flattened forehead ("square-head", arrow) from another group of about 90 whales (O19, Table 1), and indistinct lighter coloured mottling over post-fin dorsum (the saddle patch area) on two other whales; C, carcass of a fresh adult male stranded on the west coast of Taiwan (S7) showing a flattened forehead and the lack of a clear post-fin saddle patch, characteristic of the Southern form.

Skin samples from two male short-finned pilot whales from Philippine waters in 1996 (TBL 227 and 205 cm, tissue samples archived in es-BANK of Ehime University; Tanabe, 2006) were processed and examined in a separate laboratory. Their DNA was extracted with a QIAamp DNA Micro Kit (QIAGEN Inc.) and a 593-bp mtDNA control region fragment was amplified by applying primers tRpro-F.ceta (Hoelzel et al., 1998) and RCR(mod).ceta (modified from Hoelzel et al., 1998) in a 25 µL scale PCR reaction solution: 1 × PCR buffer, 0.2 mM of each dNTP, 0.2 mg ml^{-1} BSA, 0.2 mM of each primer, and 0.625 units TaKaRa Ex Taq Hot Start Version DNA polymerase (TaKaRa Bio Inc.) and 1 µL of DNA. The PCR amplification steps were: denaturation at 94°C for 60s, followed by 30 cycles of 10 s at 98°C, 45 s at 60°C, and 45 s at 72°C, and post-extension at 72°C for 60 s. The amplified mtDNA fragments were then sequenced on a CEQ2000XL DNA Sequencer (Beckman Coulter Inc.).

These new sequences, together with published worldwide pilot whale mtDNA sequences (downloaded from GenBank, see accession numbers in Table 3), were aligned manually in MEGA 5.05 (Tamura et al., 2011). The program DnaSP v5 (Librado & Rozas, 2009) was used to identify unique haplotype(s) in this mtDNA sequence data and calculate the haplotype and nucleotide diversity for Taiwanese samples. Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010) was used to assess the population differentiation among five putatively distinct geographical regions (Taiwan herein; Southern Japan, Northern Japan, South Pacific, and North Atlantic as defined in Oremus et al., 2009). The F_{ST} (estimate for the difference at allele frequency) and Φ_{ST} (estimate for the difference at genetic distance) were calculated via the Analysis of Molecular Variance (AMOVA) and pairwise comparison implemented in the program. The median-joining algorithm in the program Network 4.6.1.0 (Fluxus Technology Ltd.) was used to reconstruct the genealogical relationship of our Taiwanese and Philippine samples in the context of global short-finned pilot whale phylogeny.

RESULTS

We examined 892 photographs taken from 14 groups of pilot whales encountered off the east coast of Taiwan in 1998–2010 (Fig. 1, Table 1); 356 of them revealed a clear

Table 2. Stranding records of short-finned pilot whales along the coasts of Taiwan (2002–2012). Haplotype code referenced in Table 3.

Event	Date	Location	n	Key Biography of the Stranding Whales	Photo Record	Photos of the Body Part of Interest	DNA Sample (Haplotype)
S1	16 June 2002	Taoyuan	1	Unknown sex, 400–500 cm, live-stranded (released)			
S2	30 November 2002	Taitung	1	Unknown sex, 230 cm			
S3	1 February 2003	Tainan	1	Female, *ca.*350 cm			
S4	22 April 2003	Tainan	1	Female, 335 cm, live-stranded	Yes	Yes	
S5	7 February 2004	Tainan	2	Adult female, *ca.*400 cm, live-stranded Unknown sex, unknown body length (released)	Yes	Yes	
S6	24 February 2004	Taitung	9	Male, unknown body length Female, 415 cm Adult male, 490 cm Male, 389 cm Adult female, 322 cm Female, unknown body length Unknown sex, unknown body length (released) Unknown sex, unknown body length (released) Unknown sex, unknown body length (released)	Yes[1]	Yes[1]	Yes[2] (TWGM05)
S7	10 March 2004	Yunglin	1	Adult male, 500 cm, fresh carcass	Yes	Yes	Yes (TWGM01)
S8	28 July 2005	Taitung	1	Adult female (pregnant), 323 cm, fresh carcass	Yes	Yes	
S9	27 August 2006	Miaoli	1	Unknown sex, unknown body length (released)	Yes		
S10	9 September 2006	Miaoli	1	Unknown sex, unknown body length (the same individual in Event S9, released)			
S11	29 March 2007	Yunglin	1	Adult female (pregnant), 355 cm			Yes (TWGM02)
S12	12 June 2007	Kaoshiung	1	Unknown sex, *ca.* 300 cm			
S13	4 January 2008	Taipei	1	Unknown sex, 430–450 cm, highly decayed carcass	Yes		Yes (TWGM03)
S14	14 April 2008	Taitung	1	Female, 246 cm, fresh carcass	Yes	Yes	Yes (TWGM01)
S15	13 October 2008	Hualien	1	Sub-adult male, 355 cm, suspected by-caught, decayed carcass	Yes		Yes (TWGM01)
S16	7 September 2009	Kaoshiung	1	Adult male, 495 cm, highly decayed carcass	Yes		
S17	17 November 2010	Taoyuan	1	Adult male, 511 cm	Yes	Yes	Yes (TWGM04)
S18	17 August 2011	Hsinchu	1	Unknown sex, 370 cm, highly decayed carcass	Yes		Yes (TWGM01)
S19	15 June 2012	Orchid Island	1	Unknown sex, unknown body length, highly decayed carcass			

[1]Three of the nine whales were photographed in one photograph. The identity of each whale was undetermined.
[2]Tissue sample from one of the four whales being necropsied. Identity undetermined.

view of the dorsum of the body, and 41 of them the contour of the forehead (Table 1). At least 53 different whales were identified from a preliminary photo-identification examination (Yu, unpublished data). In none of these whales was there a distinct, lightly coloured post-fin saddle patch, instead the dorsal area caudal to the fin was uniformly dark gray to black (Fig. 2A); in a few photographs (n = 20) an indistinct mottled grayish area over the caudodorsal part of the body was observed (Fig. 2B). A 'square-shaped head' characteristic of the Southern form presumptive adult males was seen in 41 photographs from six groups of whales (Fig. 2B). Of the 14 photographed stranded whales only eight, which either stranded live or were fresh carcasses, provided photographs suitable for analysis (Table 2). As found in the whales at sea, in all eight the dorsal back was uniformly dark without any sign of distinct post-fin saddle patch. Photographs of two adult males showed a large bulbous head with a squarish contour (S7 and S17). In addition, we also examined a video-recording of a group of at least six short-finned pilot whales of undetermined sex traveling in the South China Sea (near the northeast Dongsha plateau, 21°N, 116.5–118°E, approximately 400 km southwest of southern tip of Taiwan; Supplement A, Lien, personal communication). Although the video was of low quality it was possible to discern that three of the whales that closely approached the observers' boat each had a uniformly dark dorsum without saddle patch.

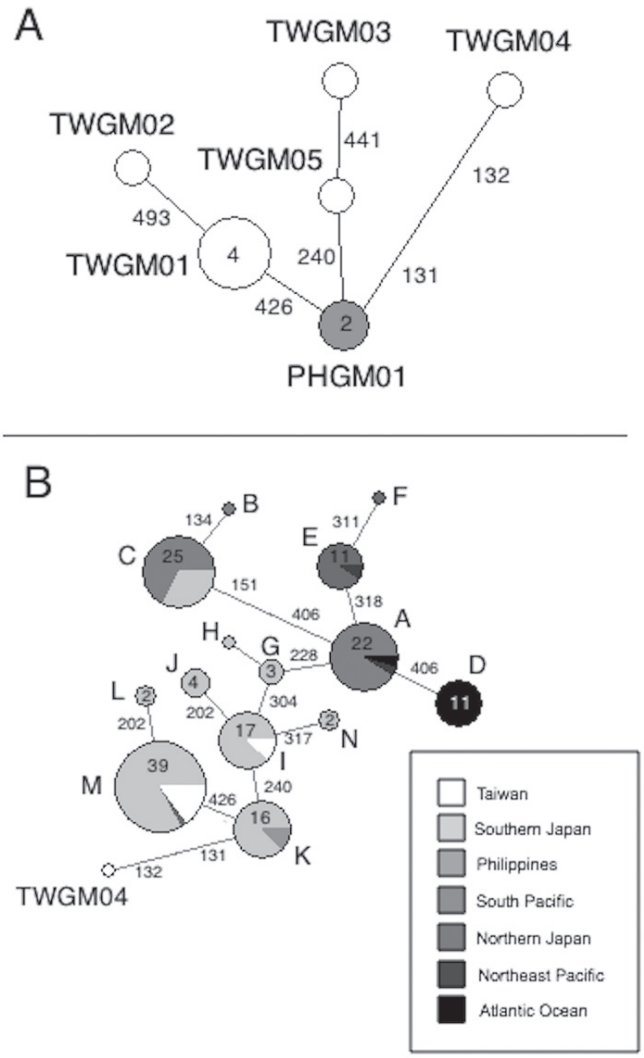

Fig. 3. Genealogical relationship reconstructed by the median-joining algorithms via the mtDNA haplotype data for short-finned pilot whales from: A, Taiwanese and Philippines waters (593-bp sequence); B, Worldwide (345-bp sequence). Each circle represents a haplotype and the number in the circle indicates the sample size (when sample size > 1). The number at the bar between each haplotype indicates the position of variable site (Table 3). Haplotypes, sample size, and origin for worldwide data in B from Oremus et al., 2009. Haplotype N is the haplotype most closely-related to those long-finned pilot whales (six mutation steps; see Oremus et al., 2009).

The total body length range for short-finned pilot whales stranded on the Taiwanese coasts was 230–511 cm (n = 19). Sexual maturity and body length data were available for six males and eight females; body lengths of adult males were 495–511 cm (n = 3) and of adult females were 322–415 cm (n = 4), and a sub-adult male had a body length at 355 cm (Table 2). The statistics suggested the body length at the onset of sexual maturity in female short-finned pilot whales in Taiwanese waters can be less than 322 cm; and in males it might be longer than 355 cm.

From the genetic analysis we found six putative haplotypes defined by six variable sites (including a 2-bp deletion site) from the 593-bp consensus mtDNA control region sequences in our samples from 10 whales salvaged from Taiwanese and Philippine waters (Table 3). Despite the small sample size (n = 10), the results suggested a rich genetic diversity (haplotype diversity, h = 0.80 ± 0.10; nucleotide diversity, p = 0.22 ± 0.05%), similar in magnitude to that seen in the short-finned pilot whales from southern Japanese waters (h = 0.86 ± 0.04 and p = 0.76 ± 0.47%; sampled 82 whales from 31 sampling events, Oremus et al., 2009). The median-joining network reconstruction showed there was always one substitution difference between each close-related haplotype, if the 2-bp deletion in TWGM04 was regarded as a result of a single mutation event (Fig. 3A). The haplotype TWGM01 was the most common haplotype among Taiwanese samples; four out of eight samples were assigned to this haplotype (Table 2). The two Philippine samples shared the same haplotype (PHGM01).

To reconstruct the phylogenetic topography among all known mtDNA haplotypes in short-finned pilot whales worldwide, a consensus 345-bp sequence data was extracted from this study and GenBank for further analysis. Due to the discordances of sequenced mtDNA fragments between our sequences and those published, the sequence truncation eventually caused all our haplotypes, except TWGM04, to lose their originality. We were unable to determine whether our haplotype PHGM01 was synonymic to the published haplotype K (GenBank accession number FJ513338), and whether haplotype M (FJ513340) was identical either to TWGM01, TWGM02, or neither of them, as well as the uncertainty among haplotypes I (FJ513340), TWGM03, and TWGM05 (Table 3). Truncation also reduced the number of potential haplotypes in short-finned pilot whales from south Pacific (Oremus et al., 2009): three variable substitution sites beyond the 345-bp consensus sequence were neglected, and it is unknown whether those three would correspond to the variable sites found in this

Table 3. Polymorphic sites within the 689-bp fragment of mitochondrial DNA control region in tissue samples from eight short-finned pilot whales salvaged from Taiwanese waters (TWGM01–05) and two whales from Philippines waters (PHGM01; 593-bp, see Materials and Methods), with comparison to published worldwide short-finned pilot whale haplotypes (A–N: 345-bp, Oremus et al., 2009). Haplotypes TWGM01, TWGM02, TWGM03 and TWGM05 resembled the published Haplotype M and I most, while PHGM01 was similar to Haplotype K. Dot (.) indicated the site was identical to the reference sequence in the top line (Haplotype A). Asterisk (*) indicates undermined substitution sites. Dash (–) indicated missing site (deletion).

Haplotypes	Variable Sites																	GenBank Accession Number
	129	131	132	134	151	202	228	240	304	311	317	318	406	426	427	441	493	
A	T	T	A	A	C	C	G	C	T	A	C	C	T	A	C	*	*	FJ513328
B	.	.	.	G	T	.	.	.	.	.	.	.	C	.	.	*	*	FJ513329
C	.	.	.	.	T	.	.	.	.	.	.	.	C	.	.	*	*	FJ513330
D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	*	*	FJ513331
E	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	*	*	FJ513332
F	.	.	.	.	.	.	.	.	.	G	.	T	.	.	.	*	*	FJ513333
G	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	*	*	FJ513334
H	C	.	.	.	.	.	A	.	.	.	.	.	.	.	.	*	*	FJ513335
I	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	*	*	FJ513336
TWGM03	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	G	T	JN390970
TWGM05	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	C	.	KF110987
J	.	.	.	.	.	T	A	.	C	.	.	.	.	.	.	*	*	FJ513337
K	.	.	.	.	.	.	A	T	C	.	.	.	.	.	.	*	*	FJ513338
PHGM01	.	.	.	.	.	.	A	T	C	.	.	.	.	.	.	C	.	JN390972
TWGM04	.	–	–	.	.	.	A	T	C	.	.	.	.	.	.	C	.	JN390971
L	.	.	.	.	.	T	A	T	C	.	.	.	.	T	.	*	*	FJ513339
M	.	.	.	.	.	.	A	T	C	.	.	.	.	T	.	*	*	FJ513340
TWGM01	.	.	.	.	.	.	A	T	C	.	.	.	.	T	.	C	.	JN390968
TWGM02	.	.	.	.	.	.	A	T	C	.	.	.	.	T	.	C	C	JN390969
N	.	.	.	.	.	.	A	.	C	.	T	.	.	.	.	*	*	FJ513341

Table 4. Tests values for significance of population genetic differentiation between different regions (pairwise comparison). The F_{ST} values listed above the diagonal, Φ_{ST} listed below the diagonal. All comparisons were statistically significant except the comparison between Taiwan and Southern Japan (** = P<0.01). Number in parentheses after the location indicates the sample size.

	Taiwan (8)	**Southern Japan (82)**	**Northern Japan (12)**	**South Pacific (38)**	**North Atlantic (12)**
Taiwan		0.00622	0.53310**	0.44094**	0.64823**
Southern Japan	0.00861		0.36179**	0.30194**	0.42208**
Northern Japan	0.72889**	0.56610**		0.53806**	0.75758**
South Pacific	0.74623**	0.57963**	0.54454**		0.56807**
Atlantic Ocean	0.84833**	0.61894**	0.74074**	0.58858**	

study. Even so the genealogical relationship inferred from the median-joining algorithm showed that all our eight Taiwanese and two Philippine samples were included in the major clade for the Southern form of short-finned pilot whales (Fig. 3B); although the genetic distance between each short-finned pilot whale haplotype was short. The haplotype M was the most common haplotype found in both Taiwanese and southern Japanese waters. The AMOVA demonstrated a significant population differentiation among pilot whales from different geographical regions ($F_{ST} = 0.402$, $p < 0.001$; $\Phi_{ST} = 0.374$, $p < 0.001$), confirming earlier results (Oremus et al., 2009), however the pairwise comparison between the samples from Taiwanese and southern Japanese waters failed to show significant differentiation (Table 4).

DISCUSSION

Our photographic data suggested that the colour pattern of the dorsal saddle area of short-finned pilot whales found in Taiwanese waters was similar to that of the Southern form of pilot whales from Japan. It could be a concern that the numbers of whales examined in this study were likely fewer than the numbers of whales encountered at sea because the photographs were collected opportunistically in the field. Even though such observation bias could be negligible given the photographs examined in this study were taken from different groups of whales over a 12-year sampling period (1998–2010), further examinations of additional photographs of whales from the same or adjacent regions is recommended for confirmation.

The measurements for the body length of adult short-finned pilot whales stranded in Taiwan closely matched the adult body lengths for adults of the Southern form (422–525 cm and 316–405 cm for male and female, respectively) rather than the longer Northern form (560–720 cm, 390–510 cm) (Kasuya & Tai, 1993). Adult short-finned pilot whales from other adjacent oceanic regions are likewise reported as being longer: female body length from the northeast Pacific was 396–480 cm, and male body length could be up to 590 cm, and from the Indian Ocean the body length for males was 508–610 cm, and for females was 405–550 cm (Perrin & Reilly, 1984). Furthermore, our data showed the body length at the onset of sexual maturity in both female (less than 322 cm) and male (longer than 355 cm) whales found in Taiwanese waters agreed with that in the Southern form (females, 300–344 cm, n = 373; males, 394–409 cm, n = 170, Kasuya & Marsh, 1984), despite the small sample size in our study. Nevertheless, these measurements, as well as the external morphological features, indicated the similarity of the pilot whales found in Taiwanese and southeast Japanese waters.

From the genetic analysis our data showed that whales from Taiwanese, Philippine and southeast Japanese waters shared a number of common, closely related haplotypes, and there was no significant population differentiation between those in Taiwanese and southeast Japanese waters. Although we did not find any whales that shared the same haplotype between Taiwanese and Philippine waters, it does not necessarily indicate that there was a population differentiation between these two regions given there were only two samples available from the Philippines. Nevertheless, our finding likely favours an earlier notion that the distribution of the Southern form pilot whales corresponds with the Kuroshio Current, which passes the east coast of Taiwan and flows northward to the southeast coast of Japan (Kasuya, 2011). However, to acquire a better resolution of the population structure of short-finned pilot whales in this region, further studies from more samples using multiple genetic markers are invincibly necessary.

Our sea survey data showed the occurrence of short-finned pilot whales in the east coast of Taiwan on a yearly basis, but these whales are not as common as other small cetaceans found in this region. Other dolphin species, including Risso's dolphins (*Grampus griseus*), spinner dolphins, pantropical spotted dolphins (*Stenella attenuata*), bottlenose dolphins (*Tursiops truncatus*), and Fraser's dolphins, comprised 83.3% of 1,907 cetacean sighting records in the shipboard surveys off the coasts of Taiwan (1994–2006), whereas short-finned pilot whale contributed only 0.5% of all sighting records (Chou, 2007). The rarity of short-finned pilot whale is also revealed in the stranding records: of the 541 stranded cetaceans during 2002–2012, only 27 were short-finned pilot whales (Chou, unpublished data). By maximising the utility of limited accessible samples and information, this study overcame the scarcity and provided handy information, which may assist further implication of conservation for the whales occurred in this region or adjacent waters.

This study has expanded the range of the Southern form of short-finned pilot whales farther south into the tropical west Pacific waters. In particular, our morphological and genetic findings have identified short-finned pilot whales in Taiwanese waters and possibly also in the Philippines and the South China Sea, that were closely similar to the

Southern form of short-finned pilot whales, which are found off the southeast coast of Japan. Such data confirm earlier brief reports of short-finned pilot whales of the Southern form distributed in more southern tropical areas of the west Pacific. For example, similar looking pilot whales have been observed in Philippines waters (Perrin et al., 2005). In addition, in photographs of 55 pilot whales mass-stranded on the north-eastern coast of Java, Indonesia in 1923, saddle patches were not seen on the two whales with observable dorsums, and another showed its square headedness (Rudolph et al., 1997: Figs. 7–8). Short-finned pilot whales in New Guinea waters likewise lack a clear saddle patch (Mitchell, 1975; cited in Kasuya, 2011). These later two observations are thus tentatively similar to those of the Southern form.

Variations in body colour patterns are not always dependable taxonomic criteria for identifying cetacean populations (Bernard & Reilly, 1999). Examination of 3,488 long-finned pilot whales from 40 schools caught off the Faroe Islands in 1986–1988, found that although these whales are commonly characterised as having a post-dorsal fin saddle-patch and a postorbital eye blaze, colour patterns varied within age and size classes (Bloch et al., 1993). The presence or absence of a saddle patch has also been observed in short-finned pilot whales in the northeast Pacific, however the character was found to be too variable to distinguish the putative tropical and the temperate forms (Leatherwood et al., 1982; Kasuya et al., 1988). Therefore, even though there were limited photographic records to support the notion that short-finned pilot whales in Philippine and Indonesian waters are likely of the Southern form, further morphological and genetic evidence derived from a reasonable sample size are necessary, since pigmentation patterns in short-finned pilot whales may vary among individuals or local populations (Kasuya, 2011), and thus the lack of saddle patch may not be a unique external characteristic for the Southern form short-finned pilot whales.

The southern range limit of the Southern form of whale remains undetermined (Kasuya, 2011). The southern range of the Southern form short-finned pilot whale was initially reported as around 25°N due to the lack of survey effort further south (Kasuya et al., 1988; Miyashita, 1993). This study now extends the range southward into the tropical West Pacific (southern Taiwan) and it is foreseeable, that the southern boundary may abut the yet to be defined "South Pacific" form: whales living in the South Pacific regions (New Caledonia, New Zealand, Samoa and French Polynesia) that are genetically distinct at some level from the Southern form (Oremus et al., 2009). This would be comparable to the occasional overlap of range between the Northern and Southern forms of pilot whale in Japanese waters, but mixed schools are suggested to be very rare (Kasuya et al*.,* 1988). In addition, Carretta et al. (2013) suggested that short-finned pilot whales in Hawaiian waters are morphologically similar to the Japanese Southern form and that this Hawaiian stock of pilot whales was reproductively isolated from the whale stock found in the northeast Pacific. However, the report did not indicate the genetic similarity between the Southern form and the Hawaiian stock, and left unresolved the eastern extent of the range for the Southern form. Current genetic evidence suggests a significant population differentiation and an insignificant phylogenetic lineage diversion in the short-finned pilot whales from Taiwan-southern Japan, northern Japan, south Pacific, and Atlantic Ocean regions (Oremus et al., 2009; current study). This possibly indicates that the initial short-finned pilot whale population dispersed world-wide in relatively recent geological history, and that there has been some oceanographic, reproductive, or ecological barriers preventing gene flow between adjacent populations which drove the sub-populations to differentiate. Current knowledge of their social structure, group composition, and gene flow is still insufficient, and thus, further long-term studies including a comprehensive photographic identification database, long-term monitoring, and genetic investigations from different regions will expand and improve our understanding of pilot whale evolution.

ACKNOWLEDGEMENTS

We are grateful to Ray Chin at the Kuroshio Ocean Education Foundation, Chang-Hong Wu and Bo-Ge Chiou at the Seawhale Whale Watching Company, and Ming-Chang Liu at the Cetacean Research Laboratory in National Taiwan University for providing high quality photographs of the whales, and to our summer intern Yuan-Ning Chen for his assistance on managing these photographs. We thank Ren-Chieh Lien and Sue Moore at the Applied Physics Laboratory, University of Washington, for supplying the video clip of pilot whales in the South China Sea. We thank the assistants and volunteers at National Taiwan University and Taiwan Cetacean Society for their assistance in collection of cetacean samples (1994–2012); I-Hua Chen and Chieh Lo for their help with the genetic experiments at National Chiayi University. We thank Malgorzata Pilot at the University of Lincoln, Robert Brownell Jr. at SWFSC, NOAA, and an anonymous reviewer for offering constructive comments to improve this manuscript. Funding for Lien-Siang Chou to maintain the Taiwan Cetacean Stranding program was provided by the Forestry Bureau, Council of Agriculture, Government of Taiwan (1994–2012).

LITERATURE CITED

Aragones LV, Roque MAA, Flores MB, Encomienda RP, Laule GE, Espinos BG, Maniago FE, Diaz GC, Alesna EB & Braun RC (2010) The Philippine marine mammal strandings from 1998 to 2009: animals in the Philippines in peril? Aquatic Mammals, 36: 219–233.

Bernard HJ & Reilly SB (1999) Pilot whales. In: Ridgway SH & Harrison R (eds.) Handbook of Marine Mammals, Volume 6: the Second Book of Dolphins and the Porpoises. Academic Press, San Diego, CA. Pp. 245–279.

Bloch D, Zachariassen M & Zachariassen P (1993) Some external characters of the long-finned pilot whale off the Faroe Islands and a comparison with the short-finned pilot whale. In: Donovan GP, Lockyer CH & Martin AR (eds.), Reports of the International Whaling Commission, Special Issue 14: Biology of Northern Hemisphere Pilot Whales. International Whaling Commission, Cambridge. Pp. 117–135.

Carretta JV, Oleson E, Weller DW, Lang AR, Forney KA, Baker J, Hanson B, Martien K, Muto MM, Lowry MS, Barlow J, Lynch

D, Carswell L, Brownell Jr. RL, Mattila DK & Hill MC (2013) U.S. Pacific Marine Mammal Stock Assessments: 2012. U.S. Department of Commerce, NOAA Technical Memorandum NOAA-TM-NMFS-SWFSC-504. 378 pp.

Chen Y (2001) Ecological Aspects of Cetaceans in Ilan Waters of Taiwan: Abundance, Distribution, Habitat Partitioning, and Acoustics. Unpublished Master's Thesis, Department of Marine Biology, University of Charleston, South Carolina. 147 pp.

Chou L-S (2007) The Population Estimation and Habitats of Cetacean in Coastal Waters of Taiwan. Final Report to Fishery Agency, Council of Agriculture, Republic of China, No. 96AS-15.1.1-FA-F3(2). 62 pp. [Text in Chinese]

Davies JL (1963) The antitropical factor in cetacean speciation. Evolution, 17: 107–116.

Dolar MLL, Perrin WF, Yaptinchay AA, Jaaman SABJH, Santos M, Alava M & Suliansa SB (1997) Preliminary investigation of marine mammal distribution, abundance, and interactions with humans in the Southern Sulu Sea. Asian Marine Biology, 14: 61–81.

Excoffier L & Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10: 564–567.

Hoelzel AR, Dahlheim M & Stern SJ (1998) Low genetic variation among killer whales (*Orcinus orca*) in the eastern North Pacific and genetic differentiation between foraging specialists. Journal of Heredity, 89: 121–128.

Kage T (1999) Study on Population Structure Analysis of Short-finned Pilot Whale Using Multiple DNA Markers. Unpublished PhD Thesis, Department of Biological Resources, Mie University. Tsu, Japan. 113 pp. [Text in Japanese]

Jefferson TA, Webber MA & Pitman RL (2008) Marine Mammals of the World: A Comprehensive Guide to Their Identification. Academic Press, San Diego, CA. 573 pp.

Kasuya T (2011) Conservation Biology of Small Cetaceans around Japan. University of Tokyo University Press, Tokyo. 640 pp. [Text in Japanese]

Kasuya T, Miyashita T & Kasamatsu F (1988) Segregation of two forms of short-finned pilot whales off the Pacific coast of Japan. Scientific Reports of the Whales Research Institute (Tokyo), 39: 77–90.

Kasuya T & Marsh H (1984) Life history and reproductive biology of the short-finned pilot whale, *Globicephala macrorhynchus*, off the Pacific coast of Japan. In: Perrin WF, Brownell Jr. RL, & DeMaster DP (eds.) Reports of the International Whaling Commission, Special Issue 6: Reproduction in Whales, Dolphins and Porpoises. International Whaling Commission, Cambridge. Pp. 259–310.

Kasuya T & Tai S (1993) Life history of short-finned pilot whale stocks off Japan and a description of the fishery. In: Donovan GP, Lockyer CH & Martin AR (eds.), Reports of the International Whaling Commission, Special Issue 14: Biology of Northern Hemisphere Pilot Whales. International Whaling Commission, Cambridge. Pp. 439–473.

Kreb D & Budiono (2005). Cetacean diversity and habitat preferences in tropical waters of East Kalimantan, Indonesia. Raffles Bulletin of Zoology, 53: 149–155.

Leatherwood S, Reeves RR, Perrin WF, Evans WE & Hobbs L (1982) Whales, Dolphins, and Porpoises of the Eastern North Pacific and Adjacent Arctic Waters: A Guide to their Identification. U.S. Department of Commerce, NOAA Technical Report NMFS Circular 444. 247 pp.

LeDuc RG, Perrin WF & Dizon AE (1999) Phylogenetic relationships among the delphinid cetaceans based on full cytochrome b sequences. Marine Mammal Science, 15: 619–648.

Librado P & Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25: 1451–1452.

Mitchell E (1975) Report of the meeting on smaller cetaceans, Montreal, April 1–11, 1974. Journal of the Fisheries Research Board of Canada, 32: 917–919.

Miyashita T (1993) Abundance of dolphin stocks in the western North Pacific taken by the Japanese drive fishery. Reports of the International Whaling Commission, 43: 417–437.

Miyazaki N & Amano M (1994) Skull morphology of two forms of short-finned pilot whales off the Pacific coast of Japan. Reports of the International Whaling Commission, 44: 499–508.

Norris KS (1961) Standardized methods for measuring and recording data on the smaller cetaceans. Journal of Mammalogy, 42: 471–476.

Oremus M, Gales R, Dalebout ML, Funahashi N, Endo T, Kage T, Steel D & Baker SC (2009) Worldwide mitochondrial DNA diversity and phylogeography of pilot whales (*Globicephala* spp.). Biological Journal of the Linnean Society, 98: 729–744.

Perrin WF & Reilly SB (1984) Reproductive parameters of dolphins and small whales of the family Delphinidae. In: Perrin WF, Brownell RL & DeMaster DP (eds.) Reports of the International Whaling Commission, Special Issue 6: Reproduction in Whales, Dolphins and Porpoises. International Whaling Commission, Cambridge. Pp. 97–133.

Perrin WF, Dolar MLL & Robineau D (1999) Spinner dolphins (*Stenella longirostris*) of the western Pacific and southeast Asia: pelagic and shallow-water forms. Marine Mammal Science, 15: 1029–1053.

Perrin WF, Dolar MLL, Amano M & Hayano A (2003) Cranial sexual dimorphism and geographic variation in Fraser's dolphin, *Lagenodelphis hosei*. Marine Mammal Science, 19: 484–501.

Perrin WF, Reeves RR, Dolar MLL, Jefferson TA, Marsh H, Wang JY & Estacion J (2005) Report of the Second Workshop on the Biology and Conservation of Small Cetaceans and Dugongs of South-East Asia. UNEP/CMS Secretariat, Bonn, Germany. 161 pp.

Rice DW (1998) Marine Mammals of the World. Systematics and Distribution. Society for Marine Mammalogy Special Publication, 4: 1–231.

Robards MD & Reeves RR (2011) The global extent and character of marine mammal consumption by humans: 1970–2009. Biological Conservation, 144: 2770–2786.

Ross HA, Lento GM, Dalebout ML, Goode M, Ewing G, McLaren P, Rodrigo AG, Lavery S & Baker CS (2003) DNA surveillance: web-based molecular identification of whales, dolphins, and porpoises. Journal of Heredity, 94: 111–114.

Rudolph P, Smeenk C & Leatherwood S (1997) Preliminary checklist of Cetacea in the Indonesian Archipelago and adjacent waters. Zoologische Verhandelingen (Leiden), 312: 1–48.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28: 2731–2739.

Tanabe S (2006) Environmental Specimen Bank in Ehime University (es-BANK), Japan for global monitoring. Journal of Environmental Monitoring, 8: 782–790.

Taylor BL, Baird R, Barlow J, Dawson SM, Ford J, Mead JG, Notarbartolo di Sciara G, Wade P & Pitman RL (2008) *Globicephala macrorhynchus*. IUCN Red List of Threatened Species. Version 2010.4. www.iucnredlist.org. (accessed 12 May 2011).

Vilstrup JT, Ho SYW, Foote AD, Morin PA, Kreb D, Krützen M, Parra GJ, Robertson KM, de Stephanis R, Verborgh P, Willerslev E, Orlando L & Gilbert MTP (2011) Mitogenomic phylogenetic analyses of the Delphinidae with an emphasis on the Globicephalinae. BMC Evolutionary Biology, 11: 65.

Wada S (1988) Genetic differentiation between two forms of short-finned pilot whales off the Pacific coast of Japan. Scientific Reports of the Whales Research Institute (Tokyo), 39: 91–101.

Wyrtki K (1975) Fluctuations of the dynamic topography in the Pacific Ocean. Journal of Physical Oceanography, 5: 450–459.

Yang W-C, Chou L-S, Jepson PD, Brownell Jr. RL, Cowan D, Chang P-H, Chiou H-I, Yao C-J, Yamada TK, Chiu J-T, Wang PJ & Fernández A (2008) Unusual cetacean mortality event in Taiwan, possibly linked to naval activities. Veterinary Record, 162: 184–185.

Yeh C-C (2001) Fauna, Distribution and Habitat Features of Cetaceans in Coastal Waters of Southeastern Taiwan. Unpublished Masters Thesis, Institute of Zoology, National Taiwan University, Taipei, Taiwan. 89 pp. [Text in Chinese]

Yonekura M, Matsui S & Kasuya T (1980) On the external characters of *Globicephala macrorhynchus* off Taiji, Pacific coast of Japan. Scientific Reports of the Whales Research Institute (Tokyo), 32: 67–95.

Supplement A

A short video-recording of a group of short-finned pilot whales encountered in the South China Sea, near the northeast Dongsha plateau (21°N, 116.5–118°E; Fig. 1). Each of the three whales that closely approached the observers' boat had a uniformly dark dorsum without saddle patch, i.e., morphologically similar to the Southern form short-finned pilot whales found in Japanese waters. The video was fi lmed by Ren-Chieh Lien (lien@apl.washington.edu). Additional details of this sighting event and behaviour of the whales are available: Moore SE & Lien RC (2007) Pilot whales follow internal solitary waves in the South China Sea. Marine Mammal Science, 23: 193–196.