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Molecular species identification of whale meat in South Korean markets

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ABSTRACT. Commercial whaling has been banned since the moratorium of the International Whaling Commission in 1986. However, domestic sale of cetaceans that are caught as bycatch is still allowed in South Korea. Although whale meat is not very popular in South Korea, it is consumed in certain areas. To identify the species composition of whale meat in South Korean markets, we collected 54 samples that were sold as minke whale meat at restaurants and markets of four cities: Seoul, Ulsan, Busan, and Pohang. Of the 54 whale meat samples, 51 were successfully identified using the partial mitochondrial cytochrome *b* gene (806–1,140 bp) amplified for species identification. Molecular species identification revealed three species among the samples: minke whale (52%), common dolphin (22%), and narrow-ridged finless porpoise (26%). We also gathered data for cetacean bycatch in South Korea. In total, 19 species were confirmed to be incidentally caught in South Korea over the past 13 years, and minke whale (13%), common dolphin (30%), and narrow-ridged finless porpoise (45%) were recorded as the most frequently caught species. The sale of "fake" minke meat in the markets may

have been due to a lack of availability of minke meat, as well as the difference in market values of meat from baleen and toothed whales. These factors that lead to the sale of “fake” minke meat are thought to be contributing causes of the illegal, unregulated, and unreported exploitation of small cetaceans. To prevent such exploitation, it is necessary to continuously monitor whale meat using molecular species identification. Our study improves the understanding of which species of whales (meat) are sold in South Korea and proposes a management policy for the conservation of small cetaceans in South Korea.

Key words: Whaling; Narrow-ridged finless porpoise; Bycatch; Whale meat

INTRODUCTION

Since the whaling moratorium of the International Whaling Commission (IWC) in 1986, the major threats to marine mammal species and populations are anthropogenic factors, such as pollution, habitat destruction, and bycatch (Lewison et al., 2004). Bycatch, one of the greatest threats to smaller-sized marine mammal populations, is the inadvertent capture and killing of non-target species; it occurs when cetaceans are unable to avoid capture or are entangled in fishing gear. To estimate the actual amount of bycatch and thus develop efficient conservation management policies, the IWC receives annual reports on bycatch from its signatories. Although the report is required to be as accurate as possible, events of illegal, unregulated, and unreported (IUU) exploitation have been found to be omitted from annual bycatch reports, thus distorting the true extent of bycatch.

The Republic of Korea (South Korea) is a member of the IWC. Direct fishing of cetaceans is prohibited in South Korea; however, cetaceans that are incidentally captured in fisheries bycatch can be sold in markets. A fisherman who unintentionally catches cetaceans has a duty to report their bycatch to the maritime police of South Korea, who then investigate whether the fisherman poached the cetacean. Only whales that are shown to have died by stranding or in bycatch are legally allowed to be traded in the market and consumed as whale bushmeat in South Korea. However, genetic monitoring has shown that IUU individuals are sold in markets as whale meat in cities of South Korea (Baker et al., 2006).

Of the 65 countries that consume whale meat, South Korea is the fifth largest consumer (Costello and Baker, 2011). In South Korea, whale meat is not very popular, but it is regarded as a local delicacy mainly in the southeastern coastal cities of Ulsan, Busan, Gyeongju, and Pohang (Tatar, 2014). Minke whale meat is the best-selling owing to its preferred flavor. More than 400 minke whale individuals are thought to be consumed annually in South Korea (Han, 2012). However, this number is presumed to be an underestimation because of the high probability of illegally caught and distributed meat in the market. In addition, Baker et al. (2006) reported that small-toothed whales, such as the narrow-ridged finless porpoise, are also consumed as whale meat; thus indicating the existence of IUU individuals. Given this situation, more systematic research is urgently needed to develop more effective management methods and conservation actions.

It is usually difficult to identify the original species of a processed wildlife product based on its external characteristics. Molecular species identification is a useful tool that can resolve this challenge. In this study, molecular species identification was used to identify the species composition of whale meat in the restaurants and markets of four South Korean cities: Seoul, Ulsan, Busan, and Pohang.

MATERIAL AND METHODS

Sampling and DNA extraction

From July 2012 to June 2015, whale meat (skin, blubber, muscle tissue, and internal organs) sold as minke whale was purchased from restaurants and markets in four cities in South Korea: Seoul, Ulsan, Busan, and Pohang. Each sample of whale meat was collected separately from one plate in each restaurant or one lot in each store. In total, 54 whale meat samples were obtained and stored at -20°C until DNA extraction.

DNA extraction, PCR amplification, and sequencing

Whole genome DNA was extracted from the whale meat samples using a DNeasy Blood & Tissue Kit (QIAGEN, Germantown, MD, USA), following the manufacturer's protocols. The mitochondrial cytochrome *b* gene is a useful marker for molecular species identification and has been used in identifying bush meat (Jain et al., 2007; Alexandra et al., 2012). We amplified the mitochondrial cytochrome *b* gene using the set of primers: L14724 and H15915 for mammal species (Irwin et al., 1991). A 30 µL amplification mixture containing 10–20 ng of the template DNA, 50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 10 pmol each primer, and 1 unit Ex Taq polymerase (iNtRON Biotechnology, Seoul, South Korea) was used. The cycling profile consisted of an initial denaturation at 94°C for 4 min, followed by 30 cycles of 94°C for 1 min, 44°C for 1 min, and 72°C for 1 min 20 s, with a final extension at 72°C for 7 min. The PCR products were confirmed using 1% agarose gel electrophoresis in 0.5X TBE and visualized by staining with ethidium bromide. The successful amplicons were purified using a Zymoclean Gel DNA Recovery Kit (Zymo Research, Irvine, CA, USA). The purified PCR products were directly sequenced with the same primers used for the PCR using an ABI3730XL sequencer (Applied Biosystems, Foster City, CA, USA), following the manufacturer's instructions.

Data analysis

Geneious Pro v6 (Kearse et al., 2012) was used to proofread and assemble the contigs. Two analyses were performed for species identification. The obtained sequences were searched within the National Center for Biotechnology Information (NCBI) database using the nucleotide-nucleotide Basic Local Alignment Search Tool (BLASTn). Alignment of 51 cytochrome *b* sequences was performed using Clustal W v2.1 (Larkin et al., 2007). A neighbor-joining (NJ) tree was constructed using a Kimura 2-parameter model in Mega v7.0 (Kumar et al., 2016) and confidence levels for internal lineages were assessed using 1,000 bootstrap replicates. Twenty-three cytochrome *b* sequences of cetaceans that had background records of bycatch in South Korea were retrieved from the GenBank database

for the phylogenetic analyses (AF084055.1, AF084057.1, AF084059.1, AF084060.1, AF084064.1, AF084067.1, AF084077.1, AF084092.1, AF084095.1, AF084103.1, AF304073.1, AY579554.1, DQ378164.1, HQ108420.1, KJ586849.1, KJ586854.1, KJ586853.1, U09679.1, U72039.1, X75583.1, X92536.1, X92540.1, and X92541.1).

RESULTS AND DISCUSSION

The mitochondrial cytochrome *b* gene (806–1,140 bp) was successfully amplified in 51 of the 54 collected whale meat samples. The remaining three samples did not amplify because of low quality DNA and were thus excluded from further analyses. The sequences obtained in this study were examined using BLASTn in NCBI (Table 1) and a phylogenetic tree was constructed using the aligned sequences. Results of the phylogenetic tree and BLASTn search demonstrated that the samples of whale meat were mostly comprised of one baleen whale (minke whale) and two toothed whale species (narrow-ridged finless porpoise and common dolphin) (Figure 1). The highest proportion of whale meat was from minke whale (52%), followed by the narrow-ridged finless porpoise (26%) and common dolphin (22%). These three species also accounted for the highest percentages of officially recorded bycatch in South Korea (Table 2). We found that the number of narrow-ridged finless porpoise individuals as bycatch had increased exponentially from 2001 (17 individuals) to 2012 (2,107 individuals) (Table 2). However, we identified fewer species than Baker et al. (2006), who identified three baleen and 13 toothed-whale species in similar samples from South Korea.

It is concerning that narrow-ridged finless porpoises and common dolphins comprised a significant proportion of whale meat sales in South Korea. These species might be disguised as minke whale meat in the markets because of the supply and demand of these species and because of the difference in the market value of the different meats. In South Korea, whale meat consumers favor minke whale meat over other cetacean species because of its flavor. Han (2012) reported that 400 individuals of minke whale are necessary to meet the annual domestic demand. However, the official, mean annual number of minke whales available from bycatch has been far less, at ~80 per year, for the last 10 years (Table 2). Conversely, a large number of narrow-ridged finless porpoises, which are not as popular with consumers, are caught as bycatch every year. The imbalance between supply and demand of minke whale meat and narrow-ridged finless porpoise meat has also resulted in a large difference in market value between these two species. The price of minke whale meat is ~60 times that of narrow-ridged finless porpoise meat in South Korean markets. This difference in market value is presumably a strong incentive for merchants to disguise a species that has a high rate of bycatch, such as narrow-ridged finless porpoise and common dolphin, as the species that has high consumer demand, i.e., minke whale.

Recently, the narrow-ridged finless porpoise population of South Korea has dramatically declined by ~70% over six years (2004/2005 to 2011) (Park et al., 2015). Bycatch is likely a major factor accelerating the decline in the population size of this species. At the end of 2016, the government of South Korea designated the narrow-ridged finless porpoise of South Korea as an endangered marine species, which thus prohibits any kind of trade of this species. Our results highlighted the possibility that IUU exploitation and trade in narrow-ridged finless porpoise persists among fishermen and merchants because of its potential economic value as disguised minke whale meat.

Table 1. Molecular species assignment of cetacean meat samples from Korean restaurants and markets, including scores and percentage sequence similarities with GenBank.

Sample ID	Collection Year	Locality	Species	Score	Max Ident (%)
WM1	2012	Ulsan	common dolphin	2054	99
WM2	2012	Ulsan	narrow-ridged finless porpoise	2102	100
WM3	2012	Ulsan	common dolphin	2061	99
WM4	2012	Ulsan	common dolphin	2058	99
WM5	2012	Ulsan	minke whale	1592	98
WM6	2012	Ulsan	common dolphin	2039	99
WM7	2012	Ulsan	narrow-ridged finless porpoise	2095	99
WM8	2012	Ulsan	common dolphin	1872	99
WM9	2012	Ulsan	common dolphin	2056	99
WM10	2012	Pohang	minke whale	2025	99
WM11	2012	Pohang	minke whale	2028	99
WM12	2012	Pohang	minke whale	2028	99
WM13	2012	Pohang	minke whale	2023	99
WM14	2012	Pohang	narrow-ridged finless porpoise	2095	100
WM15	2012	Pohang	narrow-ridged finless porpoise	2102	100
WM16	2012	Pohang	common dolphin	2061	99
WM17	2012	Pohang	narrow-ridged finless porpoise	2084	100
WM18	2012	Pohang	narrow-ridged finless porpoise	2095	100
WM19	2012	Pohang	narrow-ridged finless porpoise	1435	99
WM20	2012	Pohang	narrow-ridged finless porpoise	2095	100
WM21	2012	Busan	common dolphin	2039	99
WM22	2012	Busan	minke whale	2034	99
WM23	2012	Busan	minke whale	2025	99
WM24	2012	Busan	minke whale	1999	99
*WM25	2012	Busan	Not identified		
WM26	2012	Busan	minke whale	2001	99
*WM27	2015	Seoul	Not identified		
WM28	2015	Seoul	narrow-ridged finless porpoise	2058	100
WM29	2015	Seoul	minke whale	1591	98
WM30	2015	Ulsan	minke whale	1591	99
WM31	2015	Ulsan	narrow-ridged finless porpoise	1613	99
WM32	2015	Ulsan	narrow-ridged finless porpoise	1602	98
WM33	2015	Ulsan	minke whale	1618	99
WM34	2015	Ulsan	minke whale	1574	99
WM35	2015	Ulsan	common dolphin	1664	99
WM36	2015	Ulsan	minke whale	1574	99
WM37	2015	Ulsan	minke whale	1168	100
WM38	2015	Ulsan	minke whale	1151	99
WM39	2015	Ulsan	minke whale	1672	99
WM40	2015	Pohang	minke whale	1692	98
WM41	2015	Pohang	common dolphin	2037	99
WM42	2015	Pohang	minke whale	1445	99
WM43	2015	Pohang	minke whale	1711	98
WM44	2015	Busan	minke whale	1168	100
WM45	2015	Busan	minke whale	1613	99
WM46	2015	Busan	narrow-ridged finless porpoise	1493	98
WM47	2015	Busan	minke whale	1836	98
WM48	2015	Busan	minke whale	1753	98
WM49	2015	Busan	narrow-ridged finless porpoise	2074	100
*WM50	2015	Busan	Not identified	-	
WM51	2015	Busan	minke whale	1681	99
WM52	2015	Busan	minke whale	1807	99
WM53	2015	Pohang	narrow-ridged finless porpoise	1650	100
WM54	2015	Pohang	minke whale	1720	99

* Unsuccessfully sequenced

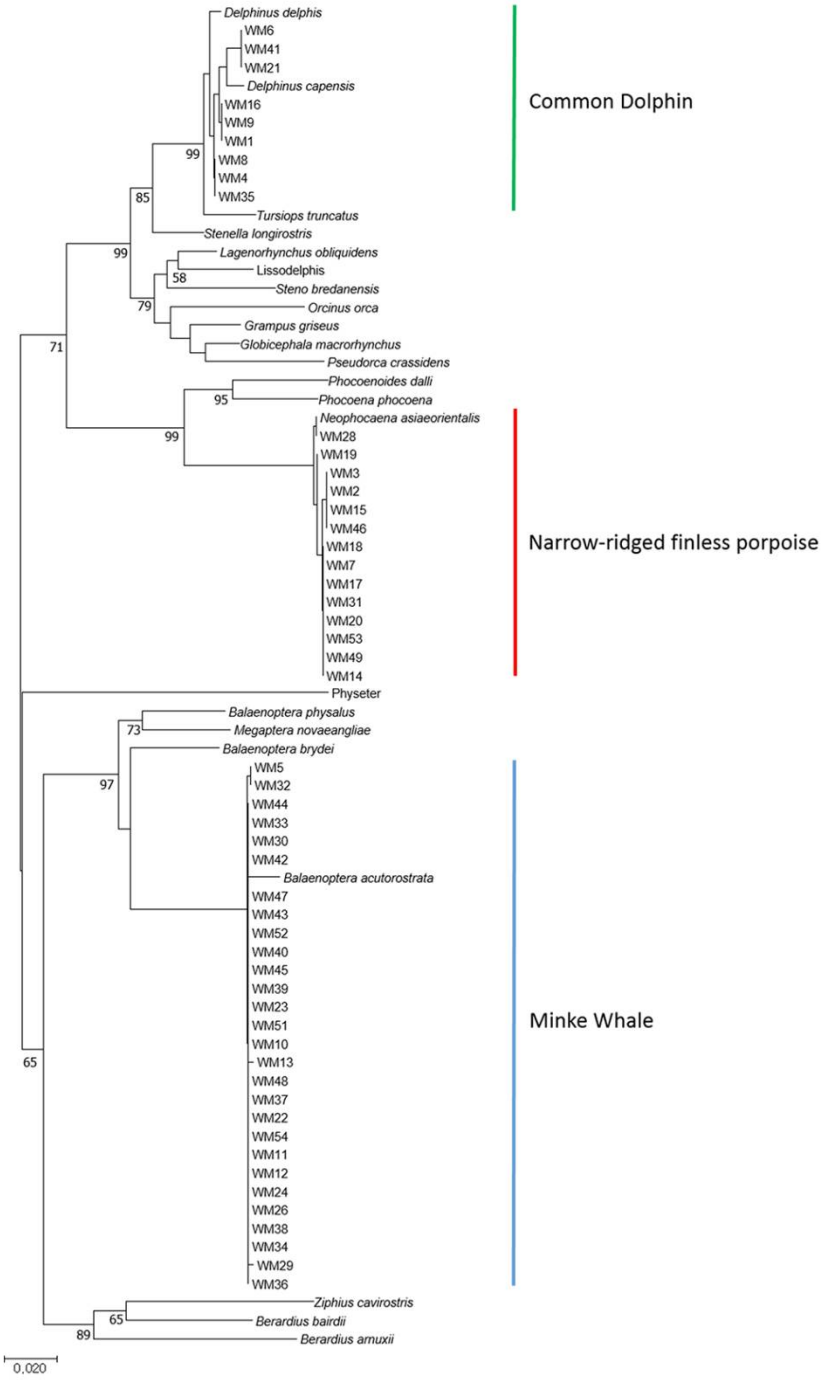

Figure 1. Phylogenetic identification of 'whale meat' products based on the mtDNA cytochrome *b* gene. Fifty-one sequences were successfully obtained, which consisted of three species: minke whale (52%), finless porpoise (26%), and common dolphin (22%).

Table 2. Summary of officially reported bycatch of baleen whales and small cetaceans from South Korea.

	2000[a]	2001[a]	2002[a]	2003[a]	2004[a]	2005[a]	2006[a]	2007[a]	2008[a]	2009[a]	2010[a]	2011[b]	2012[b]
Fin whale			1		1	1	1						
Humpback whale				1	1	1					1	1	
minke whale	77	160	83	86	61	106	81	80	81	54	70	74	73
Bryde whale											1	1	
Stejneger's beaked whale	1	4	1	3		1			1		1		
Baird's beaked whale		1		1									
Cuvier's beaked whale											1		
Risso's dolphin	20	25	2	2	4	3		1	2			1	
common dolphin	29	65	73	113	89	195	345	369	244	252	197	256	325
Pacific white sided dolphin	4	43	54	19	20	47	29	60	21	24	17	21	28
Killer whale	1		3		1				3	2			
False killer whale	1						1		1		1		
Bottlenose dolphin	12	3	4	1	4	15		18	4	3	4		3
Indo pacific bottlenose dolphin									2	9	6	1	
Rough-toothed dolphin	1	1											
Dall's porpoise	2	2	1		1	1			1				
Harbour porpoise		88	34	105	22	102	55	5	15	36	45	26	10
Finless porpoise		17	15	82	13	32	30	57	347	159	232	665	2107
Spinner dolphin												1	1
Unidentified dolphin	27		2	7	1	2							
Total	175	409	273	175	218	506	542	590	722	539	576	1047	2547

[a] data obtained from the National Progress Reports to the International Whaling Commission by the Republic of Korea.
[b] data obtained from Kim et al. (2013)

Whale meat, mainly minke whale, is a local food in South Korea, and the demand for this meat is expected to continue. Our study showed that this culture also affects the future of other whale species in South Korea. Effective and systematic monitoring is therefore required to identify species and the number of individuals that appear in the markets. Generally, it is impossible to identify the species of bushmeat samples based on their external characteristics. A molecular capture-recapture approach is a more useful method to detect illegal poaching of wildlife (Gupta et al., 2006; An et al., 2007; Dalton and Kotze, 2011). To reduce IUU exploitation of narrow-ridged finless porpoise, we propose the establishment of a systematic genetic monitoring program for whale meat, including the genetic analysis of market products.

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