Annex N

Report of the Working Group on DNA Testing

Members: Pastene (Chair), Baker, Cipriano, Danielsdottir, Funahashi, Goto, Kanda, Krivokhizhin, Lyrholm, Nagatomo, Paulus, Perrin, Perry, Rojas-Bracho, Skaug, Urban, Weinrich, Weller.

1. ELECTION OF CHAIR

Pastene was elected Chair.

2. ADOPTION OF AGENDA

The agenda is given as Appendix 1. Items 5, 6 and 7 of the agenda are in response to requirements placed on the Scientific Committee by IWC Resolution 1999-8 (IWC, 2000), which called for annual reports on progress in the following areas:

(1) Genetic methods for species, stock and individual identification.
(2) Collection and archiving of tissue samples from catches and bycatch.
(3) Status of and conditions for access to reference databases of DNA sequences or microsatellite profiles derived from directed catches, bycatch, frozen stockpiles and products impounded or seized because of suspected infractions.

3. APPOINTMENT OF RAPPORTEURS

Perrin acted as rapporteur.

4. REVIEW OF DOCUMENTS

Relevant information was contained in SC/55/SD7 and SD8, SC/55/BC2 and Ross *et al.* (2003).

5. PROGRESS ON GENETIC METHODS FOR SPECIES, STOCK, INDIVIDUAL IDENTIFICATION

Before presentation of SC/55/SD7, one of the authors noted an error in the document: in several places 'North Atlantic minke whale' should have been 'North Pacific minke whale'. SC/55/SD7 presented a new genetics method for cetacean species identification. The method is called SINE (short interspersed repetitive element) insertion analysis. It is very effective in terms of scoring data and saves cost and labour. The SINE method is recognised as one of the most powerful tools for inferring phylogeny of organisms, since sharing of the SINE insertion at an orthologous locus can be treated as a diagnostic marker at the molecular level. SC/55/SD7 characterised 13 new SINE loci, combinational use of some of which provides a simple and efficient procedure for identifying baleen whale species. The method is highly effective in that we can easily identify baleen whale species from the presence or absence of the SINE insertion by using PCR followed by agarose-gel electrophoresis. Its advantage is that there is no need to determine sequences of PCR products, allowing us to shorten several steps in the procedure of species identification. Therefore, the time and cost of species identification is considerably reduced. The loci presented here were characterised as by-products during the study of cetacean phylogeny (Nikaido *et al.*, 2001). This method opens a new attractive way for whale species identification, which should be especially useful for their management and conservation.

Members of the group agreed that this is a very powerful tool, including for quick exclusion in forensic use. It can be used for any samples from which nDNA can be extracted. The sequence analysed contains about 500bp, depending on the primer used. It was noted that the development of new SINEs is protracted and expensive, but once the method is developed application is relatively inexpensive. The method may not work for all species; shared ancestral states due to incomplete lineage sorting may be a problem for some. The hope was expressed that the method would be applied to help sort out the sei/Bryde's whale complex.

SC/55/BC2 reported on a new DNA extraction method and PCR primers used to amplify a small fragment of mitochondrial control region sequence used for species identification of whale products. The new methodology was developed in order to be able to analyse some kinds of commercial products in which the DNA has been degraded during processing and/or which contain chemical inhibitors that prevent or limit amplification. Similar difficulties may be encountered when attempting to work with tissue from highly processed whale products, a tissue specimen that has been improperly preserved, or a stranded dolphin carcass that is decomposed or naturally mummified. In addition, commercial products may contain mixtures of tissue from different individuals and different species. Mixed tissues may result in mixed PCR products – products amplified from different types of DNA and therefore having different DNA sequences. Such products can be separated out for accurate sequence analysis using molecular cloning, but this increases the time, difficulty and cost of sequence analysis. In development of the new method three improvements were attempted: (1) a more effective extraction method, able to capture and then purify degraded and chemically treated DNA from 'difficult' samples; (2) new primers that amplified a short stretch of still-informative DNA sequence; and (3) more cetacean-specific primers, able to preferentially amplify DNA from cetaceans in 'mixed' and highly processed products. The method is designed to preclude contamination through the use of pre-packaged and quality-controlled materials from molecular biology 'kits', which are supplied in a format which minimises handling.

The method has been successful in some initial tests with highly processed and canned products, and may also be applicable to poorly preserved and unpreserved samples from strandings and museum collections. The small fragment amplified using the new primers was sufficient for precise analysis of products from some whale species at high bootstrap values (e.g. Antarctic minke whales), but additional primers must be developed in order to provide enough data for precise identification of other species (e.g. sei and Bryde's whales, delphinids). One sample yielded two sequences from amplifications using two different primer sets – pig sequence was identified from the older and less-specific (dlp1.5-dlp5) amplification, while the amplification product from the new cetacean-specific primers (CTR5F-CTR3R) yielded dolphin sequence. Sequence differences (between 1 and 5 bp differences in pairwise comparisons, out of 155 bp of analysed sequence) between Antarctic minke specimens processed and analysed in the same sample set, is expected based on the high genetic diversity noted for this species, and provides evidence that the identifications were not biased by contamination, which would likely result in identical sequences for all products tested. It appears that this new approach is an effective and accurate method for species identification of some sample types which were previously difficult to analyse. The method has not yet been tested on formalin-fixed or bleached whale products (which are known to be difficult to amplify) or on cetacean tooth and bone extracts.

In response to a question, Cipriano noted that the cost of materials used for the enhanced extraction method is relatively high at around US$3 per extraction, but that additional costs for PCR and sequencing are the same as for fresh and properly preserved tissue samples. He noted that no toxic chemicals are involved in the extraction. The method can be used for samples containing products from both a cetacean and one or more non-cetaceans, but if more than one cetacean is involved, the PCR products must be cloned. It was noted that the method may be useful in SINE research; some of the SINES may be polymorphic.

Ross *et al.* (2003) and SC/55/SD8 presented updates on development and implementation of *DNA Surveillance*, a Web-based service to assist with the species identification and ultimately, population identification, of cetaceans and other taxa threatened by exploitation or protected by international agreements. The program aligns a user-submitted DNA 'test' sequence with curated datasets of pre-aligned reference sequences and returns a phylogenetic tree (including bootstrap values if desired) showing the relationship of the test sequence to the reference sequences. A table summarising the evolutionary distances between the test sequence and each of the members in the reference dataset is also provided. *DNA Surveillance* currently consists of two reference databases, for the 5' end of the mitochondrial (mt) DNA control region (285-476 base pairs depending on the dataset) and cytochrome *b* (405 bp) for cetaceans. These datasets are not yet fully validated following the criteria established in Dizon *et al.* (2000). Additional information from major contributors, regarding the provenance of specimens used in the database, will be required to complete validation. This information will be presented in an upcoming publication. A total of 87 species (in 14 families) of the 89 cetacean species currently recognised are represented in one or both databases (mtDNA control region, 285 sequences representing 78 species; mtDNA cytochrome *b*, 165 sequences representing 83 species). In most cases, species are represented by several reference sequences obtained from different populations or 'stocks' allowing potential assignment of test sequence to geographic origin. Sets of reference sequences are arranged hierarchically, from an 'All Cetaceans' set enabling initial family level identification, to Mysticete and Odontocete sets, through to family and subfamily-specific sequence sets for odontocetes (Ziphiidae and Phocoenidae, and for the Delphinidae; sets for Globicephalinae/Orcininae, Delphininae/Stenoninae, and Lissodelphininae). Comprehensive phylogeographic sets of reference sequences are also under development for many cetacean species, together with reference databases for other endangered and exploited taxa. The service, including instructions and sample data, is available at: *http://www.dna-surveillance.auckland.ac.nz.*

In response to a question, Baker noted that the taxonomic uncertainty surrounding the sei/Bryde's whale complex is not a problem; sequences can easily be identified to one of the major haplotype clades so far documented to exist in the complex.

6. PROGRESS ON COLLECTION AND ARCHIVING OF SAMPLES FROM CATCHES AND BYCATCHES

Skaug reported that all minke whales caught by Norway in 2002 have been entered in the Norwegian DNA register. The register does not include bycaught whales.

No information on collection and archiving of samples in Japan was available to the Working Group. It was noted that provision of a progress report on collection and archiving of samples would assist the Working Group in meeting its terms of reference as put forth by the Commission.

7. REFERENCE DATABASES AND STANDARDS FOR A DIAGNOSTIC REGISTER OF DNA PROFILES

No new information was available on this topic. Again, it was emphasised that progress reports on development of the databases and standards for the Norwegian and Japanese registers would aid the Working Group in fulfilling its remit as assigned by the Commission. Pastene agreed to confer with Skaug to develop a list of items that would be useful for inclusion in the annual report to the Committee.

8. WORK PLAN

The terms of references for the Working Group for the next year's meeting will remain the same, unless the Commission request other information in the interim.

9. ADOPTION OF REPORT

The report was adopted by consensus.

REFERENCES

Dizon, A., Baker, S., Cipriano, F., Lento, G., Palsbøll, P. and Reeves, R. 2000. Molecular Genetic Identification of Whales, Dolphins, and Porpoises: Proceedings of a Workshop on the Forensic Use of

Molecular Techniques to Identify Wildlife Products in the Marketplace, La Jolla, CA, USA, 14-16 June 1999. US Department of Commerce, NOAA Technical Memorandum, NOAA-TM-NMFS-SWFSC-286. 52pp+xi. [Available from: *http://www.nmfs.gov*].

International Whaling Commission. 2000. Chairman's Report of the Fifty-First Annual Meeting. Appendix 9. IWC Resolution 1999-8. Resolution on DNA testing. *Ann. Rep. Int. Whaling Comm.* 1999:55.

Nikaido, M., Matsuno, F., Hamilton, H., Brownell, R.L., Cao, Y., Ding, W., Zuoyan, Z., Shedlock, A.M., Fordyce, R.E., Hasegawa, M. and Okada, N. 2001. Retrospon analysis of major cetacean lineages: the monophyly of toothed whales and the paraphyly of river dolphins. *Proc. Natl Acad. Sci. USA* 98:7384-9.

Ross, H.A., Lento, G.M., Dalebout, M.L., Goode, M., Ewing, G., McLaren, P., Rodrigo, A.G., Lavery, S. and Baker, C.S. 2003. DNA surveillance: web-based molecular identification of whales, dolphins, and porpoises. *J. Hered.* 94(2):111-4.

Appendix 1

AGENDA

1. Election of Chair
2. Adoption of agenda
3. Appointment of rapporteurs
4. Review of documents
5. Progress on genetic methods for species, stock, individual identification
6. Progress on collection and archiving of samples from catches and bycatches
7. Reference databases and standards for a diagnostic register of DNA profiles
8. Work plan
9. Adoption of the report