

## Annex N

### Report of the Working Group on DNA

**Members:** Pastene (Chair), An, Cipriano, Gaspari, Goto, Hoelzel, Kanda, Palsbøll, Skaug, Verborgh, Víkingsson, Waples.

#### 1. ELECTION OF CHAIR

Pastene convened and chaired the Group.

#### 2. APPOINTMENT OF RAPORTEURS

Hoelzel and Pastene acted as rapporteurs.

#### 3. ADOPTION OF AGENDA

The adopted agenda is given as Appendix 1. Items 5, 6, 7 and 8 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, stocks 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.

#### 4. REVIEW OF DOCUMENTS

Relevant information was contained in SC/61/SD2 and SC/61/SD3.

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

Last year the Committee noted that for the past several years various techniques to extract and amplify DNA from 'difficult' samples have been presented and discussed and agreed to review current knowledge at the 2009 meeting (IWC, 2009a, p.60).

SC/61/SD2 reviewed current knowledge of techniques to extract and amplify DNA from 'difficult' whale samples. DNA analysis methods currently used for whale product identification are dependent on extraction and PCR amplification of cetacean nucleic acids, but certain product types and intensive processing may restrict the amount of DNA recovered or degrade the DNA and inhibit amplification. Newly developed methods developed for 'ancient DNA' and human forensic analysis may provide for more robust extraction from and amplification of cetacean tissues, including: (1) bone demineralization procedures which can release 10X more DNA than standard methods, (2) optimized silicon-capture techniques that more effectively isolate and purify DNA, (3) post-extraction purification that can remove inhibitors from degraded and chemically treated DNA isolated from 'processed' samples, (4) whole genome amplification methods which increases the amount of DNA template material prior to PCR amplification, and (5) use of a variety of different DNA polymerases which can amplify cetacean DNA with high specificity, even in a background containing degradation products and other inhibitors.

In discussion it was noted that not much success has been obtained in extracting and amplifying DNA from samples fixed in unbuffered formalin, the low pH and degradation of the DNA caused by that being the main problem. However DNA from samples fixed in buffered formalin have been successfully extracted and amplified for up to about 500bp.

The flow chart in Figure 1 of SC/61/SD2 showed various extraction/PCR options for 'difficult' cetacean product types. The Group considered this flow chart informative and appropriate to respond the recommendation from the Committee last year. The flow chart and associated references for alternative extraction techniques is included below as Appendix 2.

#### 6. SECOND ROUND OF SEQUENCE ASSESSMENT FOR SPECIES ASSIGNMENT AND PLAN FOR FUTURE SEQUENCE ASSESSMENT

Last year the Committee agreed to conduct a second round of *GenBank* sequence assessment for species assignment of baleen whale sequences deposited in *GenBank* in 2007. Specifications for this assessment were similar to those in the first round of sequence assessment (IWC, 2008 p. 339):

- (i) List the *GenBank* accession number and species identity of each mysticete control region sequence with the species identity as determined using the most recent version of the Witness for the Whale (WFTW) reference sequence alignments (see SC/59/SD5) and the DNA Surveillance software engine.
- (ii) The above list to be supported by phylogenetic trees, one per sequence, showing the placement of the *GenBank* sequence in relation to the reference sequence.
- (iii) An evaluation of the types of inconsistencies/errors as agreed by the Committee last year: quality of submitted sequences, accuracy of species identification and, where possible, accuracy of geographical location.

On behalf of the authors the Chair presented SC/61/SD3, which contained the results of the second round of sequence assessment in *GenBank*. The phylogenetic methods in *DNA Surveillance*, in conjunction with the WFTW, were used to assign species identities to the 499 sequences from baleen whales published in *GenBank* in 2007.

Regarding the accuracy of species identification all the sequences were assigned to the same species as that recorded in *GenBank*: 99 bowhead whales (*Balaena mysticetus*), 74 common minke whales (*Balaenoptera acutorostrata*), 115 Antarctic minke whales (*B. bonaerensis*), 52 Bryde's whale (*B. brydei*), 44 blue whales (*B. musculus*) and 115 humpback whales (*Megaptera novaeangliae*).

The authors of SC/61/SD3 also attempted to assign common minke whales to the sub-species recognized by Rice (1998). All the sequences labelled in *GenBank* as belonging to common minke whales were assigned to one of the three sub species, *B. acutorostrata acutorostrata* (North

Atlantic), *B. a. scammoni* (North Pacific) and *B. a. sub sp* (dwarf minke whale from the Southern Hemisphere). There was uncertainty regarding whether blue whale sequence could be assigned to a sub-species.

All the sequences appeared to be of reliable quality. No geographic information was recorded for nearly all the sequences deposited in *GenBank* in 2007.

In discussion the Group noted that there is no consistent method for submitting information on the geographic source of samples in *GenBank* and various authors entered this information in different fields in the database, if at all. The Chair pointed out that the information on the geographic origin of the large number of common minke whale sequences published in 2007 was not available at *GenBank* but that it could be obtained by crosschecking with the information available in a recent publication (Pastene *et al.*, 2007). Further he noted that two sequences from dwarf minke whale (EF113859 and EF113861) in Table 3 of SC/61/SD3 had been erroneously assigned to North Atlantic minke whale during the species assignment validation exercise. This was due to large genetic differences reported among dwarf minke whales from different regions of the Southern Hemisphere (some being closer related to North Atlantic common minke whales) (Pastene *et al.*, 2007; Pastene *et al.*, in press) that are not yet represented in the reference sequence (WFTW) database.

Evaluation of the accuracy of geographical location is complicated because very few sequences in *GenBank* are accompanied with information on location and because of the limited geographic representation currently in the reference datasets. If sample location is to be validated using the WFTW reference sequences, then species-specific alignments containing representative haplotypes from all major populations will be needed. To provide a better validation of sample origin, all major geographic regions need to be represented for each species in the reference data sets.

The Group concluded that the second round of sequence assessment was a useful exercise and confirmed no major issues with species IDs of recently submitted cetacean sequences in *GenBank*. Some disagreements were found during the first round of sequence assessment (IWC, 2009b pp. 347) but these appear to be due to a lag in the taxonomy recognized by *GenBank* or uncertainty in taxonomic distinctions currently under investigation (e.g. the number of species and appropriate names for recently described species of 'Bryde's whales'). All the sequences appeared to be of reliable quality. The Group recognized the importance of having geographic collection information associated with DNA samples and **recommended** that authors to submit this information to *GenBank* or in associated publications.

As agreed by the Committee in previous years, any anomaly detected in the species identity assessment will be shared with members of the Committee. The original submitter would be notified of the inconsistency and a suggestion made that an amendment be made to the entry. The Group nominated Pastene to carry out this work. A summary of amendments as derived from the results of the first round of sequence assessments (IWC, 2009b p. 347) is shown below:

23 labelled as *Balaenoptera acutorostrata* in *GenBank* were identified as *B. bonaerensis*.  
9 labelled as *B. edeni* in *GenBank* were identified as [...to come]  
10 labelled as *Eubalaena glacialis* in *GenBank* were identified as *E. australis* and *E. japonica*.

Given that the results of the two rounds of sequence assessment exercises indicate no major problems with the species assignment in *GenBank* the Group **agreed** that assessments of *GenBank* sequences be conducted less often, and periods of three or five years were suggested.

The Group again noted the importance of submission of DNA sequences and related specimen data to the work of the Committee and **recommended** that upon publication of their work cetacean researchers submit associated sequences to one of the linked databases: *GenBank* (at the US National Center for Biotechnology Information - <http://www.ncbi.nlm.nih.gov/>), EMBL (at the European Molecular Biology Laboratory - <http://www.ebi.ac.uk/embl/>) or DDBJ (the DNA database of Japan - <http://www.ddbj.nig.ac.jp/>).

## 7. PROGRESS ON COLLECTION AND ARCHIVING OF SAMPLES FROM CATCHES AND BYCATCHES

Skaug reported on the status of the Norwegian register (Appendix 3). The collection of samples includes commercial catches of common minke whales from 1997 to 2008. The number of samples missing from the register by year ranged from 0-11. Some of the missing samples reflect unsampled whales, while others resulted from inadvertent duplicates.

Goto reported on the status of the Japanese register (Appendix 4). The collection of samples is from scientific whaling in the Antarctic (JARPA-JARPA II) and North Pacific (JARPN II), bycatches and strandings. It includes complete coverage for 2008 and the 2008/2009 Antarctic season.

Vikingsson reported on the status of the Icelandic register (Appendix 5). Samples are presently in hand for all whales taken in 2003-2008.

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

Genetic analyses have been completed and data on mtDNA, microsatellites and sex entered in the Norwegian register for years through 2007 (Appendix 3). Laboratory work is being conducted for samples collected in 2008.

For the Japanese register (Appendix 4), the genetic analyses based on mtDNA have been completed for North Pacific common minke, Bryde's, sei and sperm whales taken by scientific whaling through 2008. Laboratory work on microsatellites for these samples is being conducted. The genetic samples of Antarctic minke whales have not been analyzed yet, except for sex and for microsatellites of 190 samples taken in 2006/07 (six loci) and 551 taken in 2007/08 (three loci). For bycatch samples, genetic analyses based on mtDNA have been completed for all samples through 2008. Laboratory work on microsatellites for these samples is being conducted. Work is ongoing for stranded animals in 2008 for both mtDNA and STR.

In response to a question it was clarified that an additional three loci are being analyzed for the Antarctic minke whales taken in the 2007/08 season, to be consistent with the six loci analyzed for earlier samples.

For the Icelandic register (Appendix 5) genetic analyses (mtDNA and microsatellites) were completed for common minke whales taken by scientific whaling in 2007. Laboratory work of samples taken under commercial whaling in 2007 and 2008 is under way.

The Chair reminded the Group that reports of updates of registers should include a list of references including the relevant documents on protocols used.

## 9. WORK PLAN

The terms of reference for the Working Group will remain the same for the next year, unless the Commission requests other information in the interim. Members of the Working Group were encouraged to submit papers relating to these terms of reference and to propose additional agenda items. Results of the 'amendment' work on sequences deposited in *GenBank* will be reported next year.

## 10. ADOPTION OF THE REPORT

The report was adopted by consensus.

## REFERENCES

- International Whaling Commission. 2000. Chairman's Report of the Fifty-First Annual Meeting. Appendix 9. IWC Resolution 1999-8. Resolution on DNA testing. *Rep. Int. Whaling Commn* 1999:55.
- International Whaling Commission. 2008. Report of the Working Group on DNA. *J. Cetacean Res. Manage.* 10 (Suppl.):336-340.
- International Whaling Commission. 2009a. Report of the Scientific Committee, *J. Cetacean Res. Manage.* 11 (Suppl.):1-406
- International Whaling Commission. 2009b. Report of the Working Group on DNA, *J. Cetacean Res. Manage.* 11 (Suppl.):344-349.
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- Pastene, L.A., Acevedo, J., Goto, M., Zerbini, A.N., Acuna, P. and Aguayo-Lobo, A. (in press). Population structure and possible migratory links of common minke whales, *Balaenoptera acutorostrata*, in the Southern Hemisphere. *Conserv. Genet.*
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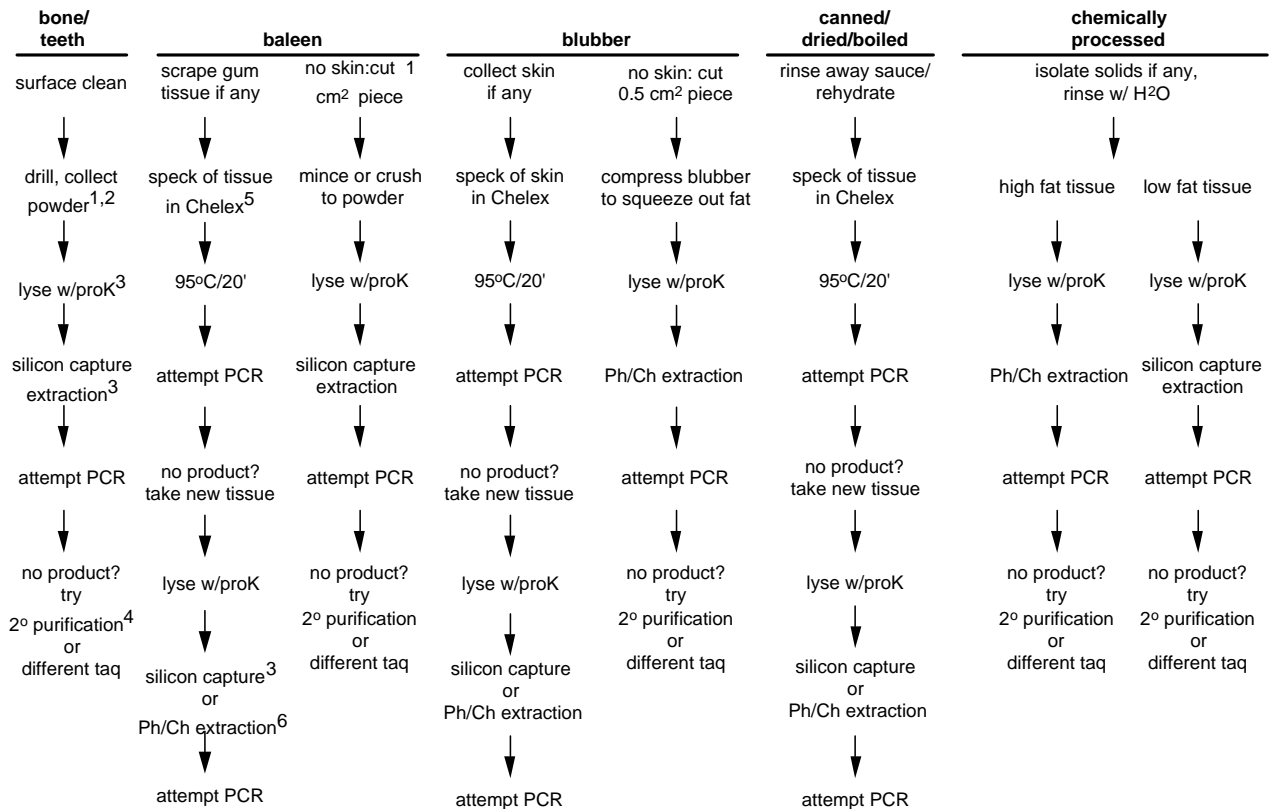
## Appendix 1

### AGENDA

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

Appendix 2

FLOW CHART FOR TECHNIQUES OF DNA EXTRACTION AND AMPLIFICATION FOR 'DIFFICULT' CETACEAN SAMPLES



Footnotes: (approximate per sample extraction cost in US \$ shown in brackets for certain extraction techniques): <sup>1</sup>Borge *et al.* 2007; <sup>2</sup>Nichols *et al.* 2007; <sup>3</sup>Rohland and Hofreiter 2007 (\$0.45 glass milk, \$4.00 column/kil); <sup>4</sup>Kim *et al.* 2008; <sup>5</sup>Baker *et al.* 1996 (\$0.15); <sup>6</sup>Sambrook *et al.* 1989 (\$2.50).

REFERENCES

Baker, C.S., Cipriano, F. and Palumbi., S.R. 1996. Molecular genetic identification of whale and dolphin products from commercial markets in Korea and Japan. *Molec. Ecol.* 5:671-685.

Borge, T., Bachmann, L., Bjørnstad, G. and Wiig, Ø. 2007. Genetic variation in Holocene bowhead whales from Svalbard. *Molec. Ecol.* 16:2223-2235.

Kim, K., Kim, K.-Y., Jeon, E., Togloom, A., Cho, Y.-O., Lee, M.-S., Lkhagvasuren, G., Choi, J.-H., Tumen, D., Park, A.-J., Kim, K.-C., Park, K.-W., Kim, J.-H., Noh, M., Yoo, K.-J. and Lee, K.-H.. 2008. Technical Note: improved ancient DNA purification for PCR using ion-exchange columns. *Amer. J. Physical Anthropology* 136:114-121.

Nichols, C., Herman, J., Gaggiotti, O.E., Dobnet, K.M., Parsons, K. and Hoelzel, A.R.. 2007. Genetic isolation of a now extinct population of bottlenose dolphins (*Tursiops truncatus*). *Proc. R. Soc. B.* (2007) 274:1611-1616.

Rohland, N. and Hofreiter, M.. 2007. Comparison and optimization of ancient DNA extraction. *BioTechniques* 42:343-352.

Sambrook J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular cloning*. Cold Spring Harbor Laboratory Press, Cold Spring Harbour, NY.

### Appendix 3

#### STATUS OF THE NORWEGIAN COMMON MINKE WHALE DNA-REGISTER BY MAY 2009

Hans Julius Skaug

The following table shows an update of the Norwegian DNA register for common minke whales.

Year	DNA-register <sup>1</sup>	IWC catch statistics <sup>2</sup>	Not landed <sup>3</sup>	Landed <sup>4</sup>	Duplicates <sup>5</sup>	Missing samples <sup>6</sup>	Lab problem <sup>7</sup>	Total missing <sup>8</sup>
1997	488	503	7	496	3	5	0	8
1998	609	625	11	614	1	4	0	5
1999	571	591	17	574	2	1	0	3
2000	470	487	6	481	3	8	0	11
2001	538	552	11	541	2	1	0	3
2002	625	634	9	625	0	0	0	0
2003	637	647	9	638	1	0	0	1
2004	530	544	7	537	7	0	0	7
2005	626	639	6	633	3	4	0	7
2006	531	545	7	538	4	2	1	7
2007	575	597	5	592	6	11	0	17
2008 <sup>9</sup>	-	536	4	532	-	-	-	-

The number of individuals contained in the DNA-register, and the number of individuals missing. For 2008 the genetic analyses are not yet completed, as indicated by the '-' in the table.

<sup>1</sup> Number of unique individuals contained in the DNA-register (not containing duplicates).

<sup>2</sup> Number of individuals caught by Norway, including individuals not landed.

<sup>3</sup> Number of individuals killed, but not taken onboard the vessel.

<sup>4</sup> Number of individuals taken onboard the vessel.

<sup>5</sup> Number of occurrences of (tissue) sample switching on board the vessel as detected by comparison of genetic profiles. The result is that two samples have been returned from one individual, and no sample has been returned for one individual.

<sup>6</sup> Number of individuals for which tissue samples are missing for other reasons than sample switching.

<sup>7</sup> Genetic laboratory not able to obtain microsatellite profile from tissue sample.

<sup>8</sup> The difference between the columns 'Landed' and 'DNA-register'.

<sup>9</sup> Laboratory analyses yet not completed.

**Appendix 4**  
**AN UPDATE OF THE JAPANESE DNA REGISTER FOR LARGE WHALES BY MAY 2009**

Mutsuo Goto and Naohisa Kanda

*The Institute of Cetacean Research*

The following table shows an update of the Japanese DNA register for large whales.

Source/Species	Period	Genetic samples	mtDNA	STRs	Sex
<b>Scientific whaling</b>					
NP minke whale	08	169	169	*	169
NP Bryde's whale	08	50	50	*	50
NP sei whale	08	100	100	*	100
NP sperm whale	08	3	3	*	3
Antarctic minke whale	05/06	853	0	0	853
	06/07	505	0	190	505
	07/08	551	0	551**	551
	08/09	679	0	0	679
Antarctic fin whale	08/09	1	0	0	1
<b>Bycatches</b>					
NP minke whale	08	133	133	*	*
NP humpback whale	08	2	2	*	*
NP fin whale	08	1	1	*	*
<b>Strandings</b>					
NP minke whale	08	4	0	0	0
NP sperm whale	08	2	0	0	0
Unidentified baleen***	08	1	0	0	0

As explained in IWC (2006), sex of the whales taken by scientific whaling was determined by scientists onboard the research vessels. 0 = not yet analyzed at the time this Appendix was prepared. STR = microsatellites, NP = North Pacific; \*: Under analysis. \*\*: Three loci. \*\*\*: DNA extraction failed due to sample condition.

**Reference**

International Whaling Commission. 2006. Report of the Working Group on DNA testing. *J. Cetacean Res. Manage. (Supple.)* 8: 252-258.

**Appendix 5**  
**STATUS OF THE ICELANDIC COMMON MINKE AND FIN WHALES DNA REGISTER\***

Gisli A. Víkingsson

*Marine Research Institute*

Practical arrangements regarding the establishment of the Icelandic DNA register were concluded in early 2007. The Marine Research Institute, Reykjavik, is responsible for the establishment and maintenance of the registry that is of the same format as the Norwegian DNA registry.

Table 1 gives the present status of the registry. Samples from all the common minke whales (*Balaenoptera acutorostrata*) landed as a part of the Icelandic research program to date (2003-2007) as well as from commercial catches of one minke whale and seven fin whales (*B. physalus*) have been archived. Genetic analyses have been completed for fin whales and for common minke whales taken under special permit. Genetic analyses of common minke whales taken for commercial purposes 2006-2008 are under way.

Table 1  
Icelandic common minke and fin whales DNA register.

Year	Type <sup>1</sup>	No. genetic samples	Microsatellites	MtDNA	Sex
<b>Common minke whales</b>					
2003	SP	36	36	36	36
2004	SP	25	25	25	25
2005	SP	34	34	34	34
2006	SP	58	58	58	58
2006	C	1	0	0	0
2007	SP	36	36	36	36
2007	C	6	0	0	0
2008	C	36	0	0	0
<b>Fin whales</b>					
2006	C	7	7	7	7

<sup>1</sup>SP= Special Permit catch; C=commercial catch.

\*Olaisen, B. 1997. Proposed specifications for the Norwegian DNA database register for minke whales. Paper SC/49/NA1 presented to the IWC Scientific Committee, September 1997, Bournemouth (unpublished). 7pp