

Annex N

Report of the Working Group on DNA

Members: Pastene (Chair), Archer, Baird, Baker, Bickham, Cipriano, George, Double, Hoelzel, Lang, Luna, Park, Skaug, Solvang, Suydam, Tiedemann, Vikingsson, Waples, Yoshida.

1. ELECTION OF CHAIR

Pastene convened and chaired the Working Group.

2. APPOINTMENT OF RAPPORTEURS

Pastene acted as rapporteur, assisted by Tiedemann.

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; and
- (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

Documents SC/66a/SD03, SC/66a/BRG12, Keane *et al.* (2015) and Seim *et al.* (2014) were relevant for the Working Group.

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

SC/66a/SD03 described a project to verifying the status, storage conditions and metadata of samples from stranded cetaceans collected by the Department of Primary Industries, Parks, Water and Environment (DPIPWE) and the Tasmanian Museum and Art Gallery (TMAG). The samples date from 1862 to the present, and the verified sample collection contains 4,349 specimens held over two collections at DPIPWE and TMAG. The composition of the sample collections reflects the functions within each institution, with an average of over four samples collected from each individual within the DPIPWE collection and an average of only 1.1 samples per individual within TMAG. There are over 27 sample types within the collection. The majority of material consists of skin samples, intended for genetic studies and confirmation of species identification and gender determination. Blubber, teeth and jaws are also routinely collected. Other less frequently collected sample types include stomach contents, parasites, major organs, dorsal fins, skeletal material and occasionally entire carcasses. A summary of previous or ongoing research using samples obtained through the Tasmanian Government sample holdings was also presented. The collection metadata set is to be made publicly available through the National Marine Mammal Database¹, DPIPWE's Natural Values Atlas², and

will also be updated on the Atlas of Living Australia³. For further information on the collection or for scoping potential collaboration contact: whales@dPIPWE.tas.gov.au.

The Working Group was pleased to receive this information and **commended** the work done. The project developed by the DPIPWE and TMGA could serve as a model for other research organisations on storage conditions and metadata from stranded cetaceans. The project would eventually optimise the use of samples and data of cetacean by the scientific community.

Keane *et al.* (2015) described the genome and transcriptome sequences of bowhead whales and analysed them for evidence of genes associated with aging and disease protection, and other adaptations. Bowhead whales are the longest-lived species of mammal and grow to 100 tons which is 1,000x the size of a human. To achieve this they must have genetic based protective mechanisms to prevent cancer, immunosenescence, neurodegenerative diseases, etc. To investigate the genetic basis for longevity and other adaptations, a single female bowhead from Greenland was sequenced for the genome and two Greenland and two Alaska bowheads were sequenced for the transcriptome. The bowhead genome assembly is approximately 2.3 Gb (billion base pairs) in length which is comparable to other cetaceans. The transcriptome (RNA sequence) of seven tissues contained 22,672 predicted protein coding genes. The frequency of Single Nucleotide Polymorphisms (SNPs) in RNA sequences is approximately 0.5 to 0.6 SNPs per 1,000 bases. High levels of sequence conservation were found in comparisons of bowhead to minke whales (96% sequence identity), dolphin (92%), and cow (91%). Using various methods of analysis, genes were identified as candidates for adaptations to aging, cancer protection, DNA repair, sensory perception of sound, growth, thermoregulation, immune system, blood homeostasis, digestive system, dentition, and adipogenesis. Although genome sequences are available for many species of importance to medical research and to agriculture, this is the first genome sequence of a species of primary importance to a subsistence diet.

Seim *et al.* (2014) described the transcriptome sequence from four B-C-B bowheads for liver ($n=4$), heart ($n=1$) and kidney ($n=3$). A total of 9,395 candidate protein coding genes were identified based upon sequence homology to genes of the minke whale, cow, yak, bat, tree shrew, monkey, mouse and rat. In liver, 45 genes were differentially expressed in the bowhead and included genes associated with insulin signaling. This is likely indicative of genetic adaptations to a lipid rich diet as compared to terrestrial relatives of whales, especially artiodactyls, which are adapted to a carbohydrate rich diet. Other genes were identified that are likely associated with hypoxic stress, vascular development, and DNA repair. Study of the bowhead heart transcriptome revealed genes associated with cardiac metabolism and likely adaptations to hypoxia, a key associate to their diving capability, and to vascular ageing. In the kidney, 53 genes were identified with differential expression in the bowhead and included known DNA repair genes. These could be key to the prevention of age-related kidney decline that is known to result from the reduced ability of kidney cells in ageing humans and other mammals to repair and proliferate.

¹<http://data.marinemammals.gov.au>.

²<https://www.naturalvaluesatlas.tas.gov.au>.

³<http://www.ala.org.au>.

The Working Group **commended** the large amount of work and valuable information produced in these two published studies. It **recognised** that the availability of both a genome (Keane *et al.*, 2015) and a transcriptome (Seim *et al.*, 2014) for the bowhead whale are a valuable resource for future investigations in an IWC Scientific Committee context, namely: (i) as a source for potentially informative markers (SNPs), which are useful in the context of stock definition/DNA registers; and (ii) to facilitate the estimation of (effective) population size.

Keane *et al.* (2015) reported inference of genes putatively under selection based on dN/dS ratio assessment. It was noted that population expansion after a bottleneck may create the same type of signal as positive selection. Previous studies on bowhead whales however did not reveal any genetic depletion attributable to a population bottleneck.

In the context of Seim *et al.* (2014), technical aspects as to immediate post-mortem access to tissue (liver) samples to prevent/minimise RNA degradation were discussed. This remains a challenge issue in cetaceans and might partially explain while only very limited transcriptome data are available for cetaceans so far.

Both papers consider the bowhead whale an interesting model species in the context of ageing and cancer research, because of its longevity. Such research (albeit hitherto not far progressed) might ultimately have medical (and hence commercial) applications. This could be an issue in an IWC context as bowheads are caught under the AWMP.

George commented on some concerns expressed by the Alaskan Eskimo whale hunting community regarding their discomfort with the use of genetic samples from subsistence harvested bowheads for human cancer and longevity studies. Their concern is primarily about the possible use of the bowhead tissues they provide (without compensation) for commercial gain. Other concerns were spiritual in nature. A workshop sponsored by the North Slope Borough on the bowhead genomics program is planned for October 2015 in which technical papers as well as these issues will be addressed.

Finally the Working Group **appreciated** that the genomic resources accumulated in Keane *et al.* (2015) and Seim *et al.* (2014) are published and hence publicly available.

SC/66a/BRG12 summarised the progress made toward two goals of the bowhead genetics project: (1) building a mtDNA database; and (2) developing a SNP panel and database. The authors continue to sequence three mitochondrial genes (control region, *cyt-b*, and *ND1*), as this combination has been shown to have more power in resolving relationships than the commonly used control region alone. To date, there is data from 711 whales: 447 sequenced for *cyt-b*, 427 for *ND1*, and 638 for the control region. Of these, 345 whales are completed for all three loci. A summary of methods used for choosing a SNP panel and assay method was given. Of the 155 previously identified bowhead SNPs, the authors chose a subset of 96 loci based on the following criteria: desire to include all sex chromosome markers, minimising linkage among loci, and ease in developing primers to amplify the SNP. The authors summarised their testing of four methods: the Illumina Miseq method, Taqman, high resolution melting analysis, and Fluidigm SNP-type assay. Pros and cons of each method were discussed, as well as relative costs for each analysis. The Fluidigm SNP-type assay was ultimately utilised in the bowhead genetics project due to low costs, low workload, and ease of data interpretation and replication. The 96-SNP panel designed with Fluidigm's system included 83 autosomal markers and 13 sex chromosome markers. The authors pointed out that this SNP panel was derived from SNPs identified in previous studies using a combination of methods, including whales from three populations. This should minimise ascertainment bias as much as possible.

Both the mtDNA and SNP data will continue to be used for monitoring stock structure, population size estimates, and historical demography of bowheads.

Last year the Working Group received information on the bowhead whale genetic project (Baird *et al.*, 2014). SC/66a/BRG12 provided additional information on this project. The Working Group again **commended** the amount of work involved in this study. In particular this information was relevant for the work of the Working Group as the SNPs could potentially replace microsatellite markers in the national DNA registers for large whales.

The Working Group also **acknowledged** that the paper presented valuable information for SNP assessment in a stock definition context, as it compares methods with regard to effectiveness, reliability, and costs of SNP identification and typing. Applicability for low-quality samples was also covered. The Working Group **agreed** that a comparison of the methods presented in SC/66a/BRG12 to SNP assessment performed by ddRAD sequencing (Lah *et al.*, 2014) would be desirable and encouraged this work for SC/66b. ddRAD-sequencing might be complicated by low DNA quality, but new methods have been developed to overcome this potential difficulty (Tin *et al.*, 2014).

6. REVIEW RESULTS OF THE 'AMENDMENTS' OF SEQUENCES DEPOSITED IN GENBANK

In previous years the Committee agreed that the list of accession numbers involving inconsistencies due to a lag in the taxonomy recognised by *GenBank* or uncertainty in taxonomic distinctions currently under investigation (IWC, 2014b) should be sent to *GenBank* with a letter explaining the background and the main reasons for the inconsistencies (IWC, 2014a, p.56).

Last year the Working Group **agreed** that Cipriano should keep in contact with *GenBank* in the next intersessional period to facilitate the work by *GenBank* staff on the correction of the inconsistencies based on the list sent.

Cipriano informed the Working Group of the work done interessionally. He had corresponded with Scott Federhen at the National Center for Biotechnology Information (NCBI)⁴ about continued progress in developing mechanisms for taxonomy updates. Two additional initiatives are planned or underway: (i) one mechanism being used (currently only for bacteria) is genome sequencing from type specimens, in order to find and correct the vast majority of the misidentified sequences in *GenBank*; and (ii) another mechanism being considered is to allow annotation of *GenBank* sequences by interested parties, in order to note taxonomic mis-assignment or questions about geographic source of the organisms involved.

The second proposed mechanism is similar to the addition of comments to information in the *PubMed Commons*, and in fact the abstracts related to some cetacean sequences (e.g. EF540867 and EF540868) in the *PubMed Commons* have already been annotated by individuals other than the original submitter, in order to highlight potential problems with taxonomic assignment.

The Group **thanked** Cipriano for his work and **encouraged** him to keep contact with NCBI in the next intersessional period to have further discussion and to make progress on the second proposed mechanism.

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

The Committee previously endorsed a new standard format for the updates of national DNA registers to assist with the

⁴National Center for Biotechnology Information, National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20889, USA.

review of such updates (IWC, 2012, p.53), and the new format has worked well. This year the update of the DNA registers by Japan, Norway and Iceland were based again on this new format.

Yoshida reported on the status of the Japanese register (see Appendix 2). The collection of samples is from scientific whaling in the North Pacific (JARPN-JARPN II) and the Antarctic (JARPA-JARPA II), and from bycatches. It includes coverage for 1994-2014 (JARPN-JARPN II), 1987/88-2013/14 (JARPA-JARPA II). In the case of bycatches it includes coverage for 2001-14.

Skaug reported on the status of the Norwegian register (see Appendix 3). The collection of samples of North Atlantic common minke whale is from commercial catches for the period 1997 to 2014.

Vikingsson reported on the status of the Icelandic register (see Appendix 4). The collection of samples is from scientific whaling and from commercial catches. It includes coverage for 2003-07 (scientific whaling) and 2006-14 (commercial catches). Samples are presently in hand for all whales taken in 2003-14.

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

An update of the Japanese register is shown in Appendix 2. For North Pacific minke whales sampled under JARPN II in 2014, mtDNA and microsatellite analyses of 100% ($n=81$) has been completed. For animals bycaught in 2014, mtDNA and microsatellite analyses has been completed for 100% ($n=140$).

For North Pacific Bryde's whales sampled under JARPN II in 2014, mtDNA and microsatellite analyses have been completed for 100% of the samples ($n=25$). No bycatch occurred in 2014. For North Pacific sei whales sampled under JARPN II in 2014, mtDNA and microsatellite analyses have been completed for 100% of the samples ($n=90$). No bycatch occurred in 2014. No sampling and bycatch of sperm whale occurred in 2014.

For Antarctic minke whales sampled under JARPA II in 2013/14 ($n=251$) 94% of the samples have been analysed by mtDNA and 100% by microsatellite. No Antarctic fin whale was sampled in the 2013/14 season.

For North Pacific humpback whales 100% of the five whales bycaught in 2014 were screened for mtDNA and microsatellites. There was no bycatch of North Pacific right whales in 2014. For North Pacific fin whales a single individual was analysed for mtDNA and microsatellite.

An update of the Norwegian register is shown in Appendix 3. After discounting for duplicates, missing samples and laboratory problems, 100% of the North Atlantic common minke whales caught in 2014 ($n=731$) were screened for mtDNA and microsatellites.

An update of the Icelandic registry is shown in Appendix 4. For North Atlantic common minke whales genetic work is ongoing for the whales caught in 2014 ($n=24$). 100% of the fin whales caught by commercial whaling in 2014 ($n=134$) were screened for both mtDNA and microsatellites.

In response to a question, the Working Group was informed that all genetic samples in the national registers are stored in ethanol solution. In response to a query, the Chair informed that the DNA data in the national registries have been previously provided to IWC Scientific Committee members for analyses on stock structure under in-depth assessments and RMP *Implementation* of some large whale species, under IWC Scientific Committee Data Access Protocols A and B.

The Group **appreciates** the efforts of Japan, Norway and Iceland in compiling and providing this detailed information of their registries.

9. OTHERS

No other matters were discussed by the Group.

10. WORK PLAN

The Terms of Reference for the Working Group will remain the same for 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. Next year a comparison of the methods presented in SC/66a/BRG12 to SNP assessment performed by ddRAD sequencing (Lah *et al.*, 2014) will be examined. Also the Working Group will examine the technical information relevant to the Terms of Reference of the Group contained in documents presented to other groups and sub-committees.

11. ADOPTION OF THE REPORT

The report was adopted by consensus.

REFERENCES

- Baird, A.B., Suydam, R.S., George, J.C. and Bickham, J.W. 2014. Update on mtDNA and SNP database for bowhead whales. Paper SC/65b/BRG13 presented to the IWC Scientific Committee, May 2014, Bled, Slovenia (unpublished). 8pp. [Paper available from the Office of this Journal].
- 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 Commn.* 1999:55.
- International Whaling Commission. 2012. Report of the Scientific Committee. *J. Cetacean Res. Manage. (Suppl.)* 13:1-74.
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- Keane, M., Semeiks, J., Thomsen, B. and Pedro de Magalhaes, J. 2015. Insights into the evolution of longevity from the bowhead whale genome. *Cell Reports* 10: 112-22.
- Lah, L., Benke, H., Berggren, P., Gunnlaugsson, T., Lens, S., Lockyer, C., Öztürk, A.A., Öztürk, B., Pawliczka, I., Roos, A., Sibert, U., Skóra, K. and Tiedemann, R. 2014. Investigating harbor porpoise (*Phocoena phocoena*) population differentiation using RAD-tag genotyping by sequencing. Paper SC/65b/SD04 presented to the IWC Scientific Committee, May 2014, Bled, Slovenia (unpublished). 12pp. [Paper available from the Office of this Journal].
- Seim, I., Ma, S., Zhou, X., Gerashchenko, M.V., Lee, S.G., Suydam, R., George, J.C., Bickham, J.W. and Gladyshev, V.N. 2014. The transcriptome of the bowhead whale *Balaena mysticetus* reveals adaptations of the longest-lived mammal. *Ageing* 6(10): 879-99.
- Tin, M.M.Y., Economo, E.P. and Mikheyev, A.S. 2014. Sequencing degraded DNA from non-destructively sampled museum specimens for RAD-tagging and low-coverage shotgun phylogenetics. *PLoS. ONE* 9: e96793.

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. Review of results of the 'amendments' of sequences deposited in *GenBank*
7. Progress on collection and archiving of tissue samples from catches and bycatches
8. Reference databases and standards for a diagnostic register of DNA profiles
9. Other
10. Work plan
11. Adoption of the Report

Appendix 2

AN UPDATE OF THE JAPANESE DNA REGISTER FOR LARGE WHALES

Mutsuo Goto¹, Hiroyuki Oikawa¹ and Hideyoshi Yoshida²¹The Institute of Cetacean Research, 4-5 Toyomi-cho, Chuo-ku, Tokyo, 104-0055, Japan.²National Research Institute of Far Seas Fisheries, 2-12-4 Fukuura, Kanazawa-ku, Yokohama 236-8648, Japan.

The status of the Japanese DNA register for large whales was presented and discussed during the 2005 IWC SC meeting (IWC, 2006). Since then, the number of genetic samples and the number of individuals analysed and registered have been reported to the IWC Scientific Committee annual meetings. The annual reports include information of whales taken by the scientific whaling in the North Pacific (JARPN/JARPNII) and the Antarctic (JARPA/JARPAII), and from bycatches and stranding. The most recent full description of the protocol used by the Institute of Cetacean Research for the genetic analyses in the context of the IWC guidelines was presented by Kanda *et al.* (2014). The update of the Japanese DNA register for large whales till 2014 is in Table 1.

REFERENCES

- International Whaling Commission. 2006. Report of the Scientific Committee. Annex N. Report of the Working Group on DNA. *Journal of Cetacean Research and Management (Supplement)* 8:252-58.
- Kanda, N., Goto, M., Oikawa, H. and Pastene, L.A. 2014. Update of note on sampling and laboratory procedure protocols of the genetic work at the Institute of Cetacean Research (SC/65b/J27rev). Paper SC/65b/DNA01 presented to the IWC Scientific Committee, May 2014, Bled, Slovenia (unpublished). 6pp. [Paper available from the Office of this Journal].

Table 1
Update of the Japanese DNA register for large whales since the last Scientific Committee meeting.

Note:	1	2	3	4	5	6	7	8	9	10	11	12
Species/year	Type	No. of whales	No. of duplicates	No. missing	No. of lab problems	No. mtDNA	% mtDNA	No. msat	% msat	Sex analysed	% sexed	Notes
North Pacific minke whale												
1994-2013	SP	2,492	0	0	8	2,484	100	2,484	100	2,492	100	
2014	SP	81	0	0	0	81	100	81	100	81	100	
2001-13	BC	1,543	0	26	2	1,543	100	1,515	98	1,513	98	
2014	BC	140	0	0	0	140	100	140	100	140	100	
North Pacific Bryde's whale												
2000-13	SP	655	0	0	3	652	100	655	100	655	100	
2014	SP	25	0	0	0	25	100	25	100	25	100	
2001-13	BC	5	0	0	0	5	100	4	80	4	80	Include three Omura's whale
2014	BC	0	0	0	0	0	0	0	0	0	0	No BC
North Pacific sei whale												
2002-13	SP	1,084	0	0	4	1,080	100	1,084	100	1,084	100	
2014	SP	90	0	0	0	90	100	90	100	90	100	
North Pacific sperm whale												
2000-13	SP	56	0	0	0	56	100	56	100	56	100	
2014	SP	0	0	0	0	0	0	0	0	0	0	No catch
2001-13	BC	2	0	0	0	2	100	2	100	2	100	
2014	BC	0	0	0	0	0	0	0	0	0	0	No BC
Antarctic minke whale												
1987/88-2004/05	SP	6,794	0	10	0	1,118	17	6,271	92	6,794	100	Incl. dwarf; 87/88-88/89; no microsats
2005/06-2010/11	SP	3,264	0	549	162	2,040	63	2,553	78	3,264	100	Some missing by the 3/11 tsunami in 2011
2011/12	SP	266	0	0	0	265	100	266	100	266	100	Analysed in 2014 after SC/65b
2012/13	SP	103	0	0	0	103	100	103	100	103	100	Analysed in 2014 after SC/65b
2013/14	SP	251	0	0	0	237	94	251	100	251	100	
Antarctic fin whale												
2005/06-2012/13	SP	18	0	0	0	18	100	18	100	18	100	
2013/14	SP	0	0	0	0	0	0	0	0	0	0	No catch
North Pacific humpback whale												
2001-13	BC	46	0	0	0	46	100	46	100	46	100	
2014	BC	5	0	0	0	5	100	5	100	5	100	
North Pacific right whale												
2001-13	BC	2	0	1	0	2	100	1	50	1	50	Missing by the 2011 tsunami, no microsats
2014	BC	0	0	0	0	0	0	0	0	0	0	No BC
NP fin whale												
2001-13	BC	9	0	0	0	9	100	9	100	9	100	
2014	BC	1	0	0	0	1	100	1	100	1	100	

Appendix 3

AN UPDATE OF THE NORWEGIAN DNA REGISTER FOR MINKE WHALES

Hans J. Skaug
Institute of Marine Research, Norway

Table 1
Update of the Norwegian DNA register for minke whales.

Note:	2	3	4	5	6	7	8	9	10	11	12	
Species/year	Type	No. of whales	No. of duplicates	No. missing	No. of lab problems	No. mtDNA	% mtDNA	No. msat	% msat	Sex analysed	% sexed	Notes
North Atlantic minke whale												
1997-2013	C	9,330	98	55	2	9,273	100	9,273	100	9,273	100	Error in numbers reported last year.
2014	C	731	3	10	0	721	100	721	100	721	100	

Appendix 4

STATUS OF THE ICELANDIC WHALE DNA REGISTER

Christophe Pampoulie and Gisli A. Víkingsson
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Practical arrangements regarding the establishment of the Icelandic DNA register were concluded in 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. An ORACLE database has now been created and contains all genotyped individuals information as well as tissue collected ID of

individuals collected but not genotyped. In parallel, a DNA tissue bank has been achieved and is now fully functional.

Table 1 gives the present status of the registry. Samples from all the common minke whales landed as a part of the Icelandic research program (2003-07) and all commercial NA fin whales have been genotyped and information stored in the database.

Table 1
Status of the Icelandic whale DNA register.

Note:	1	2	3	4	5	6	7	8	9	10	11	12
Species/year	Type	No. of whales	No. of duplicates	No. missing	No. of lab problems	No. mtDNA	% mtDNA	No. msat	% msat	Sex analysed	% sexed	Notes
North Atlantic minke whale												
2003-07	SP	189	0	0	0	189	100	189	100	189	100	
2007-13	C	331	0	0	0	272	89	276	95	276	95	
2014	C	24	0	0	0	0	0	0	0	0	0	
North Atlantic fin whale												
2006-13	C	400	0	0	0	400	100	400	100	400	100	
2014	C	134	0	0	0	134	100	134	100	134	100	

Notes to the Tables in all three appendices

- Key to sample types: SP=special permit catch, C=commercial catch, BC=bycatch, ST=stranding.
- Number of whales that potentially entered by the previous years and enters (new year) the markets.
- Number of occurrences (tissues) sample switching on board the vessels as detected by comparison of genetic profiles.
- Number of individuals for which tissue samples are missing for other reasons than sample switching.
- Genetic laboratory not able to obtain microsatellite profiles mtDNA haplotypes from tissue samples.
- Number of samples analysed for mitochondrial control region.
- % of total samples analysed for mitochondrial control region.
- Number of samples analysed for microsatellites.
- % of total samples analysed for microsatellites.
- Number of samples analysed for sex.
- % of total samples analysed for sex.
- Other problems or information.