

Short Note

Finless Porpoise (*Neophocaena phocaenoides*) Discovered at Okinawa Island, Japan, with the Source Population Inferred from Mitochondrial DNA

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The finless porpoise (*Neophocaena phocaenoides*) is a small, toothed cetacean, inhabiting tropical to warm temperate shallow waters, including estuaries and large rivers, in the Indo-Pacific region (Amano, 2002). In Eastern Asia, a number of studies on finless porpoise populations have been conducted. In Chinese waters, three populations are recognized from mitochondrial DNA (mtDNA) control-region sequence analysis: (1) the Yangtze River population in the middle and lower reaches of the Yangtze River, (2) the Yellow Sea population in the northern East China Sea and Yellow/Bohai Seas, and (3) the South China Sea population in the South China Sea and the southern East China Sea (Yang et al., 2002) (see Figure 1a). In Japan, frequent sightings of porpoises have been restricted to five geographically discrete waters (Sendai Bay-Tokyo Bay, Ise-Mikawa Bays, Inland Sea-Hibiki Nada, Omura Bay, and Ariake Sound-Tachibana Bay; Figure 1b), and animals seldom occur outside these regions (Shirakihara et al., 1992; Shirakihara et al., 1994). From distribution patterns, parturition season, skull morphology, and mtDNA control-region sequences, porpoises in Japanese waters are thought to be subdivided into at least five populations corresponding to each of the five geographic concentrations (Yoshida, 2002).

Along the coast of the Tsushima, Iki, and Nansei Islands between Japan and China-Korea (Figure 1a), finless porpoise occurrence has not been confirmed (Shirakihara et al., 1992; National Museum of Nature and Science & Institute of Cetacean Research, 2009), except in a report describing two digested animals that were found in the stomach of a great white shark (*Carcharodon carcharias*) caught in the East China Sea off of Okinawa Island (Kasuya, 1999). The rare occurrence of finless porpoise sightings suggests that animal movements are limited between Japan and

China-Korea. Here, we report the first live record of a finless porpoise in the Nansei Islands and investigate its source population using mtDNA control-region sequence.

On the morning of 17 February 2004, a finless porpoise was discovered by a local fisherman 500 m east of the northwestern corner of Motobu Peninsula of Okinawa Island in the Nansei Islands, southwestern Japan (Figure 1a). It swam feebly in the calm sea and appeared weakened. The fisherman removed the animal from the water to his boat for medical treatment at the nearby Okinawa Churaumi Aquarium. However, it quickly died on the boat. The body was transported to the aquarium and then photographed, measured, and sexed. The skin was collected and preserved in 80% aqueous ethanol, and the remaining whole body was fixed in formalin solution.

From the skin tissue collected, total cellular DNA was extracted with a Gentra Puregene Mouse Tail Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. The mtDNA control-region was amplified by polymerase chain reaction (PCR) using the primers "t-PRO" (5'-CCTCCCTAAGACTCAAGGAA-3') from Arnason et al. (1993) and "P2" (5'-TCTCGAGATTTTCAGTGTCTTGCTTT-3') described in Hoelzel et al. (1991). The PCR amplifications were performed in 25- μ l volumes of Tris buffer (67 mM, pH 8.8) containing 2 mM MgCl₂, 1 mM of each dNTP, 25 pmol of each primer, 0.6 units of the AmpliTaq Gold® DNA polymerase (Applied Biosystems, Carlsbad, CA, USA), and 50 to 100 ng template DNA. The temperature profile was 0.5 min at 96° C, 0.5 min at 60° C, and 1.5 min at 72° C for 30 cycles. The PCR product was treated with EXOSAP-IT (USB Corporation, Cleveland, OH, USA) for clean-up and subjected to direct sequencing with the BigDye® Terminator v3.1 Cycle Sequencing

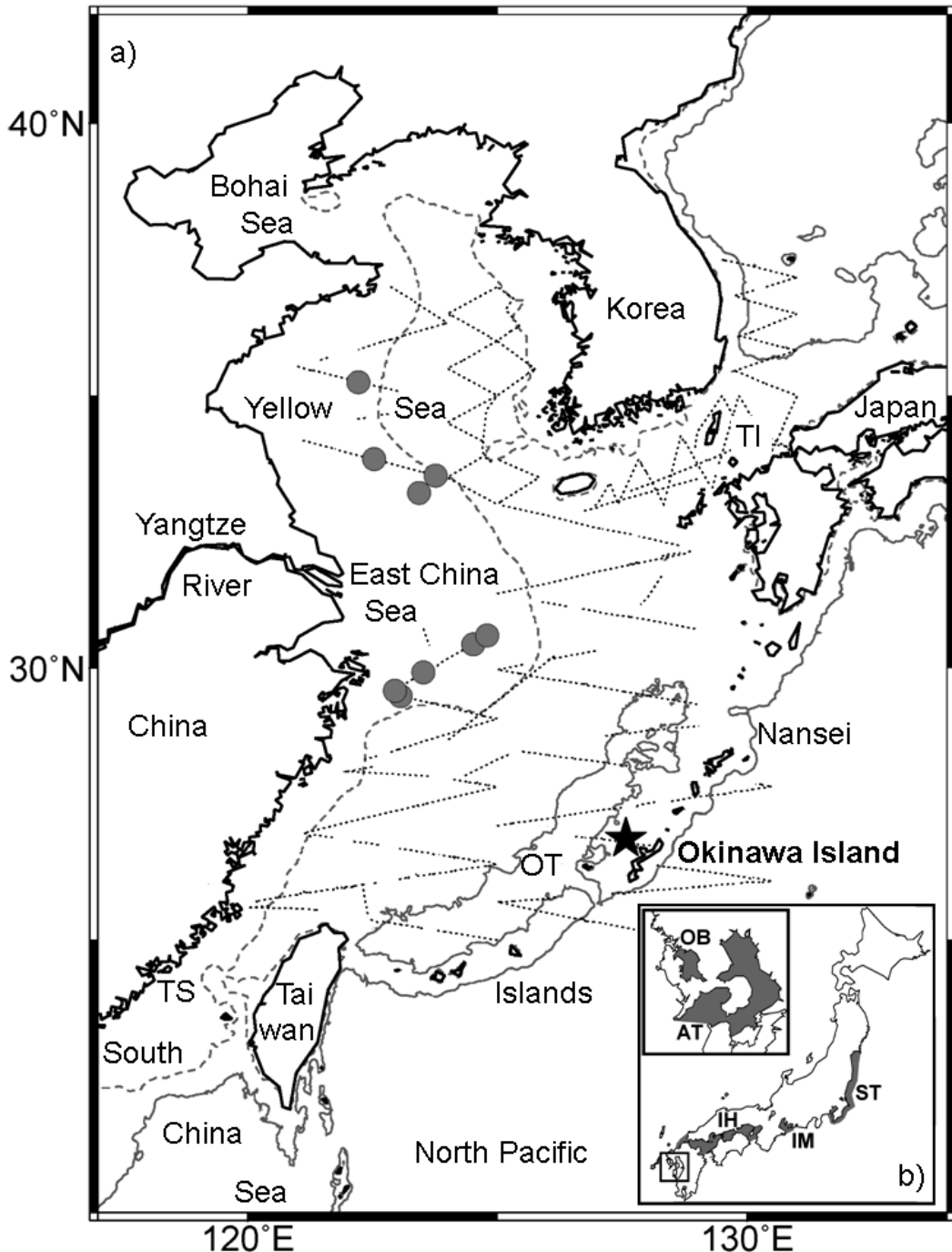


Figure 1. (a) Map of the study area, showing the location where the male finless porpoise (*Neophocaena phocaenoides*) was discovered (star); sightings of finless porpoises (circles) and cruise tracks made during ship sighting surveys (Iwasaki et al., 1995; Miyashita et al., 1995) are also shown. Isobaths are 55 m (dotted line) and 1,000 m (solid line). OT: Okinawa Trough, TI: Tsushima and Iki Islands, and TS: Taiwan Strait. (b) Five coastal waters where finless porpoise are typically distributed in Japan (Shirakihara et al., 1992); ST: Sendai Bay-Tokyo Bay, IM: Ise-Mikawa Bays, IH: Inland Sea-Hibiki Nada, OB: Omura Bay, and AT: Ariake Sound-Tachibana Bay.

Kit (Applied Biosystems) for 26 cycles (at 96° C for 0.5 min, 50° C for 0.25 min, and 60° C for 4 min), using the same primers as in the PCR reaction. Sequencing was conducted with an Applied Biosystems 3130xl Genetic Analyzer.

For haplotype matching, 27 homologous sequences of finless porpoises were downloaded from *GenBank*: 10 haplotypes obtained from 174 porpoises collected in Japanese coastal waters that were 345 base pairs (bp) long (*GenBank*

accession numbers = AF193543 to 193552; Yoshida et al., 2001) and 17 haplotypes found in 73 animals collected in Chinese waters that were 720 bp long (*GenBank* accession numbers = AF289280 to 289352; Yang et al., 2002). Sequences were aligned using the multiple sequences alignment program *CLUSTAL W* (Thompson et al., 1994).

The Okinawa specimen was a male, 129.6 cm in length, and 19.2 kg in weight, with a prominent narrow dorsal ridge (Figure 2; Table 1) as opposed



Figure 2. Dorsal (top picture) and lateral (middle picture) views of the male finless porpoise discovered in the vicinity of Okinawa Island, part of Nansei Islands in southwestern Japan; two crater wounds were found on the lateral side of the porpoise body (bottom pictures), possibly made by the largetooth cookiecutter shark (*Isistius plutodus*).

to the other variant (i.e., flat wide dorsal ridge) seen in this species (Amano, 2002). The specimen appeared to be sexually immature from its body length, as reproductive studies on finless porpoises in Japanese and Chinese waters suggest that males attain sexual maturity at > 135 cm (Shirakihara et al., 1993; Jefferson et al., 2002). Relationships between body length and weight of male finless porpoises (Shirakihara et al., 1993; Jefferson et al.,

2002) indicate that the specimen was extremely thin as its weight should have been 34.1 to 34.6 kg as determined from body length.

A total of 720 bp of the mtDNA control-region of the specimen was sequenced. When it was compared with 10 haplotypes identified from 174 Japanese porpoises (Yoshida et al., 2001), no identical sequences were found (Figure 3). However, when it was aligned with 17 haplotypes obtained from 73 Chinese animals (Yang et al., 2002), it matched the haplotype CM05 (*GenBank* accession numbers = AF289331 to 289335). Yang et al. (2002) reported that this haplotype was found in five animals: four from coastal waters of the southern Yellow Sea and one from the coast in the Taiwan Strait. Five other animals collected from the Taiwanese coast in the Taiwan Strait also possessed this mtDNA control-region haplotype (Yao et al., 2005).

In Japanese coastal waters, finless porpoise sightings are concentrated in waters with a depth of < 50 m and a distance of < 3 nmi off shore (Kasuya & Kureha, 1979; Shirakihara et al., 1992; Shirakihara et al., 1994), which suggests that the habitat of the porpoise is defined by shallow water depth and proximity to shore. However, sightings in the Yellow Sea and East China Sea indicate that proximity to shore is not an important factor for porpoise occurrence; ship sighting surveys reported porpoise sightings in waters with depths < 55 m and distances from the shore of 30 to 130 nmi (Iwasaki et al., 1995; Miyashita et al., 1995; Figure 1a). Off the west coast of Korea, recent sighting surveys observed many porpoises in waters with depth of 20 to 50 m, but few in waters > 60 m in depth (Zhang et al., 2004). These findings indicate that finless porpoises prefer shallow waters with depths less than around 55 m, regardless of distance from shore. Off the Chinese coast, such shallow waters extend eastwards, especially near the mouth of the Yangtze River. The mtDNA control-region sequence of the Okinawa specimen matched the haplotype of specimens found in China and Taiwan (Yang et al., 2002; Yao et al., 2006). From these, we presumed that the Okinawa specimen came from the Chinese or Taiwanese coast and originated from the Chinese population.

The Okinawa specimen exhibited two crater wounds on the lateral side of its body; they were fresh and elliptic-shaped, about 6.5 cm × 3 cm in diameter (Figure 2). The wounds were similar to wound shapes attributable to cookiecutter sharks (*Isistius* sp.), which are a known source for bite marks observed on whales and dolphins (e.g., Gasparini & Sazima, 1996). The largetooth cookiecutter shark (*Isistius plutodus*) has been reported in the waters off the east coast of Okinawa Island

Table 1. Body measurements of the male finless porpoise (*Neophocaena phocaenoides*) discovered in the vicinity of Okinawa Island, part of Nansei Islands in southwestern Japan

Measurement		
1.	From tip of snout to notch of fluke (total length)	129.6 cm
2.	From tip of snout to center of anus	89.8 cm
3.	From tip of snout to center of genital slits	70.2 cm
4.	From tip of snout to center of umbilicus	57.2 cm
5.	From tip of snout to anterior insertion of left flipper	26.6 cm
6.	From tip of snout to center of left eye	8.5 cm
7.	From tip of snout to center of blowhole	9.8 cm
8.	From anterior tip of melon to tip of snout	0.3 cm
9.	From eye to ear	6.2 cm
10.	Girth at anterior insertion of flipper	51.6 cm
11.	Girth at anterior point of prominent dorsal ridge	59.4 cm
12.	Girth at center of umbilicus	59.1 cm
13.	Girth at center of genital slits	48.8 cm
14.	Girth at center of anus	37.2 cm
15.	Length of left flipper, anterior insertion to tip	29.6 cm
16.	Length of left flipper, axilla to tip	22.4 cm
17.	Circumference of left flipper, from anterior insertion to tip	32.9 cm
18.	Maximum width of left flipper	11.2 cm
19.	Width of left flipper, anterior insertion to axilla	9.5 cm
20.	Fluke span	32.4 cm
21.	From anterior insertion to tip of left side fluke	25.5 cm
22.	From notch to tip of left side fluke	20.3 cm
23.	Circumference of left side fluke, from anterior insertion to tip	29.8 cm
24.	Length of mouth	5.2 cm
25.	Width of mouth	9.5 cm
26.	Length of left eye	1.6 cm
27.	Width of blowhole	2.2 cm
28.	Weight	19.2 kg

	Variable sites																											N																
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Okinawa specimen	G	A	T	A	A	T	T	A	G	A	C	T	C	C	C	T	T	C	A	A	G	C	720																					
Haplotype - a	.	.	C	T	345																					
- b	.	.	C	.	.	.	C	345																					
- c	.	.	C	345																					
- d	.	.	C	C	345																					
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- h	.	.	C	G	G	T	.	T	345																					
- i	.	.	C	G	.	.	C	345																					
- j	T	345																					
- YS01	.	.	.	G	720																					
- YS02	A	C	720																					
- YS03	.	.	C	.	.	C	T	720																					
- YS04	T	G	.	.	.	720																					
- YS05	T	C	C	.	G	720																					
- CM01	T	720																					
- CM02	.	.	C	T	720																					
- CM03	.	.	C	T	720																					
- CM04	.	.	C	T	.	.	.	T	.	.	.	G	A	.	.	.	720																					
- CM05	720																					
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- SS04	.	.	C	T	.	.	.	T	.	T	.	G	720																					
- SS05	.	.	C	T	.	.	.	G	A	.	.	.	720																					
- SS06	.	.	C	T	.	.	.	T	.	.	.	G	720																					
- SS07	T	720																					

Figure 3. Variable nucleotide sites found between mtDNA control-region of the Okinawa specimen and those of 27 haplotypes previously identified in finless porpoises collected in Japanese coastal waters (Haplotypes a through j; Yoshida et al., 2001) and Chinese waters (Haplotypes YS01 through SS07; Yang et al., 2002); dots indicate the identical nucleotides with the Okinawa specimen. N = number of base pairs of the mtDNA control-region sequenced.

(Parin, 1975). A rough-toothed dolphin (*Steno bredanensis*) stranded at an island west of Okinawa Island exhibited a fresh crater wound that was thought to be attributable to a cookiecutter shark (Akajima Marine Science Laboratory [AMSL], 2006). It is possible that the Okinawa specimen was also attacked by a cookiecutter shark in the waters around Okinawa Island.

As revealed in Japanese coastal waters, finless porpoises may be subdivided into relatively small isolated populations and possess regional genetic features such as unique mtDNA control-region haplotypes. Thus, when a porpoise is found far from the reported range, its source population can be identified from genetic information. Such analysis can provide important information on

gene flow between local isolated populations of finless porpoises.

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