

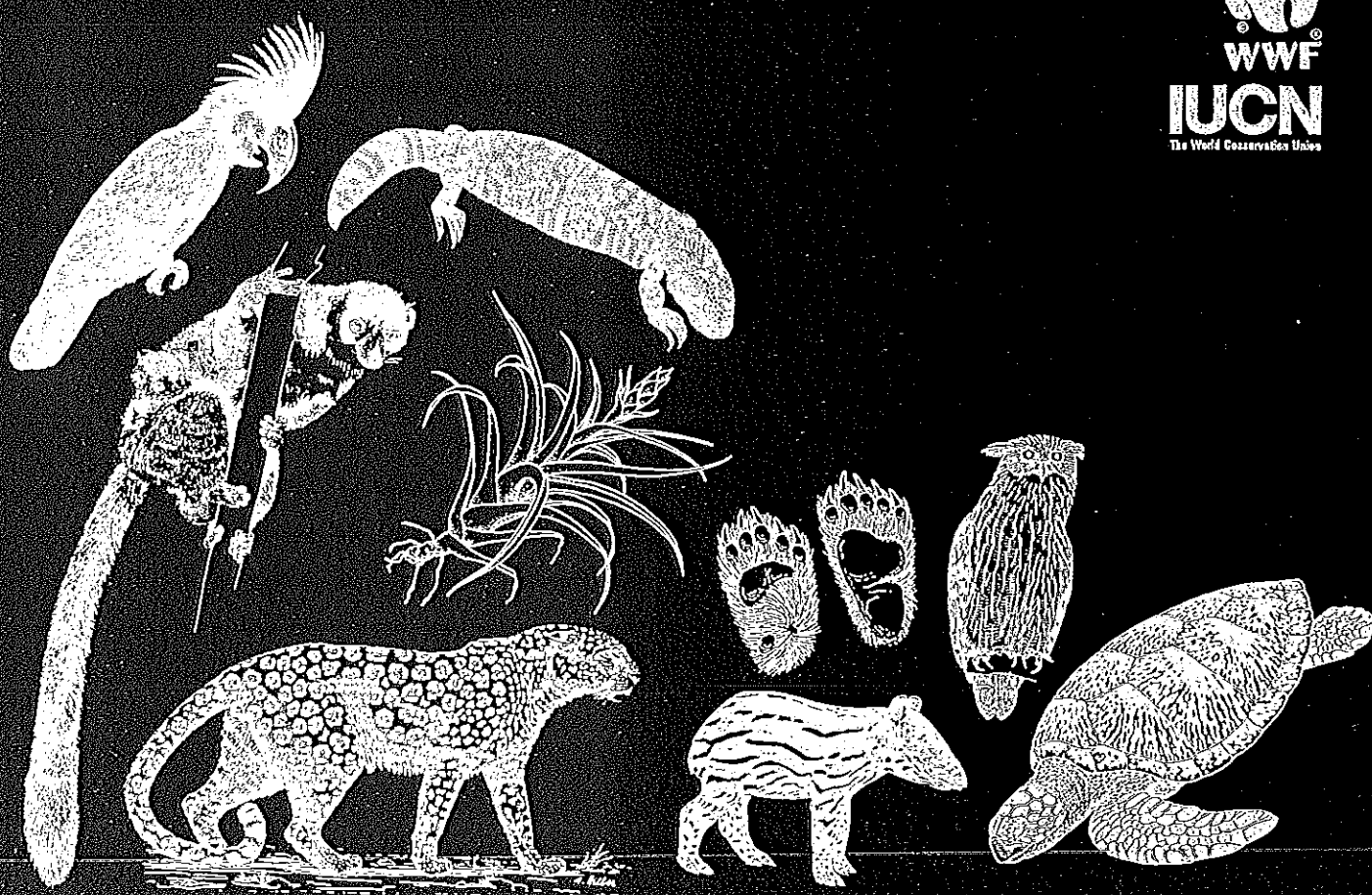
TRAFFIC EAST ASIA-JAPAN レポート

日本で収集された鯨肉および鯨肉製品のDNA分析

DNA SEQUENCE ANALYSIS OF WHALE MEAT AND WHALE MEAT

PRODUCTS COLLECTED IN JAPAN

TRAFFIC EAST ASIA-JAPAN



目次

序論		1
調査方法	サンプル収集	2
	DNA 抽出、増幅および塩基配列決定法	2
結果	分類についての注釈	3
	トラフィックによる分析の結果	3
	水産庁の結果との比較	5
考察		6
付則		7

CONTENTS

INTRODUCTION		1
METHODS	Sample collection	2
	DNA extraction, amplification and sequencing	2
RESULTS	A note on taxonomy	2
	Results of TRAFFIC's analysis	4
	Comparison with JFA results	4
DISCUSSION		6
APPENDIX		7
REFERENCE/参考文献		8

A PRELIMINARY REPORT ON DNA SEQUENCE ANALYSIS OF WHALE MEAT AND WHALE MEAT PRODUCTS COLLECTED IN JAPAN

M. Phipps, A. Ishihara, N. Kanda, and H. Suzuki

September 2000

The following article has been reprinted from *TRAFFIC Bulletin Vol.17 No.2* (1998).

INTRODUCTION

In 1982, the International Whaling Commission (IWC) adopted a moratorium on commercial whaling under the schedule of the International Convention for the Regulation of Whaling (ICRW). The moratorium entered into force in 1986 and remains in place today. All cetaceans covered by the IWC moratorium currently are listed in CITES Appendix I, effectively banning international commercial trade. Several countries have lodged objections to the IWC moratorium, entered reservations on the CITES Appendix I listings, or both. Domestic trade in cetaceans and their products, which does not fall under the jurisdiction of CITES or the IWC, remains legal in a number of countries.

The ninth (1994) and tenth (1997) meetings of the Conference of the Parties to CITES, after due consideration, voted not to accept proposals by Japan and Norway to downlist to Appendix II certain populations of Grey Whale *Eschrichtius robustus*; Minke Whale *Balaenoptera acutorostrata*, and Bryde's Whale *Balaenoptera edeni*. The same meetings adopted Resolution Conf. 9.12 (1994) *Illegal trade in whale meat* and Decisions 10.40 to 10.43 (1997) *Co-operation in monitoring illegal trade in whale parts and derivatives* in recognition of the importance of preventing illegal trade in whale meat and other whale products.

While there are no legal restrictions on the domestic buying and selling of whale meat in Japan, legal importation of whale meat products appears to have ceased since 1992 when Japan officially stopped importing whale meat (Chan *et al.*, 1995). However, reports of a number of smuggling attempts intercepted by Japan and other countries have led to concern that some level of illegal trade involving Japan may persist.

Since 1995, TRAFFIC East Asia has monitored whale meat trade in East Asia and conducted market surveys in a number of countries in the region (Chan *et al.*, 1995; Anon., 1996; Mills *et al.*, 1997). The aim of these surveys has been to document domestic control of whale meat trade; the extent to which regional trade takes place; and, where possible, to identify the species and origin of whale meat being offered for sale. To this end, in 1995 TRAFFIC researchers collected 53 samples of whale meat and whale meat products from 10 cities in Japan. However, because visual identification of whale meat products to the species level is, generally, not possible, TRAFFIC engaged scientists to carry out DNA sequence analysis of the samples. TRAFFIC also invited the Japan Fisheries Agency (JFA) to conduct its own analysis of the samples. This preliminary report describes the results of analysis by TRAFFIC's consultants to date and briefly compares TRAFFIC's results with those of the JFA.

METHODS

Sample collection

From 10 to 21 April 1995, a TRAFFIC researcher surveyed over 900 retail outlets in 13 cities in Japan (Chan *et al.*, 1995); 51 of these were found to be selling whale meat. A total of 53 samples were purchased in 10 cities during the survey. The samples were preserved in a solution of 75% ethanol and stored at room temperature prior to DNA analysis.

DNA extraction, amplification and sequencing

In this case, DNA analysis involves a three-step process: DNA extraction, amplification, and sequencing. DNA extraction was carried out at the Tokyo University of Agriculture and Technology. DNA amplification and sequencing were carried out at Hokkaido University. The entire process was carried out over a one-year period, May 1996 to May 1997.

Well-established methodologies for DNA extraction, amplification, and sequencing were used to reveal species-specific differences in a specific section of the DNA. The extraction process separates the chromosomal and extranuclear DNA from the rest of the cellular components such as fats and proteins. The amplification process utilizes the polymerase chain reaction (PCR) to produce large concentrations of the targeted section of DNA. For this study, the mitochondrial cytochrome B gene was targeted.

The sequencing process reads the series of nucleotide bases, the A's, G's, C's, and T's, which make up the information content of the DNA. Where possible, 402 nucleotide bases were sequenced for each sample. For some samples, only smaller fragments of DNA were available. For these, 180 nucleotide bases were sequenced. In either case, resulting sequences were compared to type specimen sequences from known animals.

For more detailed descriptions of methodologies, the reader is referred to Suzuki *et al.*, 1997; Kocher *et al.*, 1989; and, Hillis *et al.*, 1996.

RESULTS

A note on taxonomy

Reeves and Leatherwood (1994) state that mainstream classification of cetaceans is relatively underdeveloped below the species level, in part because of limited sampling. Of direct relevance to this report are ongoing debates over the taxonomy of the Minke Whale and Bryde's Whale. Three distinct morphological forms of the Minke Whale and a diminutive form of the Bryde's Whale have been documented, but their status as races, subspecies, or separate species needs to be clarified (Reeves and Leatherwood, 1994).

This article uses the 1996 IUCN Red List of Threatened Animals (IUCN, 1996) as its taxonomical reference. The Southern Minke Whale *Balaenoptera bonarensis* is treated therein as a separate species rather than as a subspecies of *B. acutorostrata*, as in the CITES Appendices, which refer to a single Minke Whale species, *Balaenoptera acutorostrata*. Both CITES and IWC provide for treatment of species on the basis of geographically separate populations or stocks if necessary. It should also be noted that the report of the JFA analysis (Anon., 1997) refers to the Southern Minke Whale as the Antarctic Minke Whale.

Reference number	City of origin	Product description by retailer	Scientific name	Common name
TR1	Tokyo	red meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR2	Tokyo	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR3	Tokyo	salted meat	<i>B. bonarensis</i>	Southern Minke Whale
TR4	Tokyo	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR5	Tokyo	salted blubber	<i>B. bonarensis</i>	Southern Minke Whale
TR6	Tokyo	marinated meat	No amplification	-
TR7	Tokyo	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR8	Tokyo	red meat 'gondo'	<i>Phocoenoides dalli</i>	Dall's Porpoise
TR9	Tokyo	none	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR10	Osaka	none	<i>B. bonarensis</i>	Southern Minke Whale
TR11	Osaka	none	<i>B. edeni</i>	Bryde's Whale
TR12	Osaka	none	<i>B. bonarensis</i>	Southern Minke Whale
TR13	Osaka	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR14	Osaka	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR15	Osaka	none	<i>B. bonarensis</i>	Southern Minke Whale
TR16	Osaka	none	<i>B. bonarensis</i>	Southern Minke Whale
TR17	Osaka	none	No amplification	-
TR18	Kobe	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR19	Kobe	none	<i>Phocoenoides dalli</i>	Dall's Porpoise
TR20	Nagasaki	red meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR21	Nagasaki	boiled meat	<i>B. physalus</i>	Fin Whale
TR22	Nagasaki	salted meat	<i>Lissodelphis borealis</i>	Northern Right Whale Dolphin
TR23	Nagasaki	salted meat	<i>Balaenoptera physalus</i>	Fin Whale
TR24	Nagasaki	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR25	Nagasaki	red meat	<i>B. acutorostrata</i>	Minke whale
TR26	Nagasaki	salted 'une'	<i>Phocoenoides dalli</i>	Dall's Porpoise
TR27	Miyagi	'kujira-kun'	<i>P. dalli</i>	Dall's Porpoise
TR28	Miyagi	bacon	No amplification	-
TR29	Miyagi	bacon	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR30	Miyagi	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR31	Miyagi	none	<i>B. bonarensis</i>	Southern Minke Whale
TR32	Miyagi	none	<i>B. bonarensis</i>	Southern Minke Whale
TR33	Miyagi	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR34	Miyagi	none	<i>B. bonarensis</i>	Southern Minke Whale
TR35	Fukuoka	blubber	<i>Tursiops truncatus</i>	Bottlenose Dolphin
TR36	Fukuoka	red meat	<i>Globicephala macrorhynchus</i>	Short-finned Pilot Whale
TR37	Fukuoka	salted meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR38	Fukuoka	meat steak	<i>B. bonarensis</i>	Southern Minke Whale
TR39	Fukuoka	none	<i>Phocoenoides dalli</i>	Dall's Porpoise
TR40	Fukuoka	throat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR41	Fukuoka	none	No amplification	-
TR42	Fukuoka	none	<i>B. bonarensis</i>	Southern Minke Whale
TR43	Sasebo	salted meat	<i>B. bonarensis</i>	Southern Minke Whale
TR44	Sasebo	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR45	Sasebo	red meat	<i>B. acutorostrata</i>	Minke Whale
TR46	Sasebo	salted meat	<i>Berardius bairdii</i>	Baird's Beaked Whale
TR47	Sasebo	red meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR48	Sasebo	red meat	<i>Globicephala macrorhynchus</i>	Short-finned Pilot Whale
TR49	Sasebo	salted meat	<i>Berardius bairdii</i>	Baird's Beaked Whale
TR50	Hiroshima	red meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR51	Hiroshima	salted meat	<i>Phocoenoides dalli</i>	Dall's Porpoise
TR52	Chiba	red meat	<i>Ziphius cavirostris</i>	Cuvier's Beaked Whale
TR53	Niigata	salted meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale

Table 1. Results of DNA sequence analysis by TRAFFIC of 53 whale meat samples, 1996-1997.

Results of TRAFFIC's analysis

Results were obtained for 49 of 53 samples (Table 1). Four samples could not be amplified, possibly because of the presence of impurities or because of post-mortem damage to the DNA.

Of the 49 samples identified to the species level, 36 were identified as originating from four species of Mysticetes or baleen whales; 13 samples were identified as having originated from six species of Odontocetes or toothed whales.

Baleen whales identified included Minke Whale *Balaenoptera* spp. (n = 33); Bryde's Whale *Balaenoptera edeni* (n = 1); and Fin Whale *Balaenoptera physalus* (n = 2). Thirty-one of the 33 Minke Whale samples were identified as Southern Minke Whale *B. bonarensis*. Of the toothed whale samples, the most common species identified was the Dall's Porpoise *Phocoenoides dalli* (n = 6). Other species identified were Northern Right Whale Dolphin *Lissodelphis borealis* (n = 1); Bottlenose Dolphin *Tursiops truncatus* (n = 1); Short-finned Pilot Whale *Globicephala macrorhynchus* (n = 2); Baird's Beaked Whale *Berardius bairdii* (n = 2); and Cuvier's Beaked Whale *Ziphius cavirostris* (n = 1). None of the Odontocete species identified above is under the jurisdiction of the IWC. Five species are listed in CITES Appendix II, and the sixth, *Berardius bairdii*, in Appendix I, although Japan has a reservation on this listing.

Comparison with JFA results

The JFA presented a preliminary report on its analysis of the TRAFFIC samples to the forty-ninth meeting of the IWC in October 1997 (Anon., 1997). Results from the two sets of analysis were similar for the most part, but TRAFFIC and the JFA obtained differing results from 14 samples (Table 2). Of the 14, a total of eight samples could not be amplified by one or the other laboratory. Two samples (TR38; TR40) were identified as North Pacific Minke Whale by JFA and Southern Minke Whale by TRAFFIC. TR42 was identified as Southern Minke Whale by TRAFFIC and as a dwarf form of Minke Whale by JFA. An additional three samples (TR19; TR26; TR39) were identified by each laboratory as being of two different species. Neither laboratory was able to amplify one sample (TR6).

Reference number	TRAFFIC results	JFA results
TR8	Dall's Porpoise	No amplification
TR17	No amplification	Southern Minke Whale ¹
TR19	Dall's Porpoise	Southern Minke Whale
TR26	Dall's Porpoise	Southern Minke Whale
TR27	Dall's Porpoise	No amplification
TR28	No amplification	Southern Minke Whale
TR37	Southern Minke Whale	No amplification
TR38	Southern Minke Whale	North Pacific Minke Whale
TR39	Dall's Porpoise	Southern Minke Whale
TR40	Southern Minke Whale	North Pacific Minke Whale
TR41	No amplification	Southern Minke Whale
TR42	Southern Minke Whale	Dwarf Minke Whale
TR43	Southern Minke Whale	No amplification
TR51	Dall's Porpoise	No amplification

Table 2. Samples for which TRAFFIC and JFA obtained differing results.

Source of JFA results: Fisheries Agency, Government of Japan (Anon., 1997).

¹The Southern Minke Whale is referred to in Anon., 1997 as the Antarctic Minke Whale.

The inability of one or the other of the laboratories to amplify the eight samples referred to above could be a result of problems with the samples themselves, handling prior to or during laboratory analysis, or the use of different marker genes or regions in the mtDNA. Differences in identification at the subspecies or population level may have resulted from the interpretation of results. TRAFFIC and JFA used differing identification techniques (cytochrome B and control region) and different databases as references. However, the differing species results for TRAFFIC and the JFA's analyses of samples TR19, TR26, and TR39 are perplexing.

The presence of Dall's Porpoise in the marketplace is not surprising in itself as Japan recorded catches of this species exceeding 70 000 specimens in the period 1990 to 1993 (Chan *et al.*, 1995). Several possible explanations for the differing results exist. Mixing of meat from different species in the marketplace, either accidentally or deliberately, could result in 'compound' samples. If a single sample was contaminated with tissue from two different species, results could differ between the two laboratories. Contamination during sample acquisition or in the laboratory is also possible. However, Dall's Porpoise had not previously been analysed in the laboratories where TRAFFIC's analysis of sequences was conducted, and the chances of contamination in the laboratory environment were minimal. Moreover, TRAFFIC's analysis recorded five different and unique sequences in the six samples identified as Dall's Porpoise, making it unlikely that all the samples were contaminated.

The reasons for these differing results are unclear, but the differences are more likely owing to human error than to any fundamental problem with using cytochrome B or control region sequences to identify species. TRAFFIC would like to re-examine these samples in co-operation with the JFA to determine the source of the differences.



Whale Meat ©TRAFFIC/Simba Chan

DISCUSSION

Mills *et al.* (1997) outline three avenues for whale meat of IWC-listed species to enter Japan's retail market legally: via the factory ship of Japan's scientific whaling fleet; from frozen domestic stocks originating from past whaling or importation; and from bycatch in Japan's territorial waters. The samples identified in this study conceivably could have originated from any of these sources. Should CITES in future choose to downlist any of the baleen whales to Appendix II, international trade could provide an additional legal avenue. Meat from small cetaceans, harvest of which is not regulated by the IWC, is also available in the market and may be sold under the generic term 'whale meat.'

While there has been no proof of illegal whale meat entering Japan to date, interception by Japanese and other authorities of a number of attempted illegal shipments has resulted in concern that illegal trade may take place. Under the terms of CITES Resolution Conf. 9.24, proposals to downlist species from Appendix I to Appendix II must show that adequate trade control mechanisms are in place to ensure that removal of the species from Appendix I does not jeopardize that species or other species still in Appendix I. Norway is developing domestic trade control and inspection systems that incorporate DNA profiling and creation of a DNA register of individual whale "fingerprints" as important components. Japan is considering development of a similar system.

In Japan, DNA profiling could provide an effective tool for monitoring whale meat stocks only if samples from all legitimate sources of whale meat were to be included. Under the current domestic management system, a DNA register would need to include samples of meat alleged to originate from frozen domestic stocks and legitimate bycatch as well as samples from Government-controlled whaling. A partial register would be of limited use in determining the legality of whale meat in the marketplace. In any event, DNA analysis cannot determine the length of time samples have been in storage.

An additional consideration is the reliability of this type of analysis for samples sourced from the marketplace where compounding may occur. Analysis of the 53 samples under discussion was carried out by two groups of qualified scientists utilizing differing but standard methodologies in controlled laboratory conditions. Although the results from the two sets of analysis corresponded for the most part, there were significant differences for several samples. A DNA register would need to include appropriate measures for cross-checking the results of DNA analysis.

There is the possibility that all whale species protected under the IWC moratorium on commercial whaling could enter the Japanese market either through existing frozen stocks or bycatch (Mills *et al.*, 1997). Should meat from frozen stocks and bycatch remain outside of a future DNA register, it would continue to be difficult to distinguish meat from some species as legal or illegal in origin. The Government of Japan needs to consider this when developing future trade control mechanisms to meet the requirements of CITES Resolution Conf. 9.24.

APPENDIX

Whales listed in the CITES Appendices and Reservation of Japan and covered by the IWC moratorium

Common Name	Scientific Name	CITES Appendices and Reservations	IWC moratorium
	CETACEA spp.	II	
Chinese river dolphin	<i>Lipotes vexillifer</i>	I	
	<i>Platanista</i> spp.	I	
	<i>Berardius</i> spp.	I	
Baird's Beaked Whale	<i>Berardius bairdii</i>	I R	
	<i>Hyperoodon</i> spp.	I	
Northern Bottlenose Whale	<i>Hyperoodon ampullatus</i>	I	X
Southern Bottlenose Whale	<i>Hyperoodon planifrons</i>	I	X
Sperm Whale	<i>Physeter catodon</i> ¹	I R	X
	<i>Sotalia</i> spp.	I	
	<i>Sousa</i> spp.	I	
Finless Whale	<i>Neophocaena phocaenoides</i>	I	
Cochito	<i>Phocoena siurus</i>	I	
Gray Whale	<i>Eschrichtius robustus</i> ²	I	X ³
Minke Whale	<i>Balaenoptera acutorostrata</i>	III ⁴ R ⁵	X ⁶
Sei Whale	<i>Balaenoptera borealis</i>	I R ⁷	X
Bryde's Whale	<i>Balaenoptera edeni</i>	I R	X
Blue Whale	<i>Balaenoptera musculus</i>	I	X
Fin Whale	<i>Balaenoptera physalus</i>	I R	X ⁸
Humpback Whale	<i>Megaptera novaeangliae</i>	I	X ⁹
Bowhead Whale	<i>Balaena mysticetus</i>	I	X ¹⁰
	<i>Eubalaena</i> spp.	I	X
Pygmy Right Whale	<i>Caperea marginata</i>	I	X

Notes:

- ¹ includes synonym *Physeter macrocephalus*
- ² includes synonym *Eschrichtius glaucus*
- ³ Taking of Eastern Stock in North Pacific is permitted by aborigines or a Contracting Government on behalf of aborigines exclusively for local consumption by the aborigines;
- ⁴ The West Greenland population of *Balaenoptera acutorostrata* is listed in CITES Appendix II;
- ⁵ Reservation of Japan applicable to Appendix I populations only;
- ⁶ Taking of West Greenland and Central Stocks in North Atlantic is permitted by aborigines exclusively for local consumption;
- ⁷ Reservation not applicable to stocks (A) in North Pacific and (B) in area from 0° longitude to 70° east longitude, from the equator to the Antarctic Continent;
- ⁸ Taking of West Greenland Stock in North Atlantic is permitted by aborigines exclusively for local consumption;
- ⁹ Taking by Bequians of Saint Vincent and the Grenadines is permitted exclusively for local consumption;
- ¹⁰ Taking of Bering-Chukchi-Beaufort Seas Stock is permitted by aborigines exclusively for local consumption by the aborigines;

Source: J. Mills, A. Ishihara, I. Sakaguchi, S. Kang, R. Parry-Jones and M. Phipps (1997), Anon. (1998a), Anon. (1998b)

REFERENCES/ 参考文献

- Anon.(1996). *An Update on the Whale Meat Trade in East Asia*. Traffic East Asia, Hong Kong.
- Anon.(1997). Preliminary analyses on whale products sampled by TRAFFIC Japan. Fisheries Agency, Government of Japan. *Report of the International Whaling Commission No. 49. Information 4*.
- Anon.(1998a). *CITES*, MITI Official Bulletin March 31 1998, Ministry of International Trade and Industry, Japan
- Anon.(1998b). *Annotated CITES Appendices and Reservations*. CITES Secretariat/World Conservation Monitoring Centre, UK
- Chan, S., Ishihara, A., Lu, D.J., Phipps, M., and Mills, J.A. (1995). Observations on the whale meat trade in East Asia. *TRAFFIC Bulletin* 15(3):107-115.
- Hillis, D.M., Moritz, C., and Mable, B.K. (1996). *Molecular Systematics*. Second edition. Sinauer Associates, Sunderland MA, USA. 656pp.
- IUCN (1996). *1996 IUCN Red List of Threatened Animals*. IUCN, Gland, Switzerland, and Cambridge, UK. 448pp.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. (1989). Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences, USA*. 86:6196-6200.
- Mills, J.A., Ishihara, A., Sakaguchi, I., Kang, S., Parry-Jones, R., and Phipps, M. (1997). *Whale Meat Trade in East Asia: A Review of the Markets in 1997*. TRAFFIC International, UK.
- Reeves, R.R. and Leatherwood, S. (1994). *Dolphins, Porpoises, and Whales: 1994-1998 Action Plan for the Conservation of Cetaceans*. IUCN, Gland, Switzerland.
- Suzuki, H., Minato, S., Sakurai, S., Tsuchiya, K., and Fokin, I.M. (1997). Phylogenetic position and geographic differentiation of the Japanese dormouse, *Glirulus japonicus*, revealed by variations among rDNA, mtDNA and the Sry gene. *Zoological Science* 14:167-173.
- 通産省公報(1998), ワシントン条約(絶滅のおそれのある野生動植物の種の国際取引に関する条約)について

M. Phipps, National Representative, TRAFFIC East Asia-Taipei

A. Ishihara, Programme Officer, TRAFFIC East Asia-Japan

N. Kanda, Division of Veterinary Anatomy, School of Veterinary Medicine, Tokyo University of Agriculture and Technology

H. Suzuki, Division of Biosciences, Graduate School of Environmental Earth Science, Hokkaido University.

トラフィックネットワークは、世界 20 カ国に事務所をもち、野生生物の取引をモニターする世界最大の民間機関であり、WWF（世界自然保護基金）と IUCN（国際自然保護連合）の自然保護プログラムである。

その活動目的は国内および国際的法律や協定に基づき、動植物にとって有害で違法な野生生物の取引に関して、調査、モニター、報告を通じて、野生生物取引の持続可能な利用の確立を支援することである。トラフィック・ネットワークの本部は英国・ケンブリッジにある。

トラフィックイーストアジア・ジャパンは、WWF Japan（（財）世界自然保護基金日本委員会、会長・大内照之）の野生生物取引調査部門で、トラフィックイーストアジアの日本事務所として 1982 年から活動している。

著者：マーカス・フィップス、石原明子、神田尚俊、鈴木仁

発行：2000 年 9 月 トラフィックジャパン

表紙イラスト：CITES Identification Manual

印刷：株式会社 港洋社

このレポートは TRAFFIC JAPAN Newsletter Vol.14 No.1(1998) および TRAFFIC Bulletin Vol.17 No.2(1998) の原稿を再発行したものです。

本報告書の無断転載はお断りいたします。転載の希望の際はトラフィックジャパンにご一報ください。

本誌に使用されているのは、エコマーク認定、グリーン購入ネットワークデータベース記載、環境庁推奨リスト掲載の商品で古紙配合率 100% の再生紙です。

Compiled by Marcus Phipps, Akiko Ishihara, Naotoshi Kanada and Hitoshi Suzuki

Published by TRAFFIC EAST ASIA JAPAN, Tokyo, JAPAN

© 2000 TRAFFIC EAST ASIA JAPAN

All right reserved.

Front cover illustrations: CITES Identification Manual

Reprinted from TRAFFIC JAPAN Newsletter Vol.14 No.1(1998) and TRAFFIC Bulletin Vol.17 No.2(1998).

Printed on 100% recycled paper.

A Preliminary Report on DNA Sequence Analysis of Whale Meat and Whale Meat Products Collected in Japan

M. Phipps, A. Ishihara, N. Kanda, and H. Suzuki

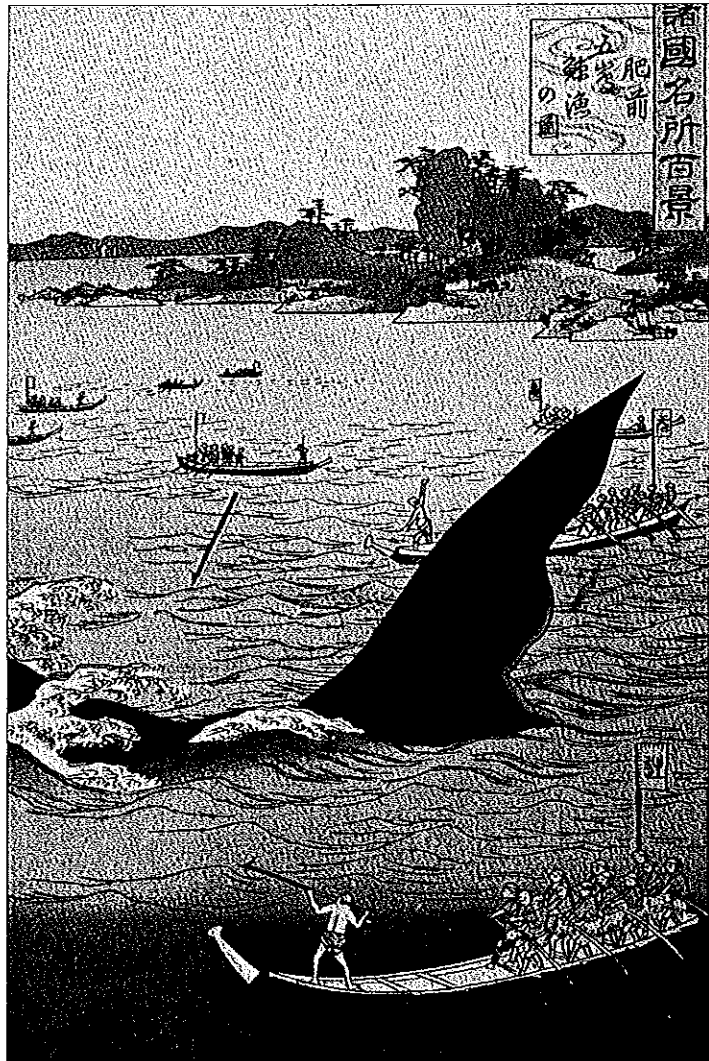
INTRODUCTION

In 1982, the International Whaling Commission (IWC) adopted a moratorium on commercial whaling under the schedule of the International Convention for the Regulation of Whaling (ICRW). The moratorium entered into force in 1986 and remains in place today. All cetaceans covered by the IWC moratorium currently are listed in CITES Appendix I, effectively banning international commercial trade. Several countries have lodged objections to the IWC moratorium, entered reservations on the CITES Appendix I listings, or both. Domestic trade in cetaceans and their products, which does not fall under the jurisdiction of CITES or the IWC, remains legal in a number of countries.

The ninth (1994) and tenth (1997) meetings of the Conference of the Parties to CITES, after due consideration, voted not to accept proposals by Japan and Norway to downlist to Appendix II certain populations of Grey Whale *Eschrichtius robustus*; Minke Whale *Balaenoptera acutorostrata*, and Bryde's Whale *Balaenoptera edeni*. The same meetings adopted Resolution Conf. 9.12 (1994) *Illegal trade in whale meat* and Decisions 10.40 to 10.43 (1997) *Co-operation in monitoring illegal trade in whale parts and derivatives* in recognition of the importance of preventing illegal trade in whale meat and other whale products.

While there are no legal restrictions on the domestic buying and selling of whale meat in Japan, legal importation of whale meat products appears to have ceased since 1992 when Japan officially stopped importing whale meat (Chan *et al.*, 1995). However, reports of a number of smuggling attempts intercepted by Japan and other countries have led to concern that some level of illegal trade involving Japan may persist.

Since 1995, TRAFFIC East Asia has monitored whale meat trade in East Asia and conducted market surveys in a number of countries in the region (Chan *et al.*, 1995; Anon., 1996; Mills *et al.*, 1997). The aim of these surveys has been to document domestic control of whale meat trade; the extent to which regional trade takes place; and, where possible, to identify the species and origin of whale meat being offered for sale. To this end, in 1995 TRAFFIC researchers collected 53 samples of whale meat and whale meat products from 10 cities in Japan. However, because visual identification of whale meat



Nineteenth century woodcut of whalers in Goto Islands, Hizen (present-day Nagasaki prefecture in Kyushu), Japan. (Hiroshige Utagawa II)

products to the species level is, generally, not possible, TRAFFIC engaged scientists to carry out DNA sequence analysis of the samples. TRAFFIC also invited the Japan Fisheries Agency (JFA) to conduct its own analysis of the samples. This preliminary report describes the results of analysis by TRAFFIC's consultants to date and briefly compares TRAFFIC's results with those of the JFA.

METHODS

Sample collection

From 10 to 21 April 1995, a TRAFFIC researcher surveyed over 900 retail outlets in 13 cities in Japan (Chan *et al.*, 1995); 51 of these were found to be selling whale meat. A total of 53 samples were purchased in 10 cities during the survey. The samples were preserved in a solution of 75% ethanol and stored at room temperature prior to DNA analysis.

Reference number	City of origin	Product description by retailer	Scientific name	Common name
TR1	Tokyo	red meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR2	Tokyo	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR3	Tokyo	salted meat	<i>B. bonarensis</i>	Southern Minke Whale
TR4	Tokyo	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR5	Tokyo	salted blubber	<i>B. bonarensis</i>	Southern Minke Whale
TR6	Tokyo	marinated meat	No amplification	-
TR7	Tokyo	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR8	Tokyo	red meat 'gondo'	<i>Phocoenoides dalli</i>	Dall's Porpoise
TR9	Tokyo	none	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR10	Osaka	none	<i>B. bonarensis</i>	Southern Minke Whale
TR11	Osaka	none	<i>B. edeni</i>	Bryde's Whale
TR12	Osaka	none	<i>B. bonarensis</i>	Southern Minke Whale
TR13	Osaka	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR14	Osaka	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR15	Osaka	none	<i>B. bonarensis</i>	Southern Minke Whale
TR16	Osaka	none	<i>B. bonarensis</i>	Southern Minke Whale
TR17	Osaka	none	No amplification	-
TR18	Kobe	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR19	Kobe	none	<i>Phocoenoides dalli</i>	Dall's Porpoise
TR20	Nagasaki	red meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR21	Nagasaki	boiled meat	<i>B. physalus</i>	Fin Whale
TR22	Nagasaki	salted meat	<i>Lissodelphis borealis</i>	Northern Right Whale Dolphin
TR23	Nagasaki	salted meat	<i>Balaenoptera physalus</i>	Fin Whale
TR24	Nagasaki	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR25	Nagasaki	red meat	<i>B. acutorostrata</i>	North Pacific Minke whale
TR26	Nagasaki	salted 'une'	<i>Phocoenoides dalli</i>	Dall's Porpoise
TR27	Miyagi	'kujira-kun'	<i>P. dalli</i>	Dall's Porpoise
TR28	Miyagi	bacon	No amplification	-
TR29	Miyagi	bacon	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR30	Miyagi	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR31	Miyagi	none	<i>B. bonarensis</i>	Southern Minke Whale
TR32	Miyagi	none	<i>B. bonarensis</i>	Southern Minke Whale
TR33	Miyagi	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR34	Miyagi	none	<i>B. bonarensis</i>	Southern Minke Whale
TR35	Fukuoka	blubber	<i>Tursiops truncatus</i>	Bottlenose Dolphin
TR36	Fukuoka	red meat	<i>Globicephala macrorhynchus</i>	Short-finned Pilot Whale
TR37	Fukuoka	salted meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR38	Fukuoka	meat steak	<i>B. bonarensis</i>	Southern Minke Whale
TR39	Fukuoka	none	<i>Phocoenoides dalli</i>	Dall's Porpoise
TR40	Fukuoka	throat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR41	Fukuoka	none	No amplification	-
TR42	Fukuoka	none	<i>B. bonarensis</i>	Southern Minke Whale
TR43	Sasebo	salted meat	<i>B. bonarensis</i>	Southern Minke Whale
TR44	Sasebo	red meat	<i>B. bonarensis</i>	Southern Minke Whale
TR45	Sasebo	red meat	<i>B. acutorostrata</i>	North Pacific Minke Whale
TR46	Sasebo	salted meat	<i>Berardius bairdii</i>	Baird's Beaked Whale
TR47	Sasebo	red meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR48	Sasebo	red meat	<i>Globicephala macrorhynchus</i>	Short-finned Pilot Whale
TR49	Sasebo	salted meat	<i>Berardius bairdii</i>	Baird's Beaked Whale
TR50	Hiroshima	red meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale
TR51	Hiroshima	salted meat	<i>Phocoenoides dalli</i>	Dall's Porpoise
TR52	Chiba	red meat	<i>Ziphius cavirostris</i>	Cuvier's Beaked Whale
TR53	Niigata	salted meat	<i>Balaenoptera bonarensis</i>	Southern Minke Whale

Table 1. Results of DNA sequence analysis by TRAFFIC of 53 whale meat samples, 1996-1997.

DNA extraction, amplification, and sequencing

In this case, DNA analysis involves a three-step process: DNA extraction, amplification, and sequencing. DNA extraction was carried out at the Tokyo University of Agriculture and Technology. DNA amplification and sequencing were carried out at Hokkaido University. The entire process was carried out over a one-year period, May 1996 to May 1997.

Well-established methodologies for DNA extraction, amplification, and sequencing were used to reveal species-specific differences in a specific section of the DNA. The extraction process separates the chromosomal and extranuclear DNA from the rest of the cellular components such as fats and proteins. The amplification process utilizes the polymerase chain reaction (PCR) to

produce large concentrations of the targeted section of DNA. For this study, the mitochondrial cytochrome B gene was targeted.

The sequencing process reads the series of nucleotide bases, the A's, G's, C's, and T's, which make up the information content of the DNA. Where possible, 402 nucleotide bases were sequenced for each sample. For some samples, only smaller fragments of DNA were available. For these, 180 nucleotide bases were sequenced. In either case, resulting sequences were compared to type specimen sequences from known animals.

For more detailed descriptions of methodologies, the reader is referred to Suzuki *et al.*, 1997; Kocher *et al.*, 1989; and, Hillis *et al.*, 1996.

RESULTS

A note on taxonomy

Reeves and Leatherwood (1994) state that mainstream classification of cetaceans is relatively underdeveloped below the species level, in part because of limited sampling. Of direct relevance to this report are ongoing debates over the taxonomy of the Minke Whale and Bryde's Whale. Three distinct morphological forms of the Minke Whale and a diminutive form of the Bryde's Whale have been documented, but their status as races, subspecies, or separate species needs to be clarified (Reeves and Leatherwood, 1994).

This article uses the 1996 IUCN Red List of Threatened Animals (IUCN, 1996) as its taxonomical reference. The Southern Minke Whale *Balaenoptera bonarensis* is treated therein as a separate species rather than as a subspecies of *B. acutorostrata*, as in the CITES Appendices, which refer to a single Minke Whale species, *Balaenoptera acutorostrata*. Both CITES and IWC provide for treatment of species on the basis of geographically separate populations or stocks if necessary. It should also be noted that the report of the JFA analysis (Anon., 1997) refers to the Southern Minke Whale as the Antarctic Minke Whale.

Results of TRAFFIC's analysis

Results were obtained for 49 of 53 samples (Table 1). Four samples could not be amplified, possibly because of the presence of impurities or because of post-mortem damage to the DNA.

Of the 49 samples identified to the species level, 36 were identified as originating from four species of Mysticetes or baleen whales; 13 samples were identified as having originated from six species of Odontocetes or toothed whales.

Baleen whales identified included Minke Whale *Balaenoptera* spp. (n = 33); Bryde's Whale *Balaenoptera edeni* (n = 1); and Fin Whale *Balaenoptera physalus* (n = 2). Thirty-one of the 33 Minke Whale samples were identified as Southern Minke Whale *B. bonarensis*. Of the toothed whale samples, the most common species identified was the Dall's Porpoise *Phocoenoides dalli* (n = 6). Other species identified were Northern Right Whale Dolphin *Lissodelphis borealis* (n = 1); Bottlenose Dolphin *Tursiops truncatus* (n = 1); Short-finned Pilot Whale *Globicephala macrorhynchus* (n = 2); Baird's Beaked Whale *Berardius bairdii* (n = 2); and Cuvier's Beaked Whale *Ziphius cavirostris* (n = 1). None of the Odontocete species identified above is under the jurisdiction of the IWC. Five species are listed in CITES Appendix II, and the sixth, *Berardius bairdii*, in Appendix I, although Japan has a reservation on this listing.

Comparison with JFA results

The JFA presented a preliminary report on its analysis of the TRAFFIC samples to the forty-ninth meeting of the IWC in October 1997 (Anon., 1997). Results from the two sets of analysis were similar for the most part, but TRAFFIC and the JFA obtained differing results from 14 samples (Table 2). Of the 14, a total of eight samples could not be amplified by one or the other laboratory. Two samples (TR38; TR40) were identified as North Pacific Minke Whale by JFA and Southern Minke Whale by TRAFFIC. TR42 was identified as Southern Minke Whale by TRAFFIC and as a dwarf form of Minke Whale by JFA. An additional three samples (TR19; TR26; TR39) were identified by each laboratory as being of two different species. Neither laboratory was able to amplify one sample (TR6).

The inability of one or the other of the laboratories to amplify the eight samples referred to above could be a result of problems with the samples themselves, handling prior to or during laboratory analysis, or the use of different marker genes or regions in the mtDNA. Differences in identification at the subspecies or population level may have resulted from the interpretation of results. TRAFFIC and JFA used differing identification techniques (cytochrome B and control region) and different databases as references. However, the differing species results for TRAFFIC and the JFA's analyses of samples TR19, TR26, and TR39 are perplexing.

The presence of Dall's Porpoise in the marketplace is not surprising in itself as Japan recorded catches of this species exceeding 70 000 specimens in the period 1990 to 1993 (Chan *et al.*, 1995). Several possible explanations for the differing results exist. Mixing of meat from different species in the marketplace, either accidentally or deliberately, could result in 'compound' samples. If a single sample was contaminated with tissue from two different species, results could differ between the two

Reference number	TRAFFIC results	JFA results
TR8	Dall's Porpoise	No amplification
TR17	No amplification	Southern Minke Whale ¹
TR19	Dall's Porpoise	Southern Minke Whale
TR26	Dall's Porpoise	Southern Minke Whale
TR27	Dall's Porpoise	No amplification
TR28	No amplification	Southern Minke Whale
TR37	Southern Minke Whale	No amplification
TR38	Southern Minke Whale	North Pacific Minke Whale
TR39	Dall's Porpoise	Southern Minke Whale
TR40	Southern Minke Whale	North Pacific Minke Whale
TR41	No amplification	Southern Minke Whale
TR42	Southern Minke Whale	Dwarf Minke Whale
TR43	Southern Minke Whale	No amplification
TR51	Dall's Porpoise	No amplification

Table 2. Samples for which TRAFFIC and JFA obtained differing results. Source of JFA results: Fisheries Agency, Government of Japan (Anon., 1997). ¹The Southern Minke Whale is referred to in Anon., 1997 as the Antarctic Minke Whale.

laboratories. Contamination during sample acquisition or in the laboratory is also possible. However, Dall's Porpoise had not previously been analysed in the laboratories where TRAFFIC's analysis of sequences was conducted, and the chances of contamination in the laboratory environment were minimal. Moreover, TRAFFIC's analysis recorded five different and unique sequences in the six samples identified as Dall's Porpoise, making it unlikely that all the samples were contaminated.

The reasons for these differing results are unclear, but the differences are more likely owing to human error than to any fundamental problem with using cytochrome B or control region sequences to identify species. TRAFFIC would like to re-examine these samples in co-operation with the JFA to determine the source of the differences.

DISCUSSION

Mills *et al.* (1997) outline three avenues for whale meat of IWC-listed species to enter Japan's retail market legally: via the factory ship of Japan's scientific whaling fleet; from frozen domestic stocks originating from past whaling or importation; and from bycatch in Japan's territorial waters. The samples identified in this study conceivably could have originated from any of these sources. Should CITES in future choose to downlist any of the baleen whales to Appendix II, international trade could provide an additional legal avenue. Meat from small cetaceans, harvest of which is not regulated by the IWC, is also available in the market and may be sold under the generic term 'whale meat.'

While there has been no proof of illegal whale meat entering Japan to date, interception by Japanese and other authorities of a number of attempted illegal shipments has resulted in concern that illegal trade may take place. Under the terms of CITES Resolution Conf. 9.24, proposals to downlist species from Appendix I to Appendix II must show that adequate trade control mechanisms are in place to ensure that removal of the species from Appendix I does not jeopardize that species or other species still in Appendix I. Norway is developing domestic trade control and inspection systems that incorporate DNA profiling and creation of a DNA register of individual whale "fingerprints" as important components. Japan is considering development of a similar system.

In Japan, DNA profiling could provide an effective tool for monitoring whale meat stocks only if samples from all legitimate sources of whale meat were to be included. Under the current domestic management system, a DNA register would need to include samples of meat alleged to originate from frozen domestic stocks and legitimate bycatch as well as samples from Government-controlled whaling. A partial register would be of limited use in determining the legality of whale meat in the marketplace. In any event, DNA analysis cannot determine the length of time samples have been in storage.

An additional consideration is the reliability of this type of analysis for samples sourced from the marketplace where compounding may occur. Analysis of the 53 samples under discussion was carried out by two groups of qualified scientists utilizing differing but standard methodologies in controlled laboratory conditions. Although the results from the two sets of analysis corresponded for the most part, there were significant differences for several samples. A DNA register would need to include appropriate measures for cross-checking the results of DNA analysis.

There is the possibility that all whale species protected under the IWC moratorium on commercial whaling could enter the Japanese market either through existing frozen stocks or bycatch (Mills *et al.*, 1997). Should meat from frozen stocks and bycatch remain outside of a future DNA register, it would continue to be difficult to distinguish meat from some species as legal or illegal in origin. The Government of Japan needs to consider this when developing future trade control mechanisms to meet the requirements of CITES Resolution Conf. 9.24.

REFERENCES

- Anon. (1996). *An Update on the Whale Meat Trade in East Asia*. Traffic East Asia, Hong Kong.
- Anon. (1997). Preliminary analyses on whale products sampled by TRAFFIC Japan. Fisheries Agency, Government of Japan. *Report of the International Whaling Commission No. 49. Information 4*.
- Chan, S., Ishihara, A., Lu, D.J., Phipps, M., and Mills, J.A. (1995). Observations on the whale meat trade in East Asia. *TRAFFIC Bulletin* 15(3):107-115.
- Hillis, D.M., Moritz, C., and Mable, B.K. (1996). *Molecular Systematics*. Second edition. Sinauer Associates, Sunderland MA, USA. 656pp.
- IUCN (1996). *1996 IUCN Red List of Threatened Animals*. IUCN, Gland, Switzerland, and Cambridge, UK. 448pp.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. (1989). Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences, USA*. 86:6196-6200.
- Mills, J.A., Ishihara, A., Sakaguchi, I., Kang, S., Parry-Jones, R., and Phipps, M. (1997). *Whale Meat Trade in East Asia: A Review of the Markets in 1997*. TRAFFIC International, UK.
- Reeves, R.R. and Leatherwood, S. (1994). *Dolphins, Porpoises, and Whales: 1994-1998 Action Plan for the Conservation of Cetaceans*. IUCN, Gland, Switzerland.
- Suzuki, H., Minato, S., Sakurai, S., Tsuchiya, K., and Fokin, I.M. (1997). Phylogenetic position and geographic differentiation of the Japanese dormouse, *Glirulus japonicus*, revealed by variations among rDNA, mtDNA and the Sry gene. *Zoological Science* 14:167-173.

M. Phipps, National Representative, TRAFFIC East Asia-Taipei; A. Ishihara, Programme Officer, TRAFFIC East Asia-Japan; N. Kanda, Division of Veterinary Anatomy, School of Veterinary Medicine, Tokyo University of Agriculture and Technology; H. Suzuki, Division of Biosciences, Graduate School of Environmental Earth Science, Hokkaido University.