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Short Note

Insights from a Gray Whale (*Eschrichtius robustus*) Bycaught in the Taiwan Strait off China in 2011

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Gray whales (*Eschrichtius robustus*) are recognized as two distinct populations in the North Pacific Ocean: the eastern North Pacific (ENP) population (also known as the California-Chukchi) and the western North Pacific (WNP) population (also known as the Korean-Okhotsk) (Andrews, 1914; Rice & Wolman, 1971; LeDuc et al., 2002; Weller et al., 2013b). Gray whales in the Atlantic were extirpated prior to the 19th century (Mead & Mitchell, 1984). The ENP population migrates from winter areas off Baja California, Mexico, to summer feeding areas in the Bering, Beaufort, and Chukchi Seas (Swartz et al., 2006; Weller et al., 2013b). This population was removed from the U.S. List of Endangered and Threatened Wildlife in 1994 and is currently estimated to number approximately 21,000 individuals (Durban et al., 2013). The WNP population feeds in the Okhotsk Sea off Sakhalin Island, Russia (Weller et al., 1999, 2002), and also off the southeastern coast of the Kamchatka Peninsula (Tyurneva et al., 2010). The wintering grounds of the WNP population remain unconfirmed but may be along the coast of southern China based on sighting, stranding, and catch records (Wang, 1984; Omura, 1988; Henderson, 1990; Kato & Kasuya, 2002; Zhu, 2002; Weller et al., 2013a). A recent population assessment using an individual-based stage-structured model resulted in a median estimate of 140 ± 6 (SE) 1+ (non-calf) individuals for the WNP population in 2012 (Cooke et al., 2013). This population is redlisted by the International Union for Conservation of Nature (IUCN) as Critically Endangered (Reilly et al., 2008).

During the winter, some gray whales seen feeding off Sakhalin and Kamchatka have been observed to migrate to the west coast of North America, including Mexico (Weller et al., 2012; Urbán et al., 2013), while others migrate to portions of the WNP off Asia, including the coasts of Japan and China (Weller et al., 2008, 2013a). Despite this level of mixing, genetic comparisons of ENP and WNP gray whales have found significant differences in both nuclear and mitochondrial DNA (LeDuc et al., 2002; Lang et al., 2010, 2011) supporting their management as distinct population stocks (Weller et al., 2013b).

Gray whales are exceptionally rare off China, with only 24 sighting, stranding, or capture records since 1933 (Weller et al., 2013a). The gray whale was listed in China as Category II of the National Key Protected Animals in 1988 and was listed under the Chinese Red List of Endangered and Threatened Wildlife and Plants in 1994. The most recent record of a gray whale from China is from 5 November 2011 when a dead female gray whale (Figure 1) was found entangled in a set gillnet off Fujian Province in the Taiwan Strait (Figure 2). This specimen was 13.1 m in length and 21,000 kg in weight, and represents the first gray whale record from Chinese waters in the 21st century. To explore the possibility of linking the 2011 Taiwan Strait gray whale to other areas prior to its death, skin tissue of the whale was collected for genetic analysis, and photographs of the whale were compared to the existing gray whale photo-identification catalogs for the WNP and ENP.

Total DNA was extracted from skin tissue of this 2011 Taiwan Strait specimen following the recommended protocol of the TIANamp Genomic DNA Kit (Catalog No. DP304, TIANGEN). The extracted DNA was stored in TE buffer (10 mM Tri-HCl, 1 mM EDTA, pH8.0). The mtDNA control region was amplified through the polymerase chain

reaction (PCR) using the following primer set: P1: 5'-GAATTCCCCGGTCTTGTAAACC-3' and P2: 5'-TCTCGAGATTTTCAGTGTCTTGCTTT-3 (Hoelzel et al., 1991). PCR was carried out in a total volume of 25 µl comprising 100 to 400 ng of DNA template, 0.2 mM of each dNTP, 0.4 µM of each primer, and 1.25 unit of ExTaq polymerase (TaKaRa). The amplification program was conducted as follows: preheating at 94° C for 5 min; cycling 38 times at 94° C for 40 s, 50° C for 30 s, and 72° C for 1 min; and extending at 72° C for 5 min.

The PCR products were electrophoresed through a 1% Agarose gel to confirm successful amplification, and then the PCR products were sent to Shanghai Invitrogen Biotechnology Co., Ltd. for direct sequencing. Cycle sequencing was performed using the above primers, and then both strands were sequenced independently on an ABI 3730 Automated DNA Analyzer. The resulting sequences were edited and spliced by *BioEdit*, Version 7.2.5 (Hall, 1999) with manual correction. PCR amplification and sequencing were repeated separately again to account for lab error. Finally, a complete control region (*D-loop*) sequence, 934 bp in length, was obtained. The full length of the *D-loop* sequence was used for the BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST results indicated that the 522 bp sequence representing the 5' end of the control region was completely identical to the haplotype R (Figure 3) described in LeDuc et al. (2002) and reported as haplotype 18 in Lang et al. (2011). Comparison of the full-length sequence generated from the China specimen with the three available gray whale sequences (AP006471.1, AJ554053.1, and X72200.1) that included the complete control region indicated that the majority of variable sites were found within the 522 bp 5' region and, thus, did not yield further insight into the origin of the 2011 Taiwan Strait specimen.

To date, mtDNA haplotype data are available for 377 gray whales considered part of the ENP population and 142 gray whales sampled in the WNP (LeDuc et al., 2002; Lang et al., 2011). The haplotype R was found in 12 ENP individuals, the majority (n = 10) of which were collected from stranded or hunted individuals prior to 2011 (Lang, pers. comm.). However, haplotype R was not identified among the gray whales sampled in the WNP, all of which were biopsied on the

Figure 1. The left side (A) and right side (B) of the female gray whale (*Eschrichtius robustus)* found bycaught in the Taiwan Strait, Fujian Province of China, on 5 November 2011

Sakhalin Island feeding ground between 1995 and 2007. This sample set included samples from 69% of the individuals identified in that region through 2011 (n = 205), suggesting that the probability of haplotype R occurring in the WNP population is relatively low.

Based on mtDNA haplotype frequency data, the 2011 Taiwan Strait gray whale specimen has a higher probability of originating from the ENP rather than the WNP. However, it is also plausible that haplotype R is carried by one or more of the individuals that utilize the Sakhalin feeding ground or other parts of the WNP but have not been sampled. To further examine this question, photographs of the 2011 Taiwan Strait specimen were compared to existing gray whale photo-identification catalogs from the WNP and ENP. Comparisons to photo-identification catalogs collected in the WNP off Sakhalin Island and southeastern Kamchatka as well as catalogs from the west coast of North America (Northern California, Washington, Oregon, and British Columbia) and the lagoons in Mexico did not produce a photographic match. Although the photographs of the 2011 Taiwan Strait specimen were suitable for photo-identification purposes, they were not considered to be ideal and were additionally limited to primarily the left side of the whale because some epidermis on the right side had already been removed as a result of decomposition (Figure 1B). Some catalogs from WNP and ENP are based on the right side; therefore, it is possible that image quality limitations contributed to the lack of a photographic match within the WNP or ENP.

Given that the photographs of the 2011 Taiwan Strait whale did not match any of the individuals photographed off Sakhalin or Kamchatka, as well as the absence of haplotype R among gray whales sampled in the WNP, our results could suggest that the 2011 Taiwan Strait whale was a vagrant from the ENP. In recent years, there have been sightings of two gray whales in the Atlantic Ocean—one that was sighted twice in the Mediterranean Sea in May 2010 (Scheinin et al., 2011) and a second individual that was sighted in Walvis Bay, Namibia, in May 2013 (Elwen & Gridley, 2013). These whales were thought to be vagrant individuals, most likely from the ENP,

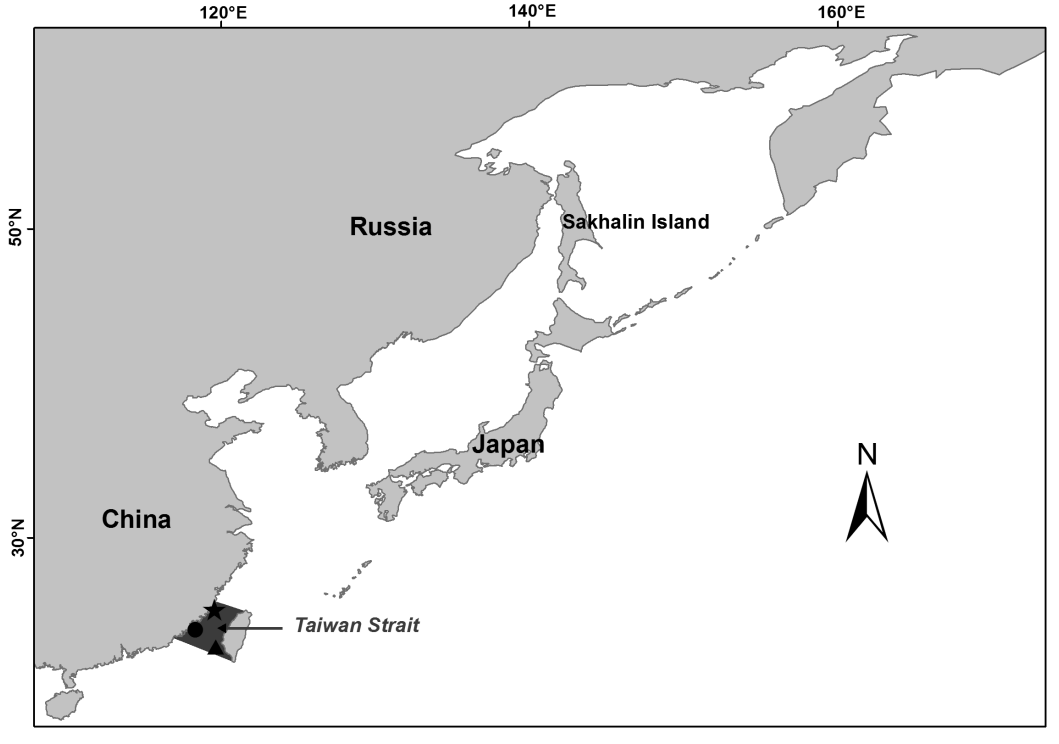

Figure 2. Map of the western North Pacific (WNP) showing (1) location of a female gray whale found bycaught in the Taiwan Strait, Fujian Province of China, on 5 November 2011 (black star); (2) location of partial skeleton of a gray whale excavated from the Quanzhou coast, Fujian Province of China, in 1958 (black circle) (Li, 1997); and (3) location of fossil specimens of two juvenile gray whales found in the Penghu Channel of Taiwan Strait, China (black triangle) (Tsai et al., 2014). The Taiwan Strait is shown in dark gray.

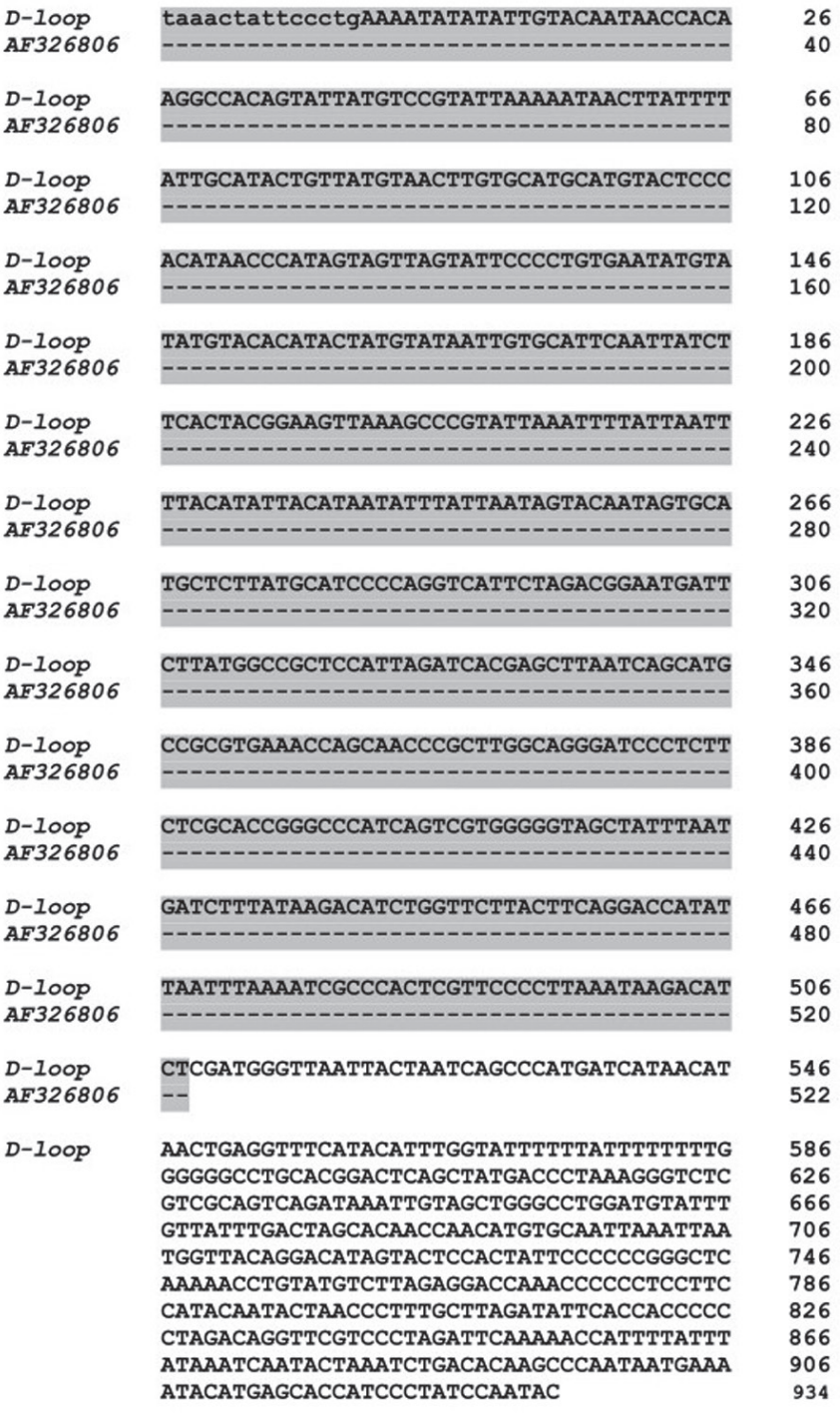

Figure 3. The *D-loop* sequence obtained in the present study was completely identical to the AF326806 (the haplotype R sequence) submitted to the GenBank by LeDuc et al. (2002) and Lang et al. (2010, 2011). An identical sequence is highlighted in gray.

that had wandered into the Atlantic as a result of diminished Arctic Sea ice cover in the northern passageways. These records suggest that environmental changes resulting from climate warming may allow gray whales to recolonize areas used historically or to disperse into new habitats suitable for living (Scheinin et al., 2011). Moreover, without needing new ice-free passages, some gray whales observed in the WNP have been confirmed to migrate to the ENP (Weller et al., 2012; Urbán et al., 2013). Therefore, it is also possible that an ENP gray whale could disperse to the Taiwan Strait where gray whales historically occurred.

Another possibility, however, is that an additional feeding ground (or grounds) exists in the WNP, but it has not yet been identified or genetically characterized. Based on records from the logbooks of 19th century ship-based whalers, gray whales appear to have had a more extensive distribution in the Okhotsk Sea, while no gray whales, and very little search effort, were recorded in the area of northeast Sakhalin Island that is currently considered the primary feeding ground (Reeves et al., 2008). While scant information is currently available to evaluate this hypothesis, the possibility that such additional WNP feeding grounds could exist and might have been used by the 2011 Taiwan Strait whale highlights the critical importance of obtaining photographic and genetic evidence, such as that presented herein, from any gray whales recorded in areas of the WNP other than the Sakhalin feeding ground.

Interestingly, a partial skeleton of a gray whale was excavated from the Quanzhou coast of Fujian Province in 1958 (Li, 1997), and fossil specimens of two juvenile gray whales have also been found in the Penghu Channel of Taiwan Strait (Tsai et al., 2014) (Figure 2). Further, Henderson (1990) summarized information from the 1869 logbooks of New Bedford whaling ships while they were on the "Chinese whale grounds" (p. 14). These logbooks reported gray whales being sighted in February at nearly an identical location as the 2011 Taiwan Strait specimen. These sources, in combination with insights from the specimen reported herein, suggest the possibility of a past (and perhaps current) gray whale wintering area in or near the Taiwan Strait region of the WNP.

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