ACCOBAMS-MOP9/2025/Inf14

REPORT OF THE ACCOBAMS WEBINAR - REGIONAL TRAINING ON GENETIC DATA COLLECTION AND ANALYSIS

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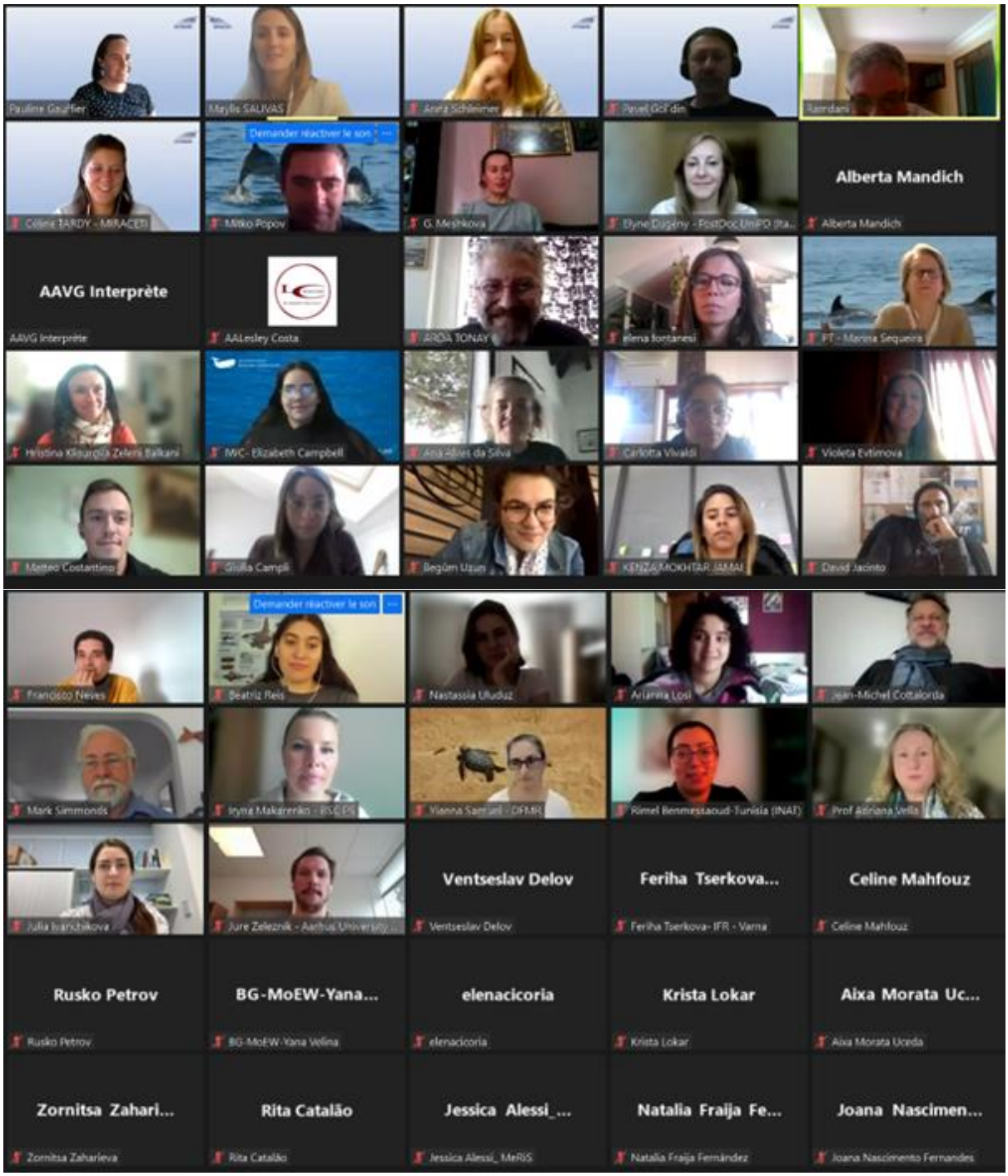

8 November 2023, online

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Background

The webinar was organised based on the 2023-2025 ACCOBAMS Working Programme (MOP8 Resolution 8.2) that foresees that the ACCOBAMS Secretariat and the Scientific Committee should improve data collection on cetacean population genetics in the ACCOBAMS Area, and develop the genetic identification of population structure (particularly in the Mediterranean Sea) by disseminating databases and guidelines to stakeholders. This webinar was organised thanks to a contribution from the Italian Ministry of the Environment and Energy Security.

The ACCOBAMS Best Practices were created collectively through a series of workshops in 2022 (ACCOBAMS-MOP8/2022/Inf25), as a road map for scientists wishing to start a cetacean population genetics study. The Best Practices detail the various aspects of cetacean population genetic studies, from study design and sample collection, to sample processing and data analyses, permits and dissemination of results. The first edition (October 2022) is currently available at https://accobams.org/population-genetics/. A new updated version (version 2, November 2023) will soon be uploaded to the ACCOBAMS website, together with a French translation.

Webinar preparation

A Google form was sent to all ACCOBAMS National Focal Points and all ACCOBAMS partners on October 16th, 2023 for participants to register to the webinar. In the registration form, participants were asked to indicate their level of experience with population genetics and their preferred language.

Fifty-six registrations were received from 15 ACCOBAMS Parties, the U.K. and participants from the International Whaling Commission and the Black Sea Commission Secretariats.

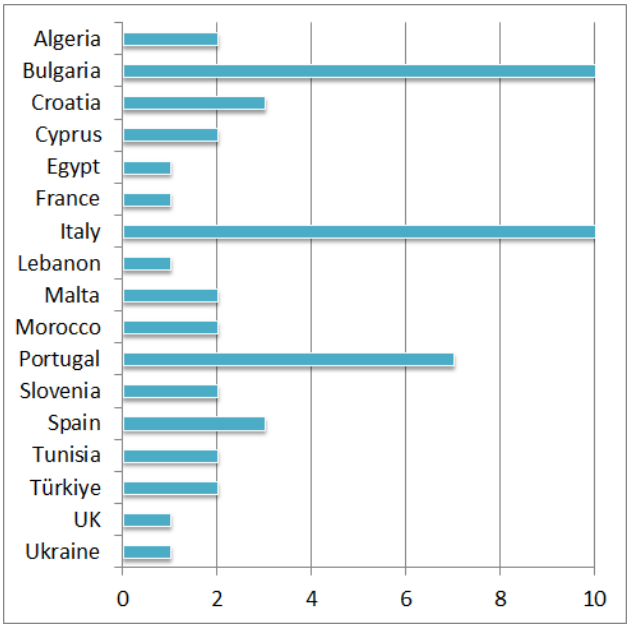

Figure 1: Participants' countries indicated on the registration form

About half of participants indicated that they had no prior experience with population genetics while the other half had some experience (Figure 2). Only 4 people indicated they had extensive experience.

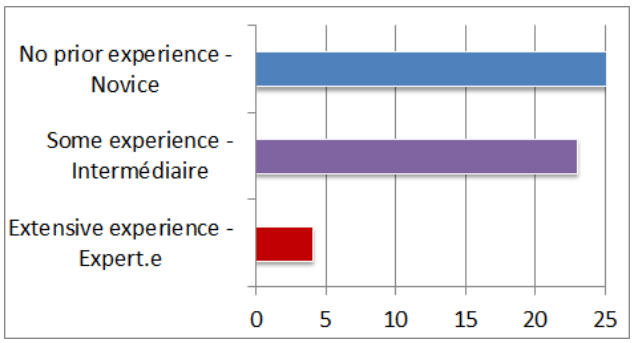

Figure 2: Prior experience of participants with population genetics

A link to the Zoom session was sent to all registered participants, together with version 1 of the ACCOBAMS Best Practices on Cetacean Population Genetics.

Webinar proceedings

The webinar started by welcome addresses from Maÿlis Salivas of the ACCOBAMS Secretariat followed by six 20-minute presentations from four trainers, Dr Anna Schleimer, Dr Céline Tardy, Dr Pavel Gol'din and Dr Pauline Gauffier covering all the sections of the ACCOBAMS Best Practices on Cetacean Population Genetics and its Appendices :

- Background of the creation of ACCOBAMS Best Practices on Cetacean Population Genetics
- Study design, including Appendix 1 of Best Practices «Existing knowledge on population genetics of cetaceans in the ACCOBAMS area»

- Permits for samples collection and Exchanges of samples, including Appendix 4 of Best Practices « Example of Biological Material Transfer Agreement » and the database of existing samples in the ACCOBAMS area
- Sample collection and preservation
- Sample processing including Appendix 2 «Suitable genetics labs in the ACCOBAMS area», Appendix 3 «Example laboratory protocols for DNA extraction from tissue» and Data analysis
- Dissemination of results, Data archiving and collecting data from published studies and Forensic science

It was divided in two parts separated by a short 20-minute coffee break, each part being followed by a Q&A session with questions asked in the chat by participants (See Appendix 1 Agenda). The webinar was held in English and French and simultaneous French/English translation was provided.

Up to 47 participants connected to the webinar on November 8th 2023 (see Appendix 2 List of participants).

Q&A

Questions from the chat were all answered during the Q&A sessions and are listed below together with their respective answers.

1. **Could environmental NGOs send cetacean samples in compliance with regulations and legislation?**

In some countries, permits for collection, transport and/or storage of cetacean samples can be delivered to NGOs under certain conditions (*e.g.* Spain). However, in other countries, only a state run organisation can be eligible for collecting and permanently storing samples of protected species (*e.g.* Ukraine). In such a case, an NGO can only act as a partner of a state run institution - doing field, analytic and logistic support. We recommend that environmental NGOs wishing to collect, store or exchange cetacean samples contact their ACCOBAMS National Focal Point (the up-to-date list of NFPs is available here: https://accobams.org/about/parties-and-range-states/) to check the procedures to be followed in their country. In case of exchanging samples, they will then need to find out about the procedures in the receiving country as well, either by contacting the National Focal Point in that country directly, or the receiving institution in that country (*e.g.* research collaborator or genetic laboratory) can take care of this.

2. We have observed from seismic vessels that several species travel in groups: common and striped dolphins, and pilot whales. Are there any existing studies about genetic homogeneity within groups of these species?

LONG–FINNED PILOT WHALES (*Globicephala melas*):

Several studies have shown that long-finned pilot whales exhibit long standing social bounds. Based on photo-identification and genetic work, long-finned pilot whales appear to live in relatively stable, maternally-based pods like those of killer whales (Amos *et al.* 1993, Fullard 2000, de Stephanis *et al.* 2008, Verborgh *et al.* 2016). Pilot whales show a hierarchical social structure with a population composed of a number of clans each containing several pods (de Stephanis *et al.* 2008). Pods will then be formed by several matrilineal units (Verborgh 2015). Using microsatellite markers, it was shown that entire families can be found within a pod, with individuals of all age classes and both sexes, but without the fathers of the calves (Amos *et al.* 1993a; Bloch *et al.* 1993). Although pods may not always show long term cohesiveness (Ottensmeyer and Whitehead 2003), matrilineal units are very stable (de Stephanis *et al.* 2008, Verborgh 2015). Mating could happen when different clans come together and form very large groups (Cañadas and Sagarminaga 2000). Reproduction would then take place between clans, and inbreeding would be avoided without the need for males to physically leave their natal group (Amos *et al.* 1993b).

Amos B, Bloch D, Desportes G, Majerus TMO, Bancroft DR, Barrett JA and Dover GA (1993a) A review of molecular evidence relating to social organisation and breeding system in the long-finned pilot whale. In: Donovan GP, Lockyer CH, Martin AR (eds) Biology of Northern Hemisphere Pilot Whales. Report of the International Whaling Commission Special Issue 14. International Whaling Commission, Cambridge, pp 209–218.

Amos B, Schlotterer C, Tautz D (1993b) Social structure of pilot whales revealed by analytical DNA profiling. Science. 260:670–672. https://doi.org/10.1126/science.8480176

Bloch D, Lockyer C, Zachariassen M (1993) Age and growth parameters of the long-finned pilot whale off the Faroe Islands. In: Donovan GP, Lockyer CH, Martin AR (eds) Biology of Northern Hemisphere Pilot Whales. Report of the International Whaling Commission Special Issue 14. International Whaling Commission, Cambridge, pp 163–208.

Cañadas A, Sagarminaga R (2000) The northeastern Alboran Sea, an important breeding and feeding ground for the long-finned pilot whale (*Globicephala melas*) in the Mediterranean Sea. Marine Mammal Science 16:513–529. https://doi.org/10.1111/J.1748-7692.2000.TB00948.X

de Stephanis R, Verborgh P, Pérez S, Esteban R, Minvielle-Sebastia L, Guinet C (2008) Long-term social structure of long-finned pilot whales (*Globicephala melas*) in the Strait of Gibraltar. Acta Ethologica 11:81–94. https://doi.org/10.1007/s10211-008-0045-2

Fullard KJ, Early G, Heide-Jørgensen MP, Bloch D, Rosing-Asvid A, Amos W (2000) Population structure of long-finned pilot whales in the North Atlantic: a correlation with sea surface temperature? Molecular Ecology 9:949–958. https://doi.org/10.1046/j.1365-294x.2000.00957.x

Ottensmeyer CA, Whitehead H (2003) Behavioural evidence for social units in long-finned pilot whales. Canadian Journal of Zoology 81:1327–1338. https://doi.org/10.1139/z03-127

Verborgh P (2015) Demografía y estructura de las poblaciones de calderones comunes (*Globicephala melas*) en el Mediterráneo español. PhD Thesis, Universidad de Las Palmas de Gran Canaria, Spain

Verborgh P, Gauffier P, Esteban R, Giménez J, Cañadas A, Salazar-Sierra JM, de Stephanis R (2016) Conservation status of long-finned pilot whales, *Globicephala melas*, in the Mediterranean Sea. In: Notarbartolo di Sciara G, Podestà M, Curry BE (eds) Advances in Marine Biology 75: Mediterranean Marine Mammal Ecology and Conservation, Academic P. Elsevier, Oxford, pp 173–203 https://doi.org/10.1016/bs.amb.2016.07.004

COMMON DOLPHINS (*Delphinus delphis*):

Patterns of kinship structure in short-beaked common dolphins were investigated by Ball *et al.* (2017) along the Portuguese coast. Overall, this species showed weak spatial genetic structure and sampled groups were not generally composed of closely related individuals. However, close kin were frequently detected in the vicinity over several years, likely the result of some level of site fidelity. While there are some studies describing the social structure of common dolphin groups in the Mediterranean Sea using mark-recapture methods, these do not allow to make inferences on the genetic relatedness among individuals (Bruno *et al.* 2004, Pace *et al.* 2009, Mussi *et al.* 2021).

Ball L, Shreves K, Pilot M, Moura AE (2017). Temporal and geographic patterns of kinship structure in common dolphins (*Delphinus delphis*) suggest site fidelity and female-biased long-distance dispersal. Behavioral Ecology and Sociobiology 71:123. https://doi.org/10.1007/s00265-017-2351-z

Bruno S, Politi E, Bearzi G (2004. Social organisation of a common dolphin community in the eastern Ionian Sea: Evidence of a fluid fission–fusion society. In P. G. H. Evans & E. O'Boyle (Eds.), Proceedings of the 15th annual conference of the European cetacean society (pp. 49–51). Liege, Belgium: European Cetacean Society.

Pace DS, Mariani M, Miragliuolo A, Venier M, Mussi B (2009). Preliminary analysis of the social structure of the short-beaked common dolphin (*Delphinus delphis*) in the Tyrrhenian Sea, Italy. In C. Vincent, G. J. Pierce, A. A. Öztürk, P. Kotnjek, M. Siemensma, & A. Tonay (Eds.), Proceedings of the 23rd annual conference of the European Cetacean Society, Istanbul, Turkey (pp. 176–177). Istanbul, Turkey: Türk Deniz Aras¸tırmaları Vakfı.

Mussi B, Vivaldi C, Zucchini A, Miragliuolo A, Pace DS (2021) The decline of short-beaked common dolphin (*Delphinus delphis*) in the waters off the island of Ischia (Gulf of Naples, Italy). Aquatic Conservation: Marine and Freshwater Ecosystems, 31(S1), 87–100.

STRIPED DOLPHINS (*Stenella coeruleoalba*):

Gaspari *et al.* (2007) reported the results of a study on social kin associations in striped dolphins in the Tyrrhenian Sea in the Mediterranean Sea. Based on microsatellite data, they found females displayed higher average kinship within than between the 12 studied groups, while no difference was observed in males. Smaller groups also tended to show higher levels of average kinship. Social cohesion of kin thus seems to be an important element in structuring groups in striped dolphins, especially among females. Another study investigated association patterns in an isolated striped dolphin population inhabiting the Gulf of Corinth, Greece (Bonizzoni *et al.* 2023). Results suggested a well-differentiated single community characterised by nonrandom associations and a loose clustering of individuals. However, identification of the driving forces behind these association patterns is hampered by lack of detailed information about the sex, age and kinship of individuals (Bonizzoni *et al.* 2023).

Gaspari S, Azzellino A, Airoldi S, Hoelzel AR (2007) Social kin associations and genetic structuring of striped dolphin populations (*Stenella coeruleoalba*) in the Mediterranean Sea. Molecular Ecology 16(14): 2922-2933. https://doi.org/10.1111/j.1365-294x.2007.03295.x

Bonizzoni, S., Santostasi, N.L., Eddy, L., Riley, M.A., Ferreira da Silveira, M., Würsig, B., and Bearzi, G. (2023). Social and community structure of striped dolphins in a semienclosed Mediterranean embayment. Marine Mammal Science, 1–21. https://doi.org/10.1111/mms.13060

3. We have also observed that humpback whales gather in breeding grounds above and below the Equator. What is known about the heterogeneity of their populations in the Mediterranean Sea?

HUMPBACK WHALES (*Megaptera novaeangliae*):

While they were once considered very rare, humpback whales have been sighted with increasing frequency in the Mediterranean Sea since the late 1980s. They have been reported almost every year in the region, sometimes up to three times (ACCOBAMS 2021). However, they are not still not a regular species in the Mediterranean Sea and they are infrequent in the contiguous Atlantic Area. To our knowledge there is no genetic study that has included samples from the Mediterranean Sea to date. There are sporadic sightings in the Mediterranean Sea, mostly of juveniles, of generally unknown origin, but probably from the North Atlantic Ocean (ACCOBAMS 2021, Espada Ruíz *et al.* 2018, Frantzis *et al.* 2004). An exceptional case is known of a female with calf sighted in 2020 in Italy and previously identified in 1986 in the Dominican Republic (Violi *et al.* 2021).

ACCOBAMS, 2021. Conserving whales, dolphins and porpoises in the Mediterranean Sea, Black Sea and adjacent areas: an ACCOBAMS status report. Monaco: ACCOBAMS. https://accobams.org/wp-content/uploads/2022/03/ACCOBAMS_ConservingWDP_web_2022.pdf

Espada Ruíz, R., Olaya-Ponzone, L., and García-Gómez, J.C., 2018. Humpback whale in the bay of Algeciras and a mini-review of this species in the Mediterranean. Regional Studies in Marine Science, 24, 156–164. https://doi.org/10.1016/j.rsma.2018.08.010

Frantzis, A., Nikolaou, O., Bompar, J., and Cammedda, A., 2004. Humpback whale (*Megaptera novaeangliae*) occurrence in the Mediterranean Sea. Journal of Cetacean Research and Management, 6 (1), 25–28. https://journal.iwc.int/index.php/jcrm/article/view/786

Violi, B., Verga, A., Jones, L.S., Calogero, G., Soldano, G., Cheeseman, T., and Wenzel, F.W., 2021. A Wanderer in the Mediterranean Sea: The Case of a Humpback Whale (*Megaptera novaeangliae*) from the West Indies. Aquatic Mammals, 599–611. https://doi.org/10.1016/10.1578/AM.47.6.2021.599

4. Concerning another potential legal enforcement issue does anyone know if ambergris (from sperm whales) can be identified using genetic techniques?

It is indeed possible to extract and sequence DNA from ambergris to identify/verify the specific nature of ambergris. The lipid-rich composition of ambergris facilitates the preservation of DNA and shotgun sequencing can be performed on the extracted DNA. Macleod *et al.* (2020) successfully aligned sequences obtained from ambergris to the sperm

whale reference genome. The authors also indicate that it may be possible to find DNA sequences from prey or the microbiome preserved in ambergris.

Macleod R, Sinding M-HS, Olsen MT, Collins MJ, Rowland SJ (2020) DNA preserved in jetsam whale ambergris. Biology Letters 16: 20190819. http://dx.doi.org/10.1098/rsbl.2019.0819

5. **It would be good to make sure all experienced and long-term genetics research labs focusing on marine wildlife including cetaceans are included in the table of Appendix 4 - Suitable genetics labs in the ACCOBAMS area.**

New additions to the list of Suitable genetics labs in the ACCOBAMS area were received in the weeks preceding the webinar, and they have been incorporated into the revised Best practices Appendix 4 and in the stand alone pdf on the dedicated page on ACCOBAMS website https://accobams.org/population-genetics/. Version 2 of the Best practices will be available soon.

6. **The genetic passport will be made for all bottlenose dolphins in captivity regardless of their origin or just for the Black Sea bottlenose dolphins?**

It will be a template of genetic passport for different cetacean species kept in captivity, not only Black Sea Bottlenose dolphins. It will be prepared in collaboration with the ACCOBAMS Advisory Committee on Captivity related issues as requested by MOP Resolution 8.2 "Work Programme & Budget for the 2023-2025 triennium" under the section CA2i "Captivity related issues".

Special case of Black Sea bottlenose dolphins
The ACCOBAMS Scientific Committee has remarked "the illegality of live removals of cetaceans from the Black Sea" and called for "an inventory and thorough assessment of individual identity of all bottlenose dolphins kept in captivity by means of genetic, morphological and photo-ID methods", as well as for the provision of "appropriate administrative measures in order to prevent substitution of dolphins that die in captivity from animals taken from the wild" (Recommendation 8.2, ACCOBAMS-MOP7/2019/Inf_09). Following recommendations by the Scientific Committee, the Fifth Meeting of ACCOBAMS Parties adopted Resolution 5.14 about Live Removals of Bottlenose Dolphins in the Black Sea. At its 17th meeting, the Conference of the Parties to CITES (Johannesburg, 2016) further dealt with this species (Recommendations 17.299-301). Parties are now encouraged to use genetic analysis to confirm the origin of the animals prior to the issuance of export permits. Furthermore, Parties are encouraged to establish national or regional repositories where relevant genetic identification data are stored and to make them accessible on-line, as well as to report to the CITES Animals Committee on exports of *Tursiops truncatus ponticus* and their origins.

In addition to CITES permits and eventually Nagoya Protocol permit/document, one should consider Customs for non-EU states, as they usually ask for at least General Customs Declaration and require value/price tag for samples.

If required by the Customs authorities, a letter from an institution setting a nominal value can be helpful. Customs require a minimum positive value so that a nominal value of 1 euro, for example, can be stated.

Appendix 1 : Agenda of the ACCOBAMS Webinar - Regional training on genetic data collection and analysis

November 8th, 2023 from 10 to 13h00 CET

Objective: to disseminate the ACCOBAMS Best practices on cetacean population genetics

10h00 – 10h05	Welcome addresses from the ACCOBAMS Secretariat	Maÿlis Salivas
10h05 – 10h20	Background, presentation of the webinar and rules for questions	Pauline Gauffier
10h20 – 10h40	**Study design,** including *Appendix 1 of Best Practices « Existing knowledge on population genetics of cetaceans in the ACCOBAMS area »*	Anna Schleimer
10h40 – 11h00	**Permits for samples** collection and **Exchanges of samples**, *including Appendix 4 of Best Practices « Example of Biological Material Transfer Agreement »* and the database of existing samples in the ACCOBAMS area	Pauline Gauffier
11h00 – 11h20	**Sample collection and preservation**	Céline Tardy
11h20 – 11h40	Questions from the chat	
11h40 – 12h00	Break	
12h00 – 12h20	**Sample processing** *including Appendix 2 Suitable genetics labs in the ACCOBAMS area*, *Appendix 3 Example laboratory protocols for DNA extraction from tissue* and **Data analysis**	Anna Schleimer
12h20 – 12h40	**Dissemination of results, Data archiving and collecting data from published studies and Forensic science**	Pavel Gol'din
12h40 – 13h00	Questions from the chat	
	Closure of the webinar	

Appendix 2: List of participants

Aixa Morata Uceda
Alberta Mandich
Ana Maria Conceição Alves da Silva
Arda Tonay
Arianna Losi
Beatriz Reis
Begüm Uzun
Biagio Violi
Carlotta Vivaldi
Celine Mahfouz
Chabane Juba
David Jacinto
Elena Fontanesi
Elena Cicoria
Elizabeth Campbell (IWC)
Elyne Dugény
Feriha Tserkova
Francisco Neves
Galina Meshkova
Giulia Campli
Hristina Klisurova
Iryna Makarenko (BSC PS)
Jean-Michel Cottalorda
Jessica Alessi
Joana Nascimento Fernandes
Julia Ivanchikova
Jure Zeleznik
Kenza Mokhtar Jamai
Lavrentios Vasiliades
Mark Simmonds
Matteo Costantino
Dimitar Popov
Krista Lokar
Nastassia Uluduz
Natalia Fraija Fernández
Noel Vella
Adriana Vella
Marina Sequeira
Mohamed Ramdani
Rimel Benmessaoud
Rita Catalão
Rusko Petrov
Ventseslav Delov
Violeta Evtimova
Yana Velina
Yianna Samuel
Zornitsa Zaharieva