Aquatic Mammals 2020, *46*(3), 301-306, DOI 10.1578/AM.46.3.2020.301

Short Note

First Record of Omura's Whale (*Balaenoptera omurai*) in the Beibu Gulf, China

Liyuan Zhao,[1,2] Mingding Zhong,[1,2] Fuxing Wu,[1,2] Yufei Dai,[1,2] Reyilamu Aierken,[1,2] Mo Chen,[3] and Xianyan Wang[1,2]

[1]*Laboratory of Marine Biology and Ecology, Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, China*
E-mail: wangxianyan@tio.org.cn
[2]*Fujian Provincial Key Laboratory of Marine Ecological Conservation and Restoration, Xiamen 361005, China*
[3]*Guangxi Beibu Gulf Marine Research Center, Guangxi Academy of Sciences, Nanning 530000, China*

Omura's whale (*Balaenoptera omurai*) was previously erroneously classified as Bryde's whale (*Balaenoptera edeni*) and, since 2003, has been described as a new species based on mitochondrial DNA sequences, osteology, and external morphology (Wada et al., 2003; Sasaki et al., 2006). With increased records of Omura's whales, additional morphological diagnostic features have been identified that distinguish them from Bryde's whales—for example, asymmetric pigmentation of the jaw (Wada et al., 2003), a single prominent rostral ridge (Wada et al., 2003; Jefferson et al., 2007; Cerchio et al., 2015), and a lesser number of baleen plates as compared with Bryde's whales (Jefferson et al., 2007). Still, it is difficult to separate this species from the other *Balaenoptera* species in the wild based only on these distinguishing features. Therefore, molecular genetic analysis is widely used to distinguish them from other baleen whales, especially for stranded specimens.

Omura's whales were previously thought to be distributed in tropical and subtropical waters between the southwestern Pacific and the eastern Indian Oceans (Wada et al., 2003; Jefferson et al., 2007). Since the discovery of the Madagascar population in 2015, the range of Omura's whales has expanded to the southwestern Indian Ocean (Cerchio et al., 2015). Stranded Omura's whales have also been reported near Mauritania (Jung et al., 2015) and Brazil (Cypriano-Souza et al., 2017) in the Atlantic Ocean. The recent geographic range of Omura's whales includes the Pacific, Indian, and Atlantic Oceans, with their core region in the eastern Indo-Pacific Ocean (Cerchio et al., 2019). To date, 15 Omura's whale records (including the present specimen) have been reported along the Chinese coast, suggesting a relatively continuous distribution from the Yangtze River Estuary to the South China Sea, mostly in the East China Sea around the Taiwan Strait (Xu et al., 2017). Therefore, Chinese coastal waters seem to be an important distribution region for Omura's whale, although there are no confirmed resident populations in the wild.

Chen et al. (2019) recently reported a Bryde's whale population frequently occurring and feeding in the northern Beibu Gulf of China. On 4 January 2019, an unidentified baleen whale was stranded at the Beilun River Estuary National Nature Reserve (21° 36' 47.7" N, 108° 13' 46.3" E) in the northern Beibu Gulf, China (Figure 1). According to macroscopic morphological characters, this stranded baleen whale was suspected to be a Bryde's whale. Therefore, the specimen was necropsied for further species identification. External morphological parameters were documented following the methods of Geraci & Lounsbury (2005) and Xu et al. (2017). Tissue samples from skin and muscle were collected and preserved in 90% ethanol for further molecular identification and analysis.

Genomic DNA was extracted from muscle tissues using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. A partial fragment of the mtDNA cytochrome c oxidase subunit 1 (*cox1*), cytochrome b (*cytb*) gene, and mitochondrial control region (D-loop) were amplified and sequenced (detailed in Supplementary Material S1; the supplementary material for this short note is available in the "Supplemental Material" section of the *Aquatic Mammals* website: https://www.aquaticmammals-journal.org/index.php?option=com_content&view=article&id=10&Itemid=147). We also amplified D-loop of the Omura's whale specimen BOM-2011-11-29, which was identified by Xu et al. (2017). The PCR products were sequenced and assembled

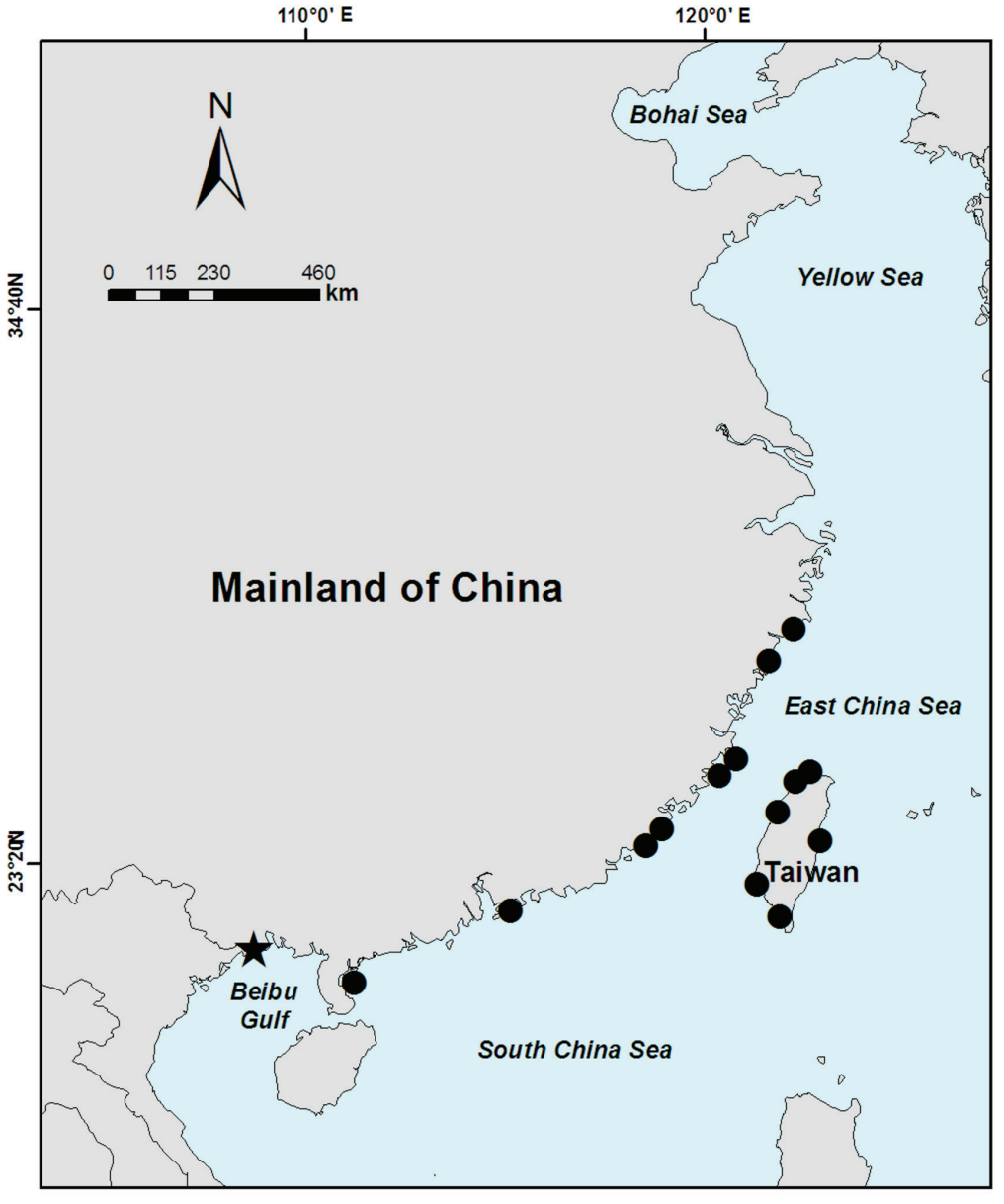

Figure 1. The present (star) and historical (circle) stranding locations of Omura's whales (*Balaenoptera omurai*) in Chinese waters

using *BioEdit* software. For species identification, significant alignments of the mtDNA sequences by the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi) were all referred to as baleen whales. The *cox1* sequence was submitted for sequence to BOLD Systems (Ratnasingham & Hebert, 2007). The *cytb* and D-loop sequences were submitted to the Web-based DNA Surveillance system (Ross et al., 2003) using the Witness for the Whale (*Mysticetes*, Version 4.3) reference database. For phylogenetic analyses, we selected all previously published sequences of the three different mtDNA markers (detailed in Supplementary Material S1). Maximum likelihood trees were built using the Kimura 2-parameter model by *MEGA*, Version 6.0, with 1,000 bootstraps

(Tamura et al., 2013). Molecular diversity parameters were estimated for the three mtDNA markers in all available *B. omurai* sequences in GenBank using *DnaSP*, Version 5.10.1 (Librado & Rozas, 2009).

The specimen (ID Code BBG01) was a stranded female juvenile. The carcass was a Code 3 with decomposition including a mild characteristic odor (Pugliares et al., 2007). Because of its small body size (4.70 m in length), when first attempting to identify this carcass, we excluded the blue (*Balaenoptera musculus*), fin (*B. physalus*), or sei whales (*B. borealis*). It also was not a minke whale (*B. acutorostrata* or *B. bonaerensis*) because of its flat and broad rostrum. A Bryde's whale population has been frequently sighted in this region (Chen et al., 2019), so the specimen was suspected to be a Bryde's whale. However, after careful morphological analysis, it was identified as an Omura's whale. Although part of the

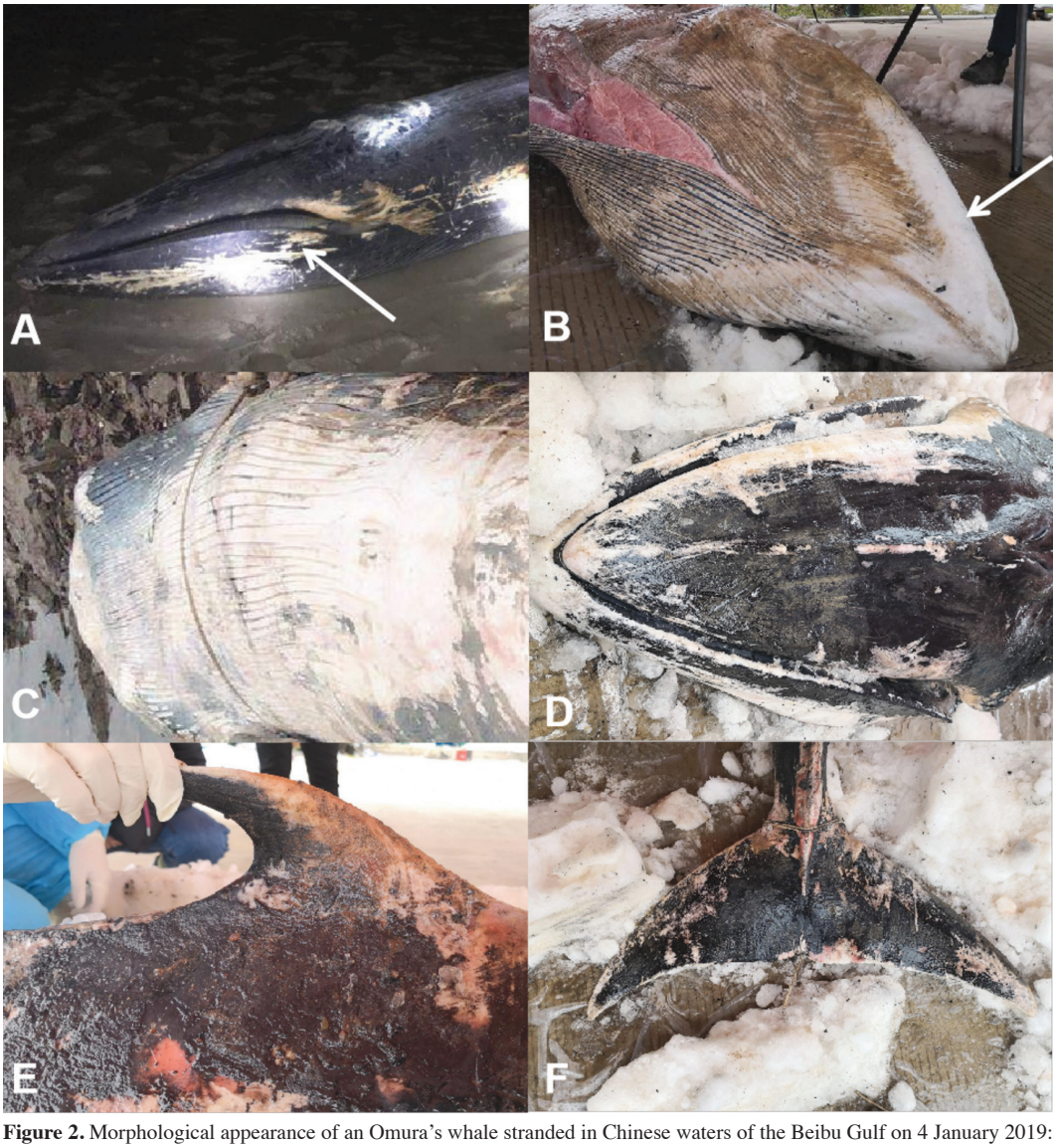

Figure 2. Morphological appearance of an Omura's whale stranded in Chinese waters of the Beibu Gulf on 4 January 2019: (A) Left side of the lower jaw with dark pigmentation; (B) right side of lower jaw, showing lightly pigmented, almost white throat, with light right mandible; (C) throat pleats reaching posterior beyond the navel; (D) dorsal view of head; (E) falcate dorsal fin; and (F) broad flukes.

external skin had fallen off, the left side of the throat was darkly pigmented (Figure 2A), while the right was mostly light colored (Figure 2B). There were 68 throat grooves that reached beyond the navel (Figure 2C). On the flat and broad rostrum, there was a prominent central ridge. A faint left lateral ridge was present on the left side of the rostrum; while on the right side, the ridge was short and faint (Figure 2D). The dorsal fin was strongly falcate with the tip pointed backwards, almost parallel to the back (Figure 2E). The fluke was broad with a relatively straight trailing edge (Figure 2F). There were approximately 223 plates hanging from the right side of the upper jaw and 216 plates on the left side. External morphometric data are presented in Table 1. All morphology data indicated this specimen to be an Omura's whale.

Though the morphology data indicated this specimen to be an Omura's whale, molecular data was required to 100% confirm the specimen to species. Three mitochondrial regions from genome DNA were successfully amplified and sequenced. After deleting the flanking and primer sequences, the 5' end of the *cox1* (700 bp; GenBank Accession Number MK676070) and *cytb* (640 bp; GenBank Accession Number MK676069), and the complete D-loop sequence (938 bp; GenBank Accession Number MK676071) were submitted to the GenBank. Sequence-significant alignments of the three mitochondrial regions by BLAST showed 99 to 100% sequence identity with *B. omurai* sequences but displayed 92 to 94% identity to the other closest neighboring species (Table 2). The use of the BOLD identification engine (Ratnasingham & Hebert, 2007) for *cox1* gene and the DNA Surveillance analysis for both the D-loop and the *cytb* gene also indicated that specimen BBG01 was a 100% match for *B. omurai* (Figure S1, detailed in Supplementary Material S2). The three ML phylogenetic trees showed the similar basic branching topology. Importantly, all three phylogenetic trees showed this specimen was clustered with all published *B. omurai*-related sequences in the same clade with 100% bootstrap (Figure 3). *B. omurai* was the basal branch in the clade that included *B. borealis*, *B. e. edeni*, and *B. e. brydei*. Due to the topology of the three gene trees being similar, we only present the D-loop tree, which includes long fragments and most of the sequences (Figure 3). All published sequences of the three different mtDNA markers (*cox1* gene, *cytb* gene, and D-loop) for Omura's whales were obtained from GenBank, and molecular diversity parameters were estimated (detailed in Supplementary Material S2).

For D-loop 402 bp sequence alignment analysis, the sequences were obtained from 29 Omura's whale specimens collected from their different

Table 1. Morphological measurements of the Omura's whale (*Balaenoptera omurai*) stranded on the Beibu Gulf, China, on 4 January 2019

Measuring parameters	
Sex	Female
Body length	470 cm
Length, tip of upper jaw to gape	87 cm
Length, tip of upper jaw to anterior insertion of flipper	165 cm
Length, tip of lower jaw to midpoint of spiracle	78 cm
Width, flipper (maximum)	14 cm
Length, flipper (anterior insertion to tip)	71 cm
Length, flipper (axilla to tip)	48 cm
Height, dorsal fin (fin tip to base)	12 cm
Length, dorsal fin base	24 cm
H/L (height/length), dorsal fin	0.5
Length, the longest throat grooves	243 cm
Length, flukes	36 cm
Width, flukes (tip to tip)	115 cm
Depth of notch between flukes	8 cm
Length, genital slit	38 cm
Maximal body-round	183 cm
Body-round at dorsal fin	53 cm
Number of throat grooves	68

Table 2. Results from GenBank BLAST searches of the three mitochondrial sequences obtained from the Beibu Gulf specimen

Mitochondrial gene		Closest matches with GenBank sequences	High similarity with other neighbor species
Marker	Length (bp)	Species (GenBank reference) and identities (percentages)	Species (GenBank reference) and identities (percentages)
Cox1	700	*B. omurai* (KM233839), (700/700), (100%) *B. omurai* (AB201256), (700/700), (100%) *B. omurai* (AB201257), (699/700), (99%)	*B. edeni* (AB201258), (654/700), (93%) *B. musculus* (MF409242), (653/699), (93%) *B. physalus* (KC572859), (653/700), (93%) *B. borealis* (MF409249), (652/699), (93%)
Cytb	640	*B. omurai* (AB201257), (640/640), (100%) *B. omurai* (AB201256), (639/640), (99%)	*B. borealis* (AP006470), (592/632), (94%) *B. edeni* (KJ586849), (587/638), (92%) *B. physalus* (KC572855), (586/638), (92%) *B. musculus* (MF409242),(587/640),(92%)
D-loop	938	*B. omurai* (KT757371), (937/938), (99%) *B. omurai* (AB201257), (937/938), (99%) *B. omurai* (AB201256), (936/938), (99%) *B. omurai* (AB116095), (936/938), (99%) *B. omurai* (AB116097), (934/938), (99%)	*B. edeni* (AB201258), (876/941), (93%) *B. borealis* (AP006470), (871/941), (93%) *B. brydei* (AP006469), (867/939), (92%) *B. musculus* (MF409242), (868/945), (92%)

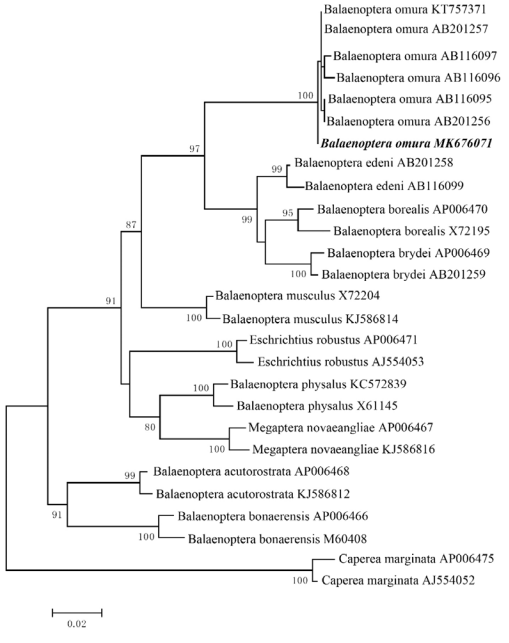

Figure 3. Maximum likelihood analysis of phylogenetic relationships of baleen whale species (including Balaenopteridae + Eschrichtiidae + Neobalaenidae) based on D-loop sequences. Bootstrap support values > 70% are shown adjacent to nodes. The bold-italic sequence "*Balaenoptera omura* MK676071" is the D-loop sequence of BBG01 obtained in this research.

distribution regions covering three oceans. The results indicated these sequences had only five haplotypes with five variable sites. Compared to the previous study (Xu et al., 2017), we found a new haplotype based on the two specimens from China. It indicated genetic diversity of Omura's whales was very low not only in one population, such as the Madagascar population, but for all global populations of Omura's whales.

Cerchio et al. (2015, 2019) suggested Omura's whales had a strong preference for coastal water distribution. When the specimen was found on the beach by fishermen, the carcass seemed to be fresh, and its condition could have been classified as Code 2 or close to Code 3 (Pugliares et al., 2007; Figure S2). Therefore, the carcass might not have been dead for a long time, possibly only 24 to 36 h. It was likely from this region and did not drift into the Beibu Gulf from the South China Sea or elsewhere. Our specimen is the first confirmed record of an Omura's whale found in the Beibu Gulf. There have been some recent reports of a Bryde's whale population frequently occurring in this region (Chen et al., 2019). Due to morphological similarity, *B. omurai*, *B. e. edeni*, and *B. e. brydei* are hard to characterize in the field. Therefore, to better understand the distribution and population of Omura's whales and Bryde's whales in this region, additional research and genetic analysis of biopsies from live animals should be considered.

Acknowledgments

This work was supported by grants from the Natural Science Foundation of China (No. 41506164); the Natural Science Foundation of Fujian Province, China (No. 2017J05062); the Fundamental Research Funds for Ministry of Natural Resources (No. 2018015); and the China-ASEAN Maritime Cooperation Fund (Nos. HX180701 and T190701). We are grateful to Su Bo of the Beilun River Estuary National Nature Reserve who helped us to collect the samples.

Literature Cited

Cerchio, S., Yamada, T. K., & Brownell, R. L., Jr. (2019). Global distribution of Omura's whales (*Balaenoptera omurai*) and assessment of range-wide threats. *Frontiers in Marine Science*, *6*(67). https://doi.org/10.3389/fmars.2019.00067

Cerchio, S., Andrianantenaina, B., Lindsay, A., Rekdahl, M., Andrianarivelo, N., & Rasoloarijao, T. (2015). Omura's whales (*Balaenoptera omurai*) off northwest Madagascar: Ecology, behaviour and conservation needs. *Royal Society Open Science*, *2*(10). https://doi.org/10.1098/rsos.150301

Chen, M., Huang, S-L., Wu, H. P., Mei, Z. G., Chen, Y. W., Zheng, J. S., Cheng, W., Deng, K., Yang, C., Chen, M., Wang, C-C., Lin, D., Peng, C., Xu, Y., & Wang, D. (2019). Occurrence of Bryde's whales, *Balaenoptera edeni*, in the northern Beibu Gulf, China. *Marine Mammal Science*, *35*(4), 1643-1652. https://doi.org/10.1111/mms.12607

Cypriano-Souza, A., Meirelles, A., Carvalho, V., & Bonatto, S. (2017). Rare or cryptic? The first report of an Omura's whale (*Balaenoptera omurai*) in the South Atlantic Ocean. *Marine Mammal Science*, *33*(1), 80-95. https://doi.org/10.1111/mms.12348

Geraci, J. R., & Lounsbury, V. J. (2005). *Marine mammals ashore: A field guide for strandings* (2nd ed.). National Aquarium in Baltimore.

Jefferson, T. A., Webber, M. A., & Pitman, R. L. (Eds.). (2007). *Marine mammals of the world: A comprehensive guide to their identification* (1st ed.). Academic Press.

Jung, J-L., Mullié, W. C., Van Waerebeek, K., Wagne, M. M., Bilal, A. S., El Abidine Ould Sidaty, Z., Toomey, L., Méheust, E., & Marret, F. (2015). Omura's whale off West Africa: Autochthonous population or inter-oceanic vagrant in the Atlantic Ocean? *Marine Biology Research*, *12*(1), 66-75. https://doi.org/10.1080/17451000.2015.1084424

Librado, P., & Rozas, J. (2009). *DnaSP* v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*(11), 1451-1452. https://doi.org/10.1093/bioinformatics/btp187

Pugliares, K. R., Bogomolni, A., Touhey, K. M., Herzig, S. M., Harry, C. T., & Moore, M. J. (2007). *Marine mammal necropsy: An introductory guide for stranding responders and field biologists*. Woods Hole Oceanographic Institution. https://doi.org/10.1575/1912/1823

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The barcode of life data systems. *Molecular Ecology Notes*, *73*(3), 355-364. www.barcodinglife.org; https://doi.org/10.1111/j.1471-8286.2007.01678.x

Ross, H. A., Lento, G. M., Dalebout, M. L., Goode, M., Ewing, G., McLaren, P., Rodrigo, A. G., Lavery, S., & Baker, C. S. (2003). DNA Surveillance: Web-based molecular identification of whales, dolphins, and porpoises. *Journal of Heredity*, *94*(2), 111-114. https://doi.org/10.1093/jhered/esg027

Sasaki, T., Nikaido, M., Wada, S., Yamada, T. K., Cao, Y., Hasegawa, M., & Okada, N. (2006). *Balaenoptera omurai* is a newly discovered baleen whale that represents an ancient evolutionary lineage. *Molecular Phylogenetic and Evolution*, *41*(1), 40-52. https://doi.org/10.1016/j.ympev.2006.03.032

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). *MEGA6*: *Molecular Evolutionary Genetics Analysis* version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725-2729. https://doi.org/10.1093/molbev/mst197

Viricel, A., & Rosel, P. E. (2012). Evaluating the utility of *cox1* for cetacean species identification. *Marine Mammal Science*, *28*(1), 37-62. https://doi.org/10.1111/j.1748-7692.2010.00460.x

Wada, S., Oishi, M., & Yamada, T. K. (2003). A newly discovered species of living baleen whale. *Nature*, *426*(6964), 278-281. https://doi.org/10.1038/nature02103

Xu, M., Wang, X., Miao, X., Wu, F., Ma, M., Tao, C., & Zhu, Q. (2017). A stranding record of Omura's whale (*Balaenoptera omurai* Yamada, 2003) in the Taiwan Strait, China. *Aquatic Mammals*, *43*(3), 289-298. https://doi.org/10.1578/AM.43.3.2017.289