

First Record of the Omura's Whale (*Balaenoptera omurai*) in Korean Waters

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ABSTRACT

To confirm the genetic identification and phylogenetic relationships of unidentified 6 baleen whales by-caught from 2002 to 2016, a partial sequence of approximately 500 base pair (bp) of the mitochondrial DNA (mtDNA) control region was analyzed and compared to published sequence from Genbank. Our results indicated that the two individuals among 6 specimens are clustered with Omura's whale clade through phylogenetic analysis, which had only a single haplotype. Omura's whale was reclassified as a new species in 2003 and they had not been previously reported in Korean waters. This study firstly revealed existence of Omura's whale in Korean waters by molecular analysis based on mtDNA control region.

Keywords: first record, Omura's whale, mtDNA control region, phylogenetic tree, Korea

INTRODUCTION

Omura's whales, *Balaenoptera omurai*, are one of the least known cetacean species because they recently described as new species (Wada et al., 2003). This species was first considered close to the Bryde's whale, *Balaenoptera brydei*. However they most closely resemble that of the fin whale, *Balaenoptera physalus*, with an asymmetrical lower jaw (Jafferson et al., 2008). Wada and Numachi (1991) introduced as "small-form Bryde's whale." "Pygmy" or "dwarf" form of Bryde's whale are also used by several researchers (Yoshida and Kato, 1999; LeDuc and Dizon, 2002). Omura's whales are currently being recognized distinct species based on concordance in genetics, osteology, and external morphology (Wada et al., 2003; Sasaki et al., 2006).

Although only limited information of the species is present, some sighting, bycatch, catch data show they are appeared to tropical and warm areas of Indian and western Pacific oceans (Yamada, 2009; Cerchio et al., 2015). They have been frequently reported in the East China Sea and coastal area of southern Japan where is located near Korean waters (Wada et al., 2003; Xu et al., 2017). Especially, the holotype of the species was collected from Tsunoshima Island in Japanese side of the Korea Strait.

In this study, we figured out the existence of the Omura's whale in Korean waters by using DNA analysis. Genetic identification and phylogenetic relationships of specimens are reconstructed using mitochondrial DNA (mtDNA) control-region sequence data. Six large baleen whales by-caught in fishing gear were collected in Korean waters between 2002 and 2016 (Fig. 1, Appendix 1). All specimens were preserved frozen (-20°C) and deposited in the Cetacean Research Institute (CRI) of National Institute of Fisheries Science. Genomic DNA was extracted from muscles or skins using the DNeasy Blood & Tissue Kit (Qiagen, USA) following manufacturer's instructions. The mtDNA control region was amplified by the polymerase chain reaction (PCR) using MTF-F, 5'-CCTCCCTAAGACTCAAGGAAG-3' primer (Arnason et al., 1993) and Dlp-5R, 5'-CCATCGWGATGTC TTATTTAAGRGGAA-3' primer (Baker et al., 1993). PCR products were sequenced in an ABI 3130xl automated DNA Sequencer using ABI's Big Dye Terminator kit (Applied Biosystems, Foster City, CA, USA). All sequences were edited in SeqMan 14 (DNASTar Inc., Madison, WI, USA) and truncated in order to have the same length determined in this study. All specimens in this study are identified by mtDNA control region sequence and compared with published sequence in Genbank. Then, all haplotype sequences for six

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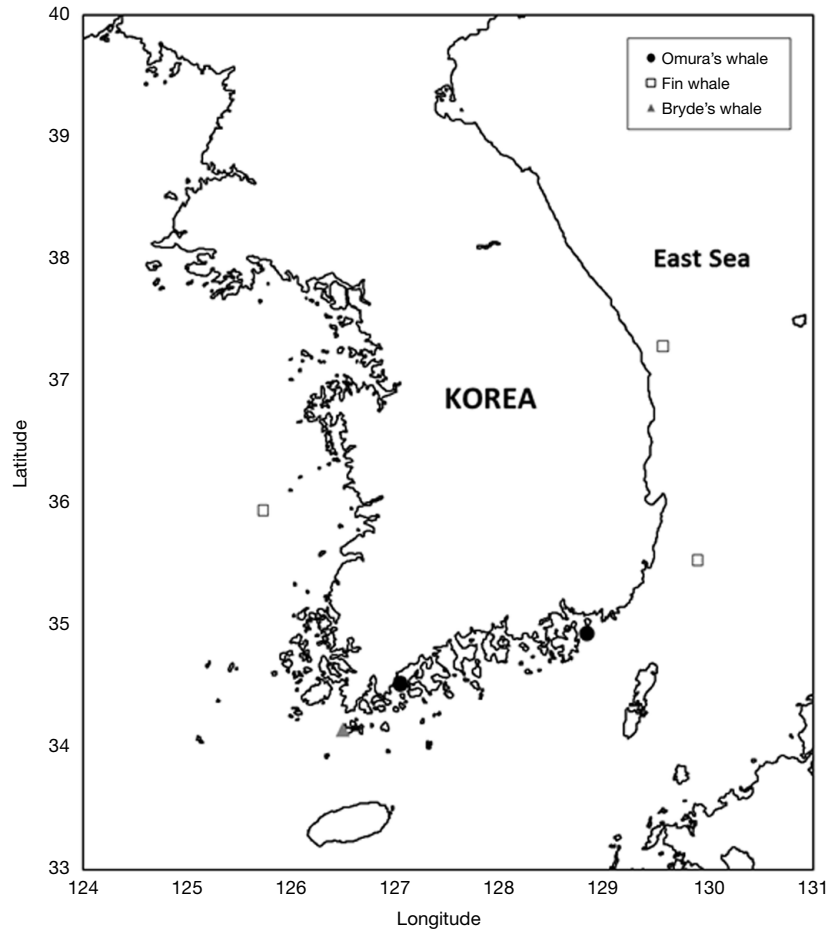


Fig. 1. Bycatch location of specimens in this study from 2002 to 2016.

specimens used in this study were deposited in Genbank under accession number (MG877682-877686 and MG877688).

Phylogenetic relationships among mtDNA control region sequences were reconstructed using neighbor-joining (NJ), maximum parsimony (MP), and Bayesian inference (BI) methods. Under both the Akaike information criterion and Bayesian information criterion criteria for the model, the HKY + G model was selected using jModeltest v. 2. 1. 1 to infer the best-fitting model. NJ tree was built using the Kimura 2 parameter distance by MEGA 7 (Kumar et al., 2016) with 1,000 bootstraps. The MP tree was conducted using PAUP v. 4 (Swofford, 1999). Support for tree nodes was assessed using 1,000 bootstrap replicates. The BI tree was estimated using MrBayes v. 3. 2.6. Four simultaneous Markov chain Monte Carlo chains were run for 100,000 generations and trees were sampled every 100 generations.

Species identification and phylogenetic analyses

A 508 base-pair (bp) fragment of the mtDNA control region

was analyzed for 6 specimens. In order to confirm the genetic identity of specimens, additional 11 reference sequence of three other baleen whale species obtained from Genbank and *Balaena mysticetus* was used as an outgroup (Table 1). The phylogenetic trees obtained for mtDNA control region with the three phylogenetic inference methods used (ML, MP, and BI) all resulted in similar branching topologies (Fig. 2).

Two of six sequences analyzed in this study were matched to the previously published sequences from *B. omurai*, which was only a single haplotype. In phylogenetic tree, Omura's whale in Korean waters was grouped with published Omura's whale clade as a monophyletic group with 99–100% value. The haplotype was shared between Omura's whale off Korea and those from Japan.

The remaining four specimens except for Omura's whale were identified as fin whale, *B. physalus* and Bryde's whale, *B. brydei*. Two haplotypes were found among three fin whales and shared with published sequence. The haplotype of Bryde's whale had a unique sequence that had not been

Table 1. List of taxa and Genbank accession numbers for mtDNA sequence used in this study

Family	Scientific name	Common name	Reg. No.	GenBank accession No.	Reference
Balaenopteridae	<i>Balaenoptera omurai</i>	Omura's whale	CRI002081	MG877682	This study
	<i>Balaenoptera omurai</i>	Omura's whale	CRI002128	MG877683	This study
	<i>Balaenoptera omurai</i>	Omura's whale	–	AB201256	Sasaki et al. (2006)
	<i>Balaenoptera omurai</i>	Omura's whale	–	AB201257	Sasaki et al. (2006)
	<i>Balaenoptera omurai</i>	Omura's whale	–	KT757371	Ottewell et al. (2016)
	<i>Balaenoptera physalus</i>	Fin whale	CRI002060	MG877685	This study
	<i>Balaenoptera physalus</i>	Fin whale	CRI004141	MG877686	This study
	<i>Balaenoptera physalus</i>	Fin whale	CRI006918	MG877688	This study
	<i>Balaenoptera physalus</i>	Fin whale	–	KC572807	Archer et al. (2013)
	<i>Balaenoptera physalus</i>	Fin whale	–	KC572766	Archer et al. (2013)
	<i>Balaenoptera physalus</i>	Fin whale	–	KC572746	Archer et al. (2013)
	<i>Balaenoptera edeni</i>	Bryde's whale	–	AB116099	Wada et al. (2003)
	<i>Balaenoptera edeni</i>	Bryde's whale	–	EF057443	Jayasankar et al. (2009)
	<i>Balaenoptera brydei</i>	Bryde's whale	CRI002136	MG877684	This study
	<i>Balaenoptera brydei</i>	Bryde's whale	–	AP006469	Sasaki et al. (2005)
<i>Balaenoptera brydei</i>	Bryde's whale	–	AB201259	Sasaki et al. (2006)	
Balaenidae	<i>Balaena mysticetus</i>	Bowhead whale	–	KT766159	Unpublished
	<i>Balaena mysticetus</i>	Bowhead whale	–	AP006472	Sasaki et al. (2005)

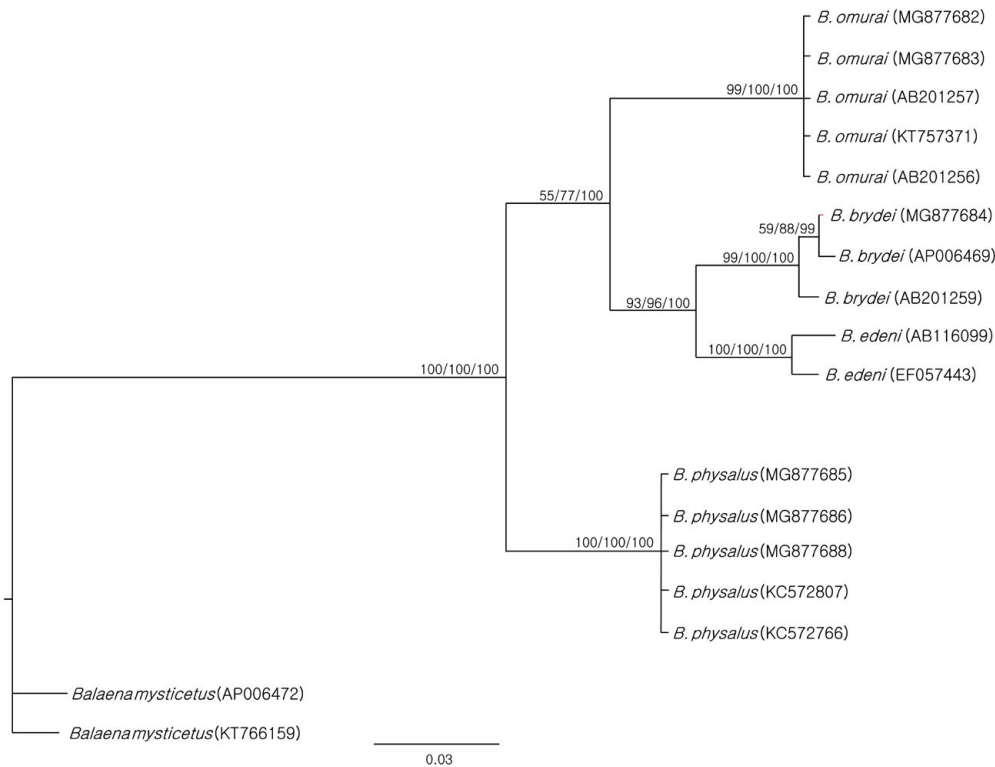


Fig. 2. Phylogenetic tree showed based on the neighbor-joining (NJ), maximum parsimony (MP), and Bayesian inference (BI) methods of phylogenetic inference for mtDNA control region sequences. Numbers above on branches correspond to NJ, MP bootstrap values, and BI posterior probabilities, respectively.

previously reported. Optimal trees indicated that fin whale and Bryde's whale were clustered with published fin and Bryde's whale clade, with the highest values, respectively.

SYSTEMATIC ACCOUNTS

Infraorder *Cetacea* Brisson, 1762

Superfamily *Mysticeti* Flower, 1864
 Family *Balaenopteridae* Gray, 1864
 Genus *Balaenoptera* Lacépède, 1804

***Balaenoptera omurai* Wada, Oishi & Yamada, 2003**

Remarks. We confirmed the existence of Omura's whale in Korean waters by molecular analysis based on mtDNA control region. The phylogenetic relationships for mtDNA control region were analyzed by NJ, ML and BI method. Our results indicate that Omura's whale is a sister taxon to the monophyletic group with *B. brydei* and *B. edeni*. The results of phylogenetic tree in this study was similar to previously documented (Wada et al., 2003; Sasaki et al., 2006; Cerchio et al., 2015). Omura's whale haplotypes in Korean waters correspond to those from Japan as one clade (Fig. 2). Although we didn't confirm the external appearance of the whales, species identification of Omura's whale is sufficient to distinguish with other baleen whales using molecular analysis. However, there is only limited morphological measurement data of the species as relatively newly described. Therefore, additional studies of external morphology, skeletal morphology and molecular biology are needed to confirm the taxonomic characteristics of the Omura's whale in Korea.

The Omura's whale was recognized as a new baleen whale species in 2003 (Wada et al., 2003). After that, current knowledge of the distribution of the species is rapidly expanding with new discoveries (Cerchio and Yamada, 2018). They mostly distribute throughout coastal tropical and subtropical waters of Indo-Pacific region (Yamada, 2009; Cerchio et al., 2015). These two bycaught Omura's whale found in southern coast of the Korean peninsula may indicate the northern limit of the range in the Pacific Ocean.

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Appendix 1. Brief information for 6 specimens of baleen whale analyzed in this study

No.	Reg. No.	Common name	Bycatch date	Bycatch area	Sex	Body length (cm)
1	CRI002081	Omura's whale	10 Jan 2004	Geoje	F	630
2	CRI002128	Omura's whale	17 Dec 2006	Goheung	M	640
3	CRI002060	Fin whale	10 Nov 2002	Samcheock	M	–
4	CRI004141	Fin whale	16 May 2014	Gunsan	–	1440
5	CRI006918	Fin whale	25 Sep 2016	Pohang	M	–
6	CRI002136	Bryde's whale	16 Dec 2007	Wando	M	–