



NOTE

Wildlife Science

First record of Ginkgo-toothed beaked whale (*Mesoplodon ginkgodens*) stranded in Korea

Sun Min KIM^{1) #}, Kyung Yeon EO^{2) #}, Jin Woo OH¹⁾, Young Ran LEE¹⁾,
Mi Hyun YOO²⁾, Shino KITAMURA³⁾, Yuko TAJIMA³⁾, Tadasu YAMADA³⁾,
Byung Yeob KIM⁴⁾, Hee Jeong YOUN^{1) *} and Junpei KIMURA^{1) *}

¹⁾College of Veterinary Medicine and Research Institute for Veterinary Medicine, Seoul National University, Seoul 08826, Korea

²⁾Conservation and Research Division, Seoul Zoo, Gwacheon 13829, Korea

³⁾National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba-shi, Ibaraki 305-0005, Japan

⁴⁾Department of Ocean Science, College of Ocean Science, Jeju National University, Jeju 63243, Korea

ABSTRACT. Two stranded whales were found dead on the coast of Jeju, South Korea. Based on the outer appearance and autopsy findings, one was determined to be an adult and the other a calf. The carcasses were dissected for species identification and pathological examination. A genetic analysis was performed, and the morphological characteristics of the skull observed. Then, 448 bp of the 5' half of the mitochondrial (mt) DNA control region and 413 bp of the mtDNA cytochrome *b* gene were sequenced. A BLAST search revealed that the whales were ginkgo-toothed beaked whales (*Mesoplodon ginkgodens*). Morphological comparison of the adult skull with the holotype specimen confirmed the result. This is the first record of a stranded ginkgo-toothed beaked whale in Korea.

KEY WORDS: ginkgo-toothed beaked whale, *Mesoplodon*, stranding

J. Vet. Med. Sci.

81(8): 1223–1228, 2019

doi: 10.1292/jvms.19-0001

Received: 9 January 2019

Accepted: 11 June 2019

Advanced Epub: 8 July 2019

The taxonomic status of beaked whales of the family Ziphiidae has been previously established; however, their habitat has not yet been precisely elucidated [3, 15, 24]. The existence of beaked whales near Korean territory is particularly uncertain, due to the lack of records in Korea [11]. According to reports from Korea from 1970 to 2009, the Ziphiidae species Baird's beaked whales (*Berardius bairdii*), Blainville's beaked whales (*Mesoplodon densirostris*), Stejneger's beaked whale (*M. stejnegeri*), and Cuvier's beaked whales (*Ziphius cavirostris*) were observed to have been stranded in Korea [19]. No beaked whale species were observed during the cetacean sighting surveys conducted within the Korean exclusive economic zone, which were undertaken 53 times between 2000 and 2010 [22]. There have been no cases of stranded, bycaught, or observed ginkgo-toothed beaked whales (*M. ginkgodens*) in Korea [10, 11, 19, 22]. Ziphiidae is the most cryptic whale family, and these whales are difficult to observe due to their long diving capacity and because their habitats remain poorly understood [3, 17].

Two stranded dead whales were found together on the coast (33°31'22.7"N, 126°56'58.80"E) of Jeju Island, South Korea on May 5, 2013. The bigger whale died 14 hr after being found alive. This indicated that the body had not drifted from the remote sea toward the Korean shore as a carcass. To enable individual identification, the whales were given the specimen numbers KJ1181 for the bigger whale and KJ1182 for the smaller whale. The carcasses were dissected and examined to obtain basic biological information on the distribution of *Mesoplodon* in Korea. The bodies were genetically and morphologically analyzed to identify their species.

For genetic identification, genomic DNA was extracted from the muscles of the whales using a G-spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Sungnam, Korea). The mitochondrial (mt) DNA markers, control region (CR), and cytochrome (*cyt*) *b* sequences that are commonly used to identify cetaceans were used. Two types of specific primers, M13-Dlp1.5-L (5'-TGAAAACGACGGCCAGTTCACCCAAAGCTGRARTTCTA-3') and Dlp5-H (5'-CCATCGWGATGTCTTATTTAAGRGGAA-3'), were used for amplification of the 448 base pairs (bp) mtDNA CR [6]. Two other specific primers, GLUDG-L (5'-TGACTTGAARAACCAAYCGTTG-3') and CB2-H (5'-CCCTCAGAATGATATTTGTCTCA-3'), were used for amplification of the 413 bp mtDNA *cyt b* gene [18]. The

*Correspondence to: Youn, H. J.: hjyoun@snu.ac.kr, Kimura, J.: kimura@snu.ac.kr

#These authors contributed equally to this work.

©2019 The Japanese Society of Veterinary Science



This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: <https://creativecommons.org/licenses/by-nc-nd/4.0/>)

polymerase chain reaction (PCR) was performed using an ABI 2720 Thermal Cycler. The amplified products were identified with ultraviolet (UV) ray (260 nm) after electrophoresis (2% agarose gel, 30 min) and dyed with EtBr. Two types of primers, Dlp4-H (5'-GCGGGWTRYTGRTTTCACG-3') [4] and Dlp10-L (5'-CCACAGTACTATGTCCGTATT-3'), were made for base sequence analysis of the mtDNA CR [1]. Three types of primers, CB1-L (5'-CCATCCAACATCTCAGCATGATGAAA-3') [18], CYBMF-L (5'-GAACTATAAGAACACTAATGACCAA-3'), and CYBMR-H (5'-TGATTCAGCCATAGTTAACGTCTCGAC-3'), were also made for base sequence analysis of the mtDNA *cyt b* gene [5]. Nucleotide sequence analysis was then performed. PCR products for nucleotide sequence analysis were dried after removal of remaining BigDye using a QIAGEN DyeEx 2.0 spin kit (Qiagen, Redwood City, CA, U.S.A.), and 20 μ l of HiDi Foramide was added and the reaction proceeded at 95°C for 2 min. After these processes were complete, the base sequences were assayed using an ABI PRISM 310 Genetic Analyzer. The nucleotide sequences were combined using CLC Main Workbench 5.0 (CLC Bio), and were then compared and analyzed using NCBI Nucleotide BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) [2]. We obtained partial sequences (448 bp) of mtDNA CR and partial sequences (413 bp) of mtDNA *cyt b* gene from the two specimens. There was no variable site between the two sequences in both CR and *cyt b*. From the BLAST search, the two sequences were related to *M. ginkgodens* with 99% match to accession no. KR534596 in both CR and *cyt b*. These mtDNA CR and *cyt b* haplotypes was new in the INSD (the International Nucleotide Sequence Databases). Two novel sequences, MgCR1 and MgCytb1, were deposited in INSD with accession nos. MH019963 and MH019964, respectively. Between the two haplotypes, MgCR1 and KR534596 had four variable sites in 448 bp and MgCytb1 and KR534596 had five variable sites in 413 bp, and these were all transitions. For the molecular phylogenetic analysis, one to four sequences of five *Mesoplodon* species (*M. ginkgodens*, *M. hotaula*, *M. stejnegeri*, *M. carlhubbsi*, and *M. densirostris*, with *Ziphius cavirostris* as an outgroup; see Appendix for accession nos. for each species sequences) were used to test the position of MgCR1 and MgCytb1 in the trees. Alignment of the CR and *cyt b* sequences was performed using the CLUSTAL X program [23], with visual inspection of the output based on the multiple alignment parameters in the program. A molecular phylogenetic tree was constructed using the Neighbor Joining algorithm [20] in MEGA version 7 [13], based on the Kimura 2-parameter model [12] for the CR (consensus length 434 bp) and *cyt b* (consensus length 384 bp) separately. Bootstrap values for both data sets were computed using 1,000 replicates [9]. All the *Mesoplodon* species included in the study were monophyletic in both trees (Fig. 1a and 1b). *M. ginkgodens* formed a sister clade to *M. hotaula* in both trees, with >60% statistical bootstrap support (Fig. 1a and 1b). MgCR1 and MgCytb1 clustered with other *M. ginkgodens* reference sequences in both trees (Fig. 1a and 1b). Thus, the stranded two whales were strongly identified as *M. ginkgodens* based on molecular genetic analyses.

The total body length of the adult specimen was 456.5 cm (Fig. 2). Numerous scars were observed on the body surface, particularly round scars that were likely caused by cookie-cutter shark attacks. The total body length of the female calf was 199.0 cm (Fig. 2). Both whales were suspected to be of the genus *Mesoplodon* (family Ziphiidae) because of their outer appearance. Their appearance included a pronounced rostrum that blends into a high forehead without a break, a v-shaped throat groove, a crescent-shaped blowhole, flippers that are relatively small in comparison to entire body size, a small triangular dorsal fin that is placed far back on the body, and the absence of notch at the middle of caudal fin.

The whales were identified as being a sexually and physically matured female adult and a female calf based on observation of their external genital organs, mammary slits, and the absence of tusks. Beaked whales have reduced teeth: males have one pair of teeth and females have only one pair of vestigial teeth [15]. These individuals are thought to be a dam and her calf, based on evidence

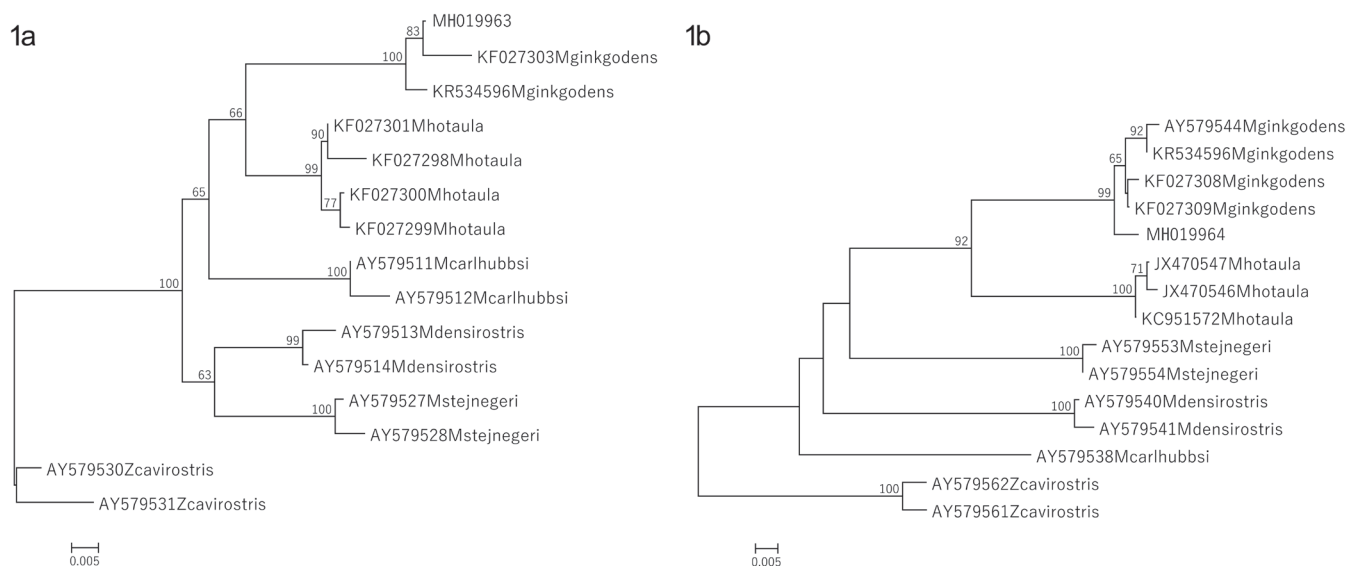


Fig. 1. Neighbor joining phylogenetic tree of 15 taxa from six species in the Ziphiidae family based on (a) mtDNA CR sequences (434 bp) and (b) cytochrome *b* sequences (384 bp). Bootstrap values (1,000 replicates) greater than 60% are shown on the nodes of branches. All taxa are included with accession numbers and scientific names. Abbreviations for genera: M, *Mesoplodon*; Z, *Ziphius*.

including the stranding situation, their body sizes, the highly developed mammary glands of the adult whale (Fig. 3a and 3b) that had plenty of milk in the right gland (Fig. 3c), the stomach contents of the smaller whale, which consisted only of milk (Fig. 3d), and the results of the genetic analysis did not contradict this hypothesis because the maternal mtDNA haplotype was identical for both individuals.

The adult skull specimen was prepared and subsequently compared with the holotype specimen of *M. ginkgodens* stored in the National Museum of Nature and Science, Japan (NSMT-M8744, Male). There are several variations in skull structure among species of the genus *Mesoplodon* [16]. Among those variations, five distinguishing characteristics of the skull of the holotype specimen of *M. ginkgodens* were compared with the skull of KJ1181. Firstly, the angle between the frontopremaxillary suture and the frontonasal suture was compared. In the dorsal view of the vertex of the skull where the right frontal bone meets the right nasal bone, the suture between the frontal bone and premaxillary bone formed a sharp angle with the frontonasal suture in the holotype of *M. ginkgodens* (Fig. 4a). In the same manner, the two sutures also made an acute angle in the skull of KJ1181 (Fig. 4b). Secondly, in the dorsal view of the vertex of the skull, the right nasal bone and the right premaxilla ended similarly in the holotype of *M. ginkgodens* (Fig. 4a). This was also observed in the skull of KJ1181 (Fig. 4b). Thirdly, the right antorbital notch made an acute angle of less than 90 degrees in the holotype of *M. ginkgodens* (Fig. 4c). Similarly, the angle of the right antorbital notch was sharp in KJ1181 (Fig. 4d). The features of a specimen of *M. stejnegeri* stored in the American Museum of Natural History (AMNH No. 143829) [16] were also used to compare the structural differences between *M. ginkgodens* and *M. stejnegeri* skulls. In the holotype of *M. ginkgodens*, the proximal butt-end of the two premaxillae were ascended and truncated dorsally. These dorsally projected ends were relatively flat surfaces on a plane in the lateral view that was approximately parallel to the long axis of the beak (Fig. 5a). This was also observed in the skull of KJ1181 (Fig. 5b). In some species of *Mesoplodon*, such as *M. stejnegeri*, there is a crest formed due to the change direction of the butt-end of the premaxillary [16]. This crest, called a premaxillary brow



Fig. 2. Stranded dead whales suspected to be of the genus *Mesoplodon*. (a) An adult whale (KJ1181). (b) A juvenile (KJ1182) whale.

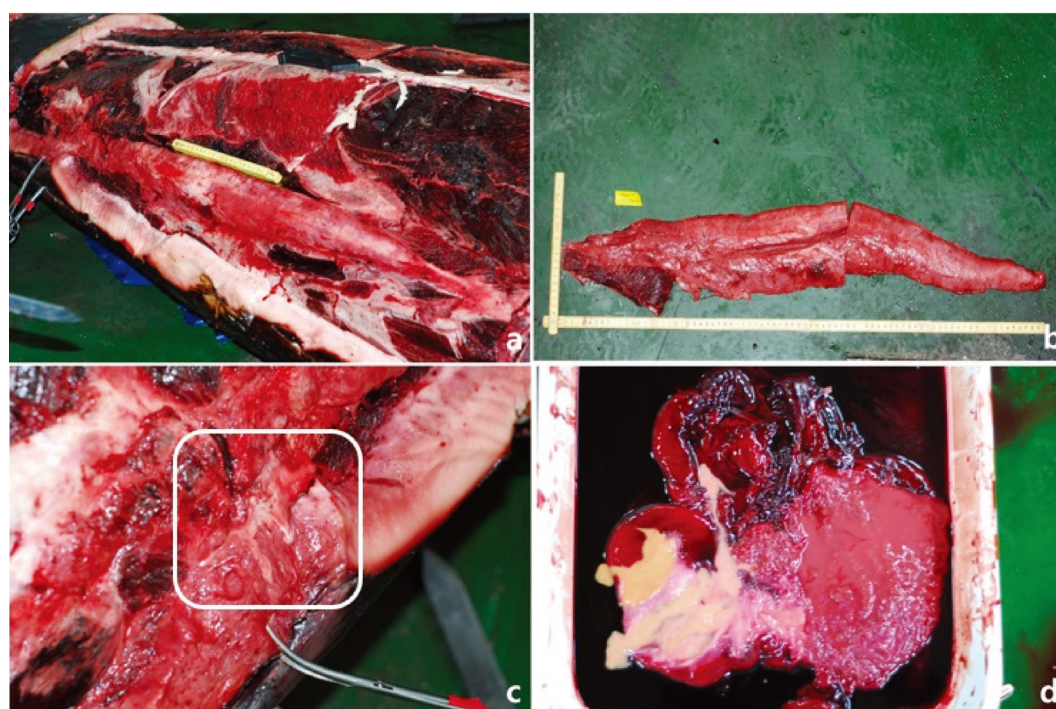


Fig. 3. Highly developed mammary gland of adult specimen (a, b). Presence of milk inside the right mammary gland of adult specimen (c). Stomach contents of calf specimen, which only included milk (d).

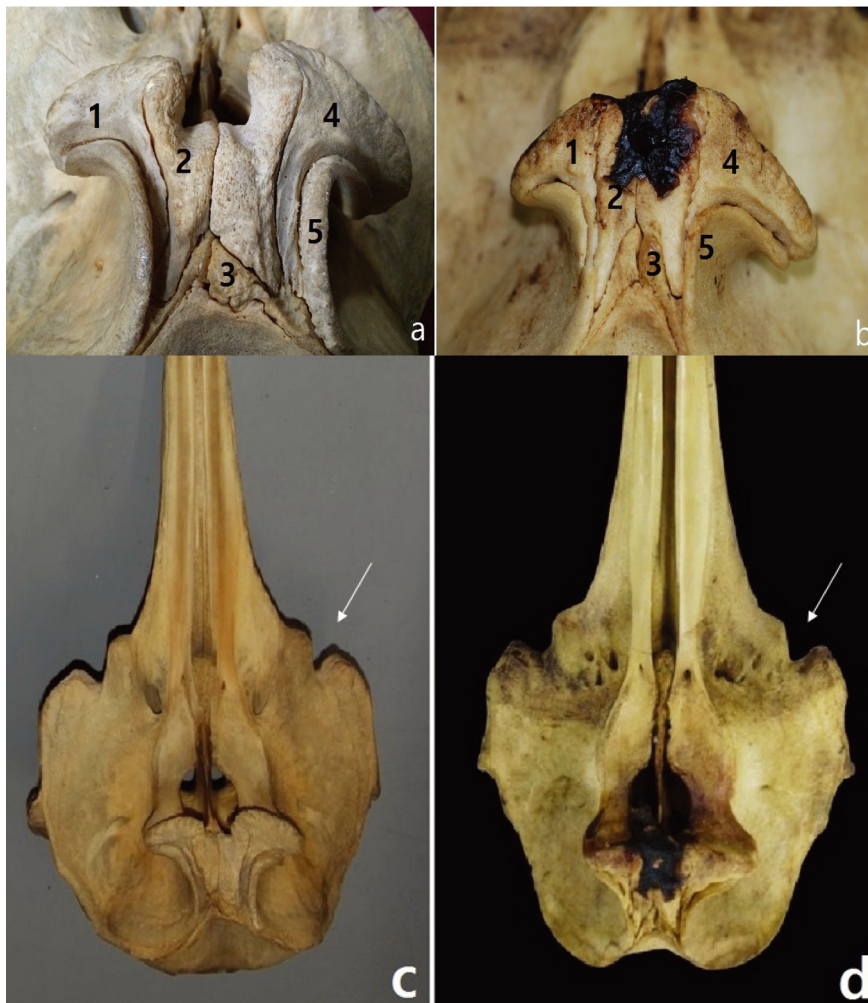


Fig. 4. Dorsal views of skulls of the holotype of *Mesoplodon ginkgodens* (NSMT-M8744) (a, c) and KJ1181 (b, d). Figures a and b are magnified from c and d, respectively. 1: Left premaxillary bone; 2: left nasal bone; 3: right frontal bone; 4: right premaxillary bone; 5: right maxilla bone. c, d: Arrows indicate antiorbital notch.



Fig. 5. Morphological comparison of the holotype of *Mesoplodon ginkgodens* (NSMT-M 8744) (a, c) and KJ1181 (b, d). a, b: Lateral view. Upper arrow: ascended and dorsally truncated proximal butt-end of two premaxillae. Lower arrow: absence of premaxillary brow crease due to dorsally projected ends. c, d: Frontal view. Arrows indicate absence of premaxillary brow crease.

crease, was not present in either the holotype of *M. ginkgodens* or KJ1181 (Fig. 5a and 5b). The absence of the premaxillary brow crease was also observed in the front view of the skull of *M. ginkgodens* and KJ1181 (Fig. 5c and 5d). In addition to the genetic analysis, these morphological findings confirmed that the adult specimen belonged to *M. ginkgodens*. These two bodies were first identified as beaked whales based on their outer appearance and previous references, and the identification was then confirmed by morphological comparison of the adult skull specimen and by genetic analysis.

Mesoplodon species are identified based on features of the skull, such length of the rostrum, as well as the placement, shape, and size of the teeth, particularly in adult males. However, specimens have often been misidentified due to anatomical similarities between species, and molecular genetic analyses have become increasingly important for the identification of individual specimens [15]. Nowadays, identification by genetic testing is considered to be the most reliable method.

Based on the results of genetic and anatomical analyses, the stranded whales were identified as ginkgo-toothed beaked whales (*M. ginkgodens*). No pathological lesions that could be related to their deaths were observed.

This is the first record of a stranded ginkgo-toothed beaked whale in Korea. The ginkgo-toothed beaked whale has not been previously considered to be an inhabitant of the seas near Korea [25]. This should be reconsidered based on the results of this study. One previous paper described a stranded ginkgo-toothed beaked whale in China in 1980 [21]. The identification was made only on the basis of body length and descriptions of the external features, due to the unavailability of genetic analysis at that time. Variations in each part of the body, including total body length, that can occur within the same species were not considered in that paper. Additionally, an analysis of the skull, which is essential for morphological identification, was not conducted. Thus, the result of the identification cannot be considered to be reliable. Re-identification of this specimen using genetic analysis of bone specimens is required. If this specimen is determined not to be *M. ginkgodens* on reanalysis, the current specimens from Jeju Island are the first accurate records of *M. ginkgodens* not only in the Korean Sea, but also in the whole Yellow Sea. Further research will be required to obtain more information about whales of this genus, including their habitat, abundance, and ecological characteristics.

ACKNOWLEDGMENT. This research was supported by the 2017 Scientific Promotion Program funded by Jeju National University and the Research Institute for Veterinary Science, Seoul National University.

REFERENCES

1. Baker, C. S., Perry, A., Bannister, J. L., Weinrich, M. T., Abernethy, R. B., Calambokidis, J., Lien, J., Lambertsen, R. H., Ramírez, J. U., Vasquez, O., Clapham, P. J., Alling, A., O'Brien, S. J. and Palumbi, S. R. 1993. Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proc. Natl. Acad. Sci. U.S.A.* **90**: 8239–8243. [Medline] [CrossRef]
2. Baker, C. S., Dalebout, M. L., Lavery, S. and Ross, H. A. 2003. www. DNA-surveillance: applied molecular taxonomy for species conservation and discovery. *Trends Ecol. Evol.* **18**: 271–272. [CrossRef]
3. Carpenter, K. E. and Niem, V. H. 2001. FAO species identification guide for fishery purposes. p.4022. In: The living marine resources of the Western Central Pacific, Vol. 6: Bony fishes, part 4(Labridae to Latimeriidae), estuarine crocodiles, sea turtles, sea snakes and marine mammals. Rome: FAO.
4. Dalebout, M. L., Baker, C. S., Mead, J. G., Cockcroft, V. G. and Yamada, T. K. 2004. A comprehensive and validated molecular taxonomy of beaked whales, family Ziphiidae. *J. Hered.* **95**: 459–473. [Medline] [CrossRef]
5. Dalebout, M. L., Mead, J. G., Baker, C. S., Baker, A. N. and Van Helden, A. L. 2002. A new species of beaked whale *Mesoplodon perrini* sp. n. (Cetacea: Ziphiidae) discovered through phylogenetic analyses of mitochondrial DNA sequences. *Mar. Mamm. Sci.* **18**: 577–608. [CrossRef]
6. Dalebout, M. L., Helden, A. V., van Waerebeek, K. and Baker, C. S. 1998. Molecular genetic identification of southern hemisphere beaked whales (Cetacea: Ziphiidae). *Mol. Ecol.* **7**: 687–694. [Medline] [CrossRef]
7. Dalebout, M. L., Baker, C. S., Steel, D., Robertson, K. M., Chivers, S. J., Perrin, W. F., Mead, J. G., Grace, R. V. and Schofield, T. D. Jr. 2007. A divergent mtDNA lineage among *Mesoplodon* beaked whales: Molecular evidence for a new whale in the Tropical Pacific? *Mar. Mamm. Sci.* **23**: 954–966. [CrossRef]
8. Dalebout, M. L., Baker, C. S., Steel, D., Thompson, K., Robertson, K. M., Chivers, S. J., Perrin, W. F., Goonatilake, M., Charles Anderson, R., Mead, J. G., Potter, C. W., Thompson, L., Jupiter, D. and Yamada, T. K. 2014. Resurrection of *Mesoplodon hotaula* Deraniyagala 1963: A new species of beaked whale in the tropical Indo-Pacific. *Mar. Mamm. Sci.* **30**: 1081–1108. [CrossRef]
9. Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**: 783–791. [Medline] [CrossRef]
10. Kim, D. N., Sohn, H., An, Y. R., Park, K. J., Kim, H. W., Ahn, S. E. and An, D. H. 2013. Status of the Cetacean Bycatch near Korean Waters. *Korean J. Fisheries Aquat. Sci.* **46**: 892–900. [CrossRef]
11. Kim, Z. G., Choi, S. G., An, Y. R., Kim, H. W. and Park, K. J. 2009. Whales, Dolphins and Porpoises off Korean Peninsula, 2nd ed. National Fisheries Research and Development Institute.
12. Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**: 111–120. [Medline] [CrossRef]
13. Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**: 1870–1874. [Medline] [CrossRef]
14. Lacsamana, J. K. M., Ventolero, M. F. H., Blatchley, D. and Santos, M. D. 2015. First record of a rare beaked whale *Mesoplodon hotaula* in the Philippines. *Mar. Biodivers. Rec.* **8**: e77.
15. Mead, J. G. 2009. Beak Whales, Overview Ziphiidae. pp. 94–97. In: Encyclopedia of Marine Mammals, 2nd ed. (Perrin, W. F., Wursig, B. and Thewissen J. G. M.), Elsevier, Academic Press.
16. Moore, J. C. 1963. Recognizing certain species of beaked whales of the Pacific Ocean. *Am. Midl. Nat.* **70**: 396–428. [CrossRef]
17. Perrin, W. F. and Geraci, J. R. 2009. Stranding. pp. 1118–1123. In: Encyclopedia of Marine Mammals, 2nd ed. (Perrin, W. F., Wursig, B. and Thewissen J. G. M.), Elsevier, Academic Press.
18. Palumbi, S. R. 1996. Nucleic acid II: the polymerase chain reaction. pp. 205–247. In: Molecular systematics (Hillis D.M., Moritz C. and Mable

- B.K. eds.), Sunderland, Sinauer Associates.
19. Robards, M. D. and Reeves, R. R. 2011. The global extent and character of marine mammal consumption by humans: 1970–2009. *Biol. Conserv.* **144**: 2770–2786. [[CrossRef](#)]
 20. Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425. [[Medline](#)]
 21. Shi, Y. and Wang, X. 1984. On the ginkgo-toothed beaked whale found in the northern part of the Yellow Sea. *T. Liaoning Zool. Soc.* **5**: 111–116.
 22. Sohn, H., Park, K. J., An, Y. R., Choi, S. G., Kim, Z. G., Kim, H. W., An, D. H., Lee, Y. R. and Park, T. G. 2012. Distribution of whales and dolphins in Korean waters based on a sighting survey from 2000 to 2010. *Korean J. Fish. Aquat. Sci.* **45**: 486–492.
 23. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**: 4876–4882. [[Medline](#)] [[CrossRef](#)]
 24. The IUCN Red List of Threatened Species Version 2016–1. Available via <http://www.iucnredlist.org/> [accessed on July 24, 2016].
 25. Yamada, T. K. 2009. Cetacea. pp. 313–395. In: *The Wild Mammals of Japan* (Ohdachi, S. D., Ishibashi, Y., Iwasa, M. A. and Saitoh, T.), Shoukadoh book sellers and the mammalogical society of Japan.
 26. Yao, C. J., Chen, C. H. and Hsiao, C. D. 2016. The complete mitogenome of Ginkgo-toothed beaked whale (*Mesoplodon ginkgodens*) (Chordata: Ziphiidae). *Mitochondrial DNA A. DNA Mapp. Seq. Anal.* **27**: 2846–2847. [[Medline](#)]

Appendix

Appendix Table 1. List of Accession Numbers in GenBank/DDBJ used in the molecular phylogenetic analyses

Species name	CR	cyt <i>b</i>	Reference
<i>Mesoplodon ginkgodens</i>	KR534596	KR534596	[26]
	KF027303		[8]
		AY579544	[4]
		KF027308	[8]
		KF027309	[8]
<i>M. hotaula</i>	KF027298		[8]
	KF027299		[8]
	KF027300		[8]
	KF027301		[8]
		KC951572	[14]
<i>M. carlhubbsi</i>		JX470546	[7]
		JX470547	[7]
	AY579511		[4]
	AY579512		[4]
		AY579538	[4]
<i>M. densirostris</i>	AY579513		[4]
	AY579514		[4]
		AY579540	[4]
<i>M. stejnegeri</i>		AY579541	[4]
	AY579527		[4]
	AY579528		[4]
		AY579553	[4]
		AY579554	[4]
<i>Z. cavirostris</i>	AY579530		[4]
	AY579531		[4]
		AY579561	[4]
	AY579562	[4]	