An Acad Bras Cienc (2024) 96(Suppl. 2): e20240502 DOI 10.1590/0001-3765202420240502
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ANIMAL SCIENCE

Molecular identification of whales remains from the Keller Peninsula, Admiralty Bay, King George Island, Antarctica

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Abstract: At the beginning of the 20th century, intense whaling activity took place in the South Shetland Islands, which is represented today in the form of ruins and numerous whale bones scattered along several Antarctic beaches. Despite being exposed to a harsh environment throughout the last decades, the present manuscript tried to answer if these bone remains still have viable DNA to allow species' identification using molecular methods. Several individuals were collected from the shores of Keller Peninsula, Admiralty Bay, Antarctica, and submitted to DNA extraction, amplification and Sanger sequencing. The challenging identification of these bone fragments proved to be still feasible. Mitochondrial DNA was successfully extracted, amplified and sequenced. A database with 43 sequences including previously published and newly determined sequences were built and enabled the precise identification to species level for some of the collected samples, therefore shedding light on the whales species that inhabited the region and how their overexploitation seems to have affected modern day presence of these species within the study area.

Key words: Antarctica, Whales, Identification, Molecular.

INTRODUCTION

Throughout the 19th and 20th centuries, whaling in the Southern Ocean led some cetacean populations to the brink of extinction, and these populations are today still in the process of recovery and returning to their grounds (Clapham et al. 1999, Thomas et al. 2016, Jackson et al. 2020). Hundreds of thousands of baleen whales were caught around the Antarctic Peninsula throughout the 'Modern Whaling' years (17th to 19th century), when advances of technology from Industrial revolution were applied in whaling (Clapham & Baker 2018, Vighi et al. 2020). The place was considered an ideal hunting hotspot, as it comprises feeding grounds for several cetaceans (Friedlaender & Modest 2018, Bierlich et al. 2022), due to the abundance of planktonic organisms present in the diet of most of these marine mammals (Flores et al. 2012, McBride et al. 2021). In the early years of Antarctic whaling, Humpback whales were the first and main targets of whalers, but it changed from about 1914, with the implementation of more advanced hunting technologies, such as the steam engine and explosive harpoons, increased the capacity number and size of captures, making Blue whales and Fin whales became the most exploited species when whaling activities became possible in the Admiralty Bay (Tønnessen & Johnsen 1982, Kittel 2001, Clapham & Baker 2018).

Remains from this period can be found in the South Shetland Islands in the form of whaling stations' ruins, rusting ships, minor

objects of wood and metal; oil storage barrels and littered whale bone remains still deposited on several Antarctic shores (Hacquebord 1992, Kittel 2001). The Admiralty Bay, King George Island, is one of the most important areas for research about the whaling period in Antarctica (Kittel 2001). Kittel (2001), Rakusa-Suszczewski & Nedzarek (2002) counted 158 to 175 whale skulls still deposited in Admiralty Bay beaches, with variable disponibility due to eventual burying. Almost half of them, 89 skulls, are located in Keller Peninsula, near the 'Comandante Ferraz' Brazilian Antarctic Station (Kittel 2001, Rakusa-Suszczewski 1998). The abundance of these bone fragments is related to its sheltered waters, which provided an ideal base for floating factories (Tønnessen & Johnsen 1982), where the carcasses were flensed. In the present, these bones may be an unique source for retrieving genetic information that would aid in the study of historical whale populations prior to human exploitation in the Southern Ocean (Lindqvist et al. 2009). However, morphological species identification is not easy since most remains are found disarticulated and affected by severe weathering, some of them represented only as small bone fragments present in the soil (Kittel 2001, Szabo 2008), all extensively affected by the nearby marine environment.

In this sense, molecular methods may be a key tool and have been used in the identification of different marine mammals, including whale remains (Foote et al. 2012, Lindqvist et al. 2009, Solazzo et al. 2017, Speller et al. 2016). However, the process of DNA profiling bone samples is highly challenging due to several factors that can either facilitate or impede successful testing (Jakubowska et al. 2012, Campos et al. 2012, Andelinovic et al. 2005). Additionally, if these remains came from aquatic environments, the associated difficulties for DNA typing grow exponentially.

The exposure of bone specimens to fresh water has been demonstrated to be significantly damaging to the remaining DNA (Crainic et al. 2002, Courts & Madea 2011). The same is true for seawater and there is limited research on DNA profiling of remains submerged in seawater, particularly when only bone samples are available for analysis (Staiti et al. 2004, D'Errico et al. 1998, Kapinska & Szczerkowska 2004), and to date, there is no universal method for DNA profiling bone samples exposed to prolonged seawater immersion, prompting ongoing efforts to develop and enhance new methodologies.

The present study aimed to investigate if even after about a hundred years of exposure on a harsh marine environment, eventually submerged, eventually buried in sediments/snow, there is still available DNA to accomplish the first molecular identification of an old, degraded, and heavily weathered whale bone samples from Admiralty Bay, shedding light on the whales species that inhabit the region and the impact of their overexploitation along the modern whaling years in Antarctica.

MATERIALS AND METHODS

For molecular analyses, bone fragments were isolated from the remains deposited on the shores of Keller Peninsula, Admiralty Bay, King George Island, Antarctica (Figures 1 and 2). 38 samples were collected, a triplicate collection was carried from each skull bone, under the license MMA 02000.000277/2021-09 and IBAMA 02001.013446/2019-38. In this work, we chose to use only fragments related to skull remains as reference because vertebrae and ribs could be from the same individual. The collections were carried out during the Antarctic summer of 2021 and 2022. Although bone pieces can be moved along the time from their original deposition locations by the sea waves and anthropogenic

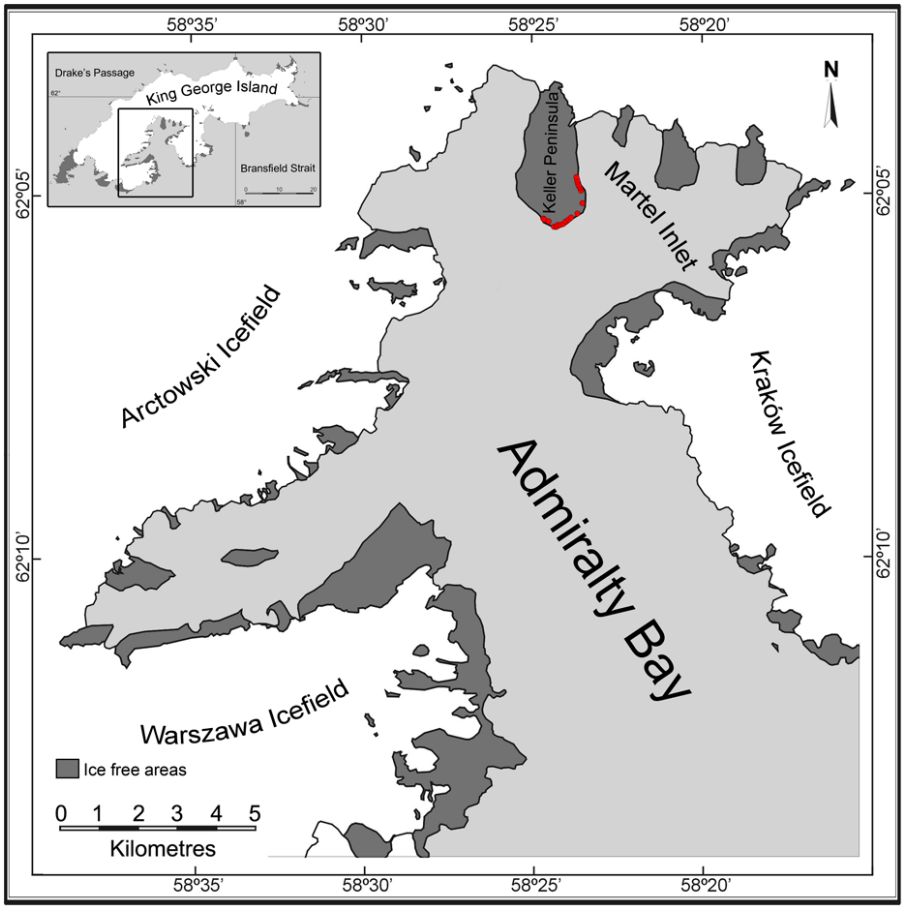

Figure 1. Samples localities (in red) on the shores of Keller Peninsula, Admiralty Bay, King George Island, Antarctica.

activity, the choice of the sites followed previous studies of whale bones distribution (Kittel 2001) and was limited by the available access at the beginning of the austral summer. All sample sites were plotted over the Admiralty Bay map using QGIS 3.22.8 software (QGIS Development Team, http://qgis.osgeo.org) (Figure 1).

DNA extractions were done using the ExtractMe RNA & DNA Kit (BLIRT S.A., Poland) following the manufacturer's protocol, with an additional time on the first step, where the samples were incubated with the Lysis Buffer overnight in an Eppendorf ThermoMixer C at 56°C, to increase the bone digestion (Arismendi et al. 2004). DNA quantification was estimated via spectrophotometry using a Multiskan SkyHigh Microplate and a Nanodrop 2000 (ThermoFisher Scientific) spectrophotometers.

After extraction and quantification, the PCR amplification step was made using the universal 16Sar (5'-CGCCTGTTTATCAAAAACAT-3) and 16Sbr (5'-CCGGTCTGAACTCAGATCACGT-3') primers (Milinkovitch et al. 1994, Palumbi et al. 1991, Palumbi 1996). The protocol followed was of 2 min at 95°C, 35 cycles of 30 sec at 94°C, 40 sec at 52°C, and 1 min at 72°C, with a final extension step for 10 min at 72°C. The PCR products were purified using the enzyme ExoSAP-IT® (USB Corporation) and Sanger sequencing method was performed in the Laboratory of Diagnosis by DNA (UERJ- RJ), using the BigDye Terminator v.3.1 Cycle Sequencing kit (Applied Biosystems), with 25 cycles of 10 sec at 95°C, 5 sec at 50ºC and 4 min at 60°C. Sequencing products were processed in an ABI 3500 capillary system (Applied Biosystems). The results of the capillary electrophoresis were visually checked, trimmed

Figure 2. Details about sampling in field (a) Whale bone remains densely covered by snow ice during the first months of the Antarctic winter; (b) Sampling sites at Keller Peninsula; (c and d) heavily weathered bone remains.

and analyzed using Geneious v4.8.2. (Drummond et al. 2010).

A database was built including 39 DNA sequences from Antarctic commonly

found whales were downloaded from GenBank and added to the four newly generated sequences and aligned in a single matrix using Muscle.The database included the Antarctic Peninsula occurring species of baleen whales and large odontocetes, such as: *Balaenoptera acutorostrata*, *Balaenoptera borealis*, *Balaenoptera musculus*, *Balaenoptera physalus*, *Eubalaena australis*, *Megaptera novaeangliae*, *Physeter macrocephalus*, and *Orcinus orca* (Table I). All sequences were aligned using the default parameters of MUSCLE on the software Geneious v.4.8.2 (Kearse et al. 2012, Edgar 2004).

The species identification was inferred using the Neighbor-Joining method (Saitou & Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (x 1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. This analysis involved 43 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 199 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018).

Table I. GenBank accession numbers for the previously published sequences used in our analyses.

Species	Accession	Species	Accession
Balaenoptera acutorostrata	MT410935	*Balaenoptera physalus*	KV572860
Balaenoptera acutorostrata	AP006468	*Eubalaena australis*	OP205178
Balaenoptera acutorostrata	NC00527	*Eubalaena australis*	OP205179
Balaenoptera borealis	MF409248	*Eubalaena australis*	OP205180
Balaenoptera borealis	MF409249	*Eubalaena australis*	AP006473
Balaenoptera borealis	AP006470	*Megaptera novaeangliae*	U13117
Balaenoptera musculus	ON257891	*Megaptera novaeangliae*	MF409246
Balaenoptera musculus	ON257892	*Megaptera novaeangliae*	PP475430
Balaenoptera musculus	ON257894	*Megaptera novaeangliae*	AP006467
Balaenoptera musculus	ON257896	*Physeter macrocephalus*	KU891393
Balaenoptera musculus	ON257900	*Physeter macrocephalus*	KU891394
Balaenoptera musculus	ON257903	*Physeter macrocephalus*	MT410874
Balaenoptera musculus	ON257908	*Physeter macrocephalus*	U13119
Balaenoptera musculus	ON257909	*Physeter macrocephalus*	NC002503
Balaenoptera musculus	ON257910	*Orcinus orca*	GU187211
Balaenoptera physalus	U13103	*Orcinus orca*	GU187215
Balaenoptera physalus	KC572854	*Orcinus orca*	GU187217
Balaenoptera physalus	KC572857	*Orcinus orca*	GU187218
Balaenoptera physalus	KC572858	*Orcinus orca*	GU187219
Balaenoptera physalus	KC572859		

The molecular identification was verified by using the Maximum Likelihood method and General Time Reversible model (Nei & Kumar 2000), chosen after the model selection carried out on MEGA X (Kumar et al. 2018) using the AKAIKE criterion. Support for nodes was evaluated using the Bootstrap test with 1000 replicates (Felsenstein 1985). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.2930)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 41.84% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 199 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018).

Maximum likelihood verification results are presented as Supplementary Material.

RESULTS AND DISCUSSION

Molecular Identification

The most usual approach for molecular species identification is by far the DNA Barcoding method (Hebert et al. 2003). Used for vertebrates, invertebrates, plants, fungii, the DNA Barcoding based on cytochrome C oxidase and alternatively using the cytochrome b for mammals, may lead to misidentification for some cetaceans species, including two target species of this study, *Eubalaena spp.* and *Megaptera novaeangliae* (Viricel & Rosel 2012, Silva et al. 2021). Anjos et al. (2023) recently suggested based on *in silico* data the 16S rRNA could be used as a reliable marker for the molecular identification of Antarctic cetaceans. However, although we opted for the use of 16S rRNA in the present manuscript mainly due to its mitochondrial inheritance and the ease of obtaining high quality sequences, a consensus on what molecular marker should be used for cetaceans identification is still under debate, with several studies use different molecular markers for cetaceans identification such as the COI, the D-Loop, the cytochrome b, and the 16S (Falcão et al. 2017, Silva et al. 2021, Dalebout et al. 1998, Kamal et al. 2021, Jayasankar 2020). Additionally, the high copy number that characterize the mitochondrial markers made them a first choice for degraded samples such as the bone fragments herein analyzed.

We successfully obtained genomic DNA from all bone fragments recovered and submitted to extraction with acceptable quantity values (Table SI). However, among the 34 samples obtained from Admiralty Bay, only four of them amplified the partial 16S rRNA region (samples B13, B23, B31, and B33) while all the remaining failed in amplification. Obtained sequences were deposited in the GenBank database under the accession numbers PP790983 (B13), PP790984 (B23), PP790985 (B31), and PP790986 (B33). Among the new retrieved sequences we successfully identified three of them as *Balaenoptera musculus*, the Blue Whale (samples B13, B31, B33), and the remaining one as *Balaenoptera physalus*, the Fin Whale (sample B23) (Figure 3 and Figure S1), based on 16S rRNA.

All the material analyzed in the present manuscript had been exposed on the shores of Keller Peninsula for decades, since they may be related to whaling remains (Kittel 2001). Although the Antarctic environment provides specific conditions favorable for the conservation of these samples, considering the low temperatures during all the year and the thawing cycle during the winter, handling these samples still demands a specific and careful approach (Cavalcanti et al. 2015, 2017).

The recovery of DNA from bones submerged/partially submerged in saltwater presents a complex challenge due to potential modifications to the bone structure caused by environmental and biological factors (Bertolini et al. 2022). Salt ions absorbed during submersion can increase the bone's porosity, leading to the breakdown of collagen from the hydroxyapatite matrix. This increased porosity and collagen degradation can accelerate DNA degradation, as the nucleic acid loses protection and stability normally provided by the inorganic matrix (Bertolini et al. 2022). Additionally, microscopic marine organisms can contribute to bone structure alteration through marine microboring, which partially dissolves the mineral portion of the bone (Bertolini et al. 2022). It is common that research groups working with such challenging samples test several extraction protocols and kits, such as the QIAamp DNA Investigator Kit, based on the use of silica columns to remove PCR inhibitors and the purified DNA is then eluted with an

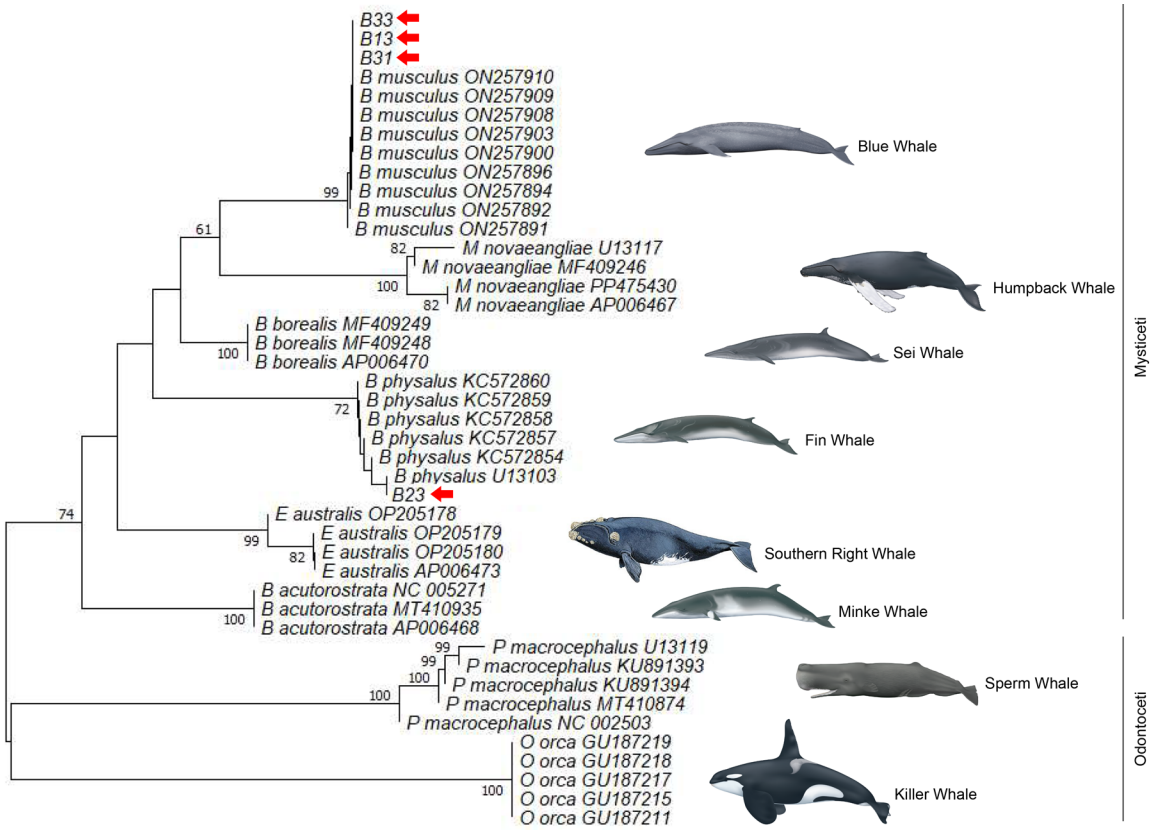

Figure 3. Neighbor-Joining tree (K2P) of Antarctic occurring whales based on partial 16S rRNA (bootstrap x1000) and newly determined samples (red arrows).

elution buffer under alkaline conditions and low salt concentration. Modifications based on this protocol are also common but the obtained results are usually used for small fragment profiling such as STR methods rather than sequencing.

The exposure effects of bone remains to degrading environmental conditions have been investigated including different agents, such as UV exposition, high levels of humidity, temperature, and prolonged burial (Perry et al. 1988, Schwartz et al. 1991, Alaeddini et al. 2010). In addition, the difference in bone structure, such as those rich in oil, large rigid bones or waterlogged, may cause differences in DNA conservation state (Cartozzo et al. 2018, Alaeddini et al. 2010, Krestoff et al. 2021).

Although the cold climate in Antarctica may aid the conservation of DNA in old bones, the exposure to degrading agents could fragment the mitochondrial regions commonly used for species identification (*cf*. COI, 16S, 12S, and the cytochrome b gene) might not be simple to sequence (Hebert et al. 2003, Milinkovitch et al. 1994, Mitani et al. 2009, Speller et al. 2016, Willerslev et al. 2004, Vences et al. 2005). This seems to be the case for our challenging samples regarding the difficulty observed for amplification and sequencing.

Whaling activities in Admiralty Bay and its relation to the bone fragments found in Keller Peninsula

The present whale remains analyzed represent only a portion of whales caught near King George Island, given that there is no register

of whaling stations inside the Admiralty Bay and the processing of whales initially were only possible with facilities provided by shore stations. Until the first operation of a factory ship in the region, in 1906, with the *Admiralen* (Rakusa-Suszczewski 1998), availing that the bay provided ideal conditions for floating factories (Tønnessen & Johnsen 1982). The capture of large rorquals became possible with the advance of technologies and techniques of whaling, such as steam engines and explosive harpoons. These elements improved the capacity to catch larger and faster rorquals, allied with the refinement of whaling techniques, such as the injection of compressed air in the head to increase the carcasse buoyancy and avoid losing the hunt product sinking after death (Vighi et al. 2020). The air compression and the decapitation before flensing could be the enablers for big and heavy pieces being washed ashore, although many others must be in the seabed.

The obtained sample identifications are consistent with the massive capture of rorquals in Antarctica during the whaling period, from 1904, until its prohibition in 1982, and the Soviet illegal hunt, until 1972 (Branch 2007a, Clapham et al. 1999, Thomas et al. 2016, Tønnessen & Johnsen 1982). In addition, the absence of Humpback is also reasonable, since they were no longer the main targets when the whalers settled in Admiralty Bay (Tønnessen & Johnsen 1982, Kittel 2001, Clapham & Baker 2018). It is estimated that during the 20th century, a high number of rorquals were caught, with over 360.000 blue whales and 725.000 fin whales, in the southern hemisphere (Clapham & Baker 2001). These numbers may be underestimated, considering that a great number of catches was inefficient, ending on the whale being 'struck but lost', or severely injured, and these whales killed in failure capturings were not reported (Vighi et al. 2020).

Recent observation registers and acoustic detection of Antarctic Blue whales for the region are lacking, making its distribution and abundance poorly understood (Bassoi et al. 2019, Branch 2007a), still, the main feeding grounds known for Antarctic Blue whales are in the circumpolar belt (Rice 1998). The scarce observation of these giant animals near South Shetland Islands and its presence in samples probably reflects the impact of the exploitation on their abundance in the region. Despite these whales being under protection (Clapham et al. 1999, Ivashchenko & Clapham 2014), Branch (2004, 2007a, 2007b) estimated that the species' current abundance is still less than 1% of the original, with about 1700 individuals (Stacey 2022). However, studies to evaluate the return of Blue whales to this historical hotspot are still needed, such as it have been made for other species and locations (Jackson et al. 2020). Regardless of being one of the most exploited species, Fin whales' current abundance is about 38,200 individuals in Antarctica and they are commonly observed and registered seasonally in Antarctica peninsula (Burkhardt & Lanfredi 2012, Širović et al. 2004, 2009). These cosmopolitan whales were hunted in the region until the level of exploitation far exceeded the sustainable yield of the populations, resulting in the collapse of whaling operations.

CONCLUSIONS

Degraded and aged samples have always been a challenge for DNA typing. In the present manuscript we successfully extracted, amplified and sequenced DNA from bone fragments left on a nearby marine environment since the modern whaling period in Antarctica. The specific identification of two species of *Balaenoptera* (*B. musculus* and *B. physalus*) from heavily weathered fragments itself is remarkable

regarding the harsh environment faced by these bone remains along the last decades. The record of these species do also support the historical occurrence of them in the Admiralty Bay or nearby areas.

Blue and fin whales' current presence in Admiralty Bay still needs more observations and studies to be conclusive. However, it is clear that their abundance and distribution has reduced in the whole world due to whaling, and also within the Admiralty Bay, as illustrated in the number of bones in Antarctic shores and herein confirmed with the use of molecular techniques. Although the degraded samples represent an obstacle for DNA typing, methods using small fragments such as STR-CE or those using massive parallel sequencing (MPS) could be used to enhance recovery of DNA fragments from these samples.

Finally, this study represents one more step to understand the history of Antarctic whaling on Admiralty Bay, showing that we can still scientifically gather information from the biological remains of this period, showing how the impacts of these past anthropogenic activities over the hunted whales species still influence the present day distribution of these species in the study area.

Acknowledgments

We thank Salvatore Siciliano and an anonymous reviewer for the precious comments on the previous version of this paper. This work was supported by Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We thank the Brazilian Navy and the Brazilian Antarctic Program (PROANTAR) for the logistic support offered during the OPERANTAR XL. The authors declare that there are no conflicts of interest.

REFERENCES

ANDELINOVIC S ET AL. 2005. Twelve-year experience in identification of skeletal remains from mass graves. Croat Med J 46(4): 530-539.

ALAEDDINI R, WALSH SJ & ABBAS A. 2010. Molecular studies of time and environment dependent effects on bone DNA survival. Aust J Forensic Sci 42: 211-220. doi: 10.1080/00450611003758264.

ANJOS DAA, GOLDENBERG-BARBOSA R, DONATO ALS, CARVALHO EF & AMARAL CRL. 2023. Probing the potential of InDel polymorphisms on the molecular identification of cetaceans. Acad Biol 1. doi: 10.20935/AcadBiol6032.

ARISMENDI JL, BAKER LE & MATTESON KJ. 2004. Effects of processing techniques on the forensic DNA analysis of human skeletal remains. J Forensic Sci 49(5): JFS2003314-5.

BASSOI M, ACEVEDO J, SECCHI ER, AGUAYO-LOBO A, ROSA LD, TORRES D, SANTOS MCO & AZEVEDO AS. 2019. Cetacean distribution in relation to environmental parameters between Drake Passage and Northern Antarctic Peninsula. Polar Biol 43: 1-15.

BERTOLINI E, GRIGNANI P, BERTOGLIO B, MARRUBINI G, MAZZARELLI D, LUCHESCHI S, BOSETTI A, FATTORINI P, CATTANEO C & PREVIDERÉ C. 2022. Dead migrants in the Mediterranean: genetic analysis of bone samples exposed to seawater. Forensic Sci Int 340: 111421. doi: 10.1016/j.forsciint.2022.111421.

BIERLICH KC ET AL. 2022. Seasonal gain in body condition of foraging humpback whales along the Western Antarctic Peninsula. Front Mar Sci 9: 1036860.

BRANCH TA, MATSUOKA K & MIYASHITA T. 2004. Evidence for increases in Antarctic blue whales based on Bayesian modelling. Mar Mamm Sci 20: 726-754.

BRANCH TA ET AL. 2007a. Past and present distribution, densities and movements of blue whales Balaenoptera musculus in the Southern Hemisphere and northern Indian Ocean. Mamm Rev 37.2: 116-175.

BRANCH TA, ALLISON C, MIKHALEV YA, TORMOSOV D & BROWNELL JR RL. 2007b. Historical catch series for Antarctic and pygmy blue whales. Report (SC/60/SH9) to the Scientific Committee of the International Whaling Commision, available on request from the Secretariat, International Whaling Commission, The Red House 135.

BURKHARDT E & LANFREDI C. 2012 Fall feeding aggregations of fin whales off Elephant Island (Antarctica). In Paper SC/64/SH9 presented at the Scientific Committee Meeting of the IWC p. 1-6.

CAMPOS PF, CRAIG OE, TURNER-WALKER G, PEACOCK E, WILLERSLEV E & GILBERT MTP. 2012. DNA in ancient bone - where is it located and how should we extract it? Ann Anat 194: 7-16.

CARTOZZO C, SINGH B, BOONE E & SIMMONS T. 2018. Evaluation of DNA extraction methods from waterlogged bones: a pilot study. J Forensic Sci 63(6): 1830-1835.

CAVALCANTI P, CARVALHO F, CARVALHO EF & SILVA DA. 2017. A mini-primer set in a multiplex PCR fashion covering the MTDNA control region from submerged skeletal remains. Forensic Sci. Int. Genet.Supplement Series 6: e469-e470.

CAVALCANTI P, FREITAS H, CARVALHO EF & SILVA DA. 2015. Optimized DNA extraction method from skeletal remains using different typing methodologies in forensics. J Forensic Sci Int Genet Supplement

CLAPHAM PJ & BAKER CS. 2001. How many whales were killed in the Southern Hemisphere in the 20th century? Report of the International Whaling Commission 53: 3.

CLAPHAM PJ & BAKER CS. 2018. Modern whaling. Encyclopedia of marine mammals 3rd ed., 1328-1332.

CLAPHAM PJ, YOUNG SB & BROWNELL RL JR. 1999. Baleen whales: conservation issues and the status of the most endangered populations. Mamm Rev 29(1): 37-62.

COURTS C & MADEA B. 2011. Full STR profile of a 67-year-old bone found in a fresh water lake. J Forensic Sci 56: 172-175.

CRAINIC K, PARAIRE F, LETERREUX M, DURIGON M & DE MAZANCOURT P. 2002. Skeletal remains presumed submerged in water for three years identified using PCR-STR analysis. J Forensic Sci 47(5): 1-3.

DALEBOUT ML, VAN HELDEN A, VAN WAEREBEEK K & BAKER CS. 1998. Molecular genetic identification of southern hemisphere beaked whales (Cetacea: Ziphiidae). Mol Ecol 7: 687-694.

D'ERRICO G, ZANON C, LAGO G, VECCHIO C & GAROFANO L. 1998. DNA analysis on skeletal remains after months of permanence in sea-water. In: Olaisen B, Brinkmann B, Lincoln PJ, editors. Progress in Forensic Genetics No. 7. Amsterdam, The Netherlands: Elsevier, p. 106-108.

DRUMMOND AJ ET AL. 2010. Geneious v4.8.2 http://www.geneious.com (last accessed in November 2013).

EDGAR RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput Nucl Acids Res 32: 1792-1797.

FALCÃO LHO, CAMPOS AS, FREITAS JEP, FURTADO-NETO MAA & FARIA VV. 2017. Molecular identification of cetaceans from the West Atlantic using the E3-I5 region of COI. Genet Mol Res Apr 20 16(2). doi: 10.4238/gmr16029607. PMID: 28437554.

FELSENSTEIN J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evol 39: 783-791.

FLORES H, VAN FRANEKER JA, SIEGEL V, HARALDSSON M, STRASS V, MEESTERS EH, BATHMANN U & WILLEM JW. 2012. The Association of Antarctic Krill Euphausia superba with the Under-Ice Habitat. PLOS ONE 7(2): 31775.

FOOTE AD, HOFREITER M & MORIN PA. 2012. Ancient DNA from marine mammals: Studying long-lived species over ecological and evolutionary timescales. Ann Anat - Anatomischer Anzeiger 194 (1): 112-120.

FRIEDLAENDER A & MODEST M. 2018. Whales of the Antarctic Peninsula - Science and Conservation for the 21st Century. A report for policymakers from the University of California, Santa Cruz and WWF.

HACQUEBORD L. 1992. Hector station on Deception Island (South Shetlands Islands, Antarctica), an environmental assessment study of a whalingstation. Int J Circumpolar Health 1-2(6): 72-97.

HERBERT PDN, CYWINSKA A, BALL SL & DEWAARD JR. 2003. Biological identifications through DNA barcodes. Proc Royal Soc B 270: 313-321.

IVASHCHENKO YV & CLAPHAM PJ. 2014. Too Much Is Never Enough: The Cautionary Tale of Soviet Illegal Whaling. Mar Fish Rev 76 (1-2) 1-21.

JACKSON JA ET AL. 2020. Have whales returned to a historical hotspot of industrial whaling? The pattern of southern right whale *Eubalaena australis* recovery at South Georgia. Endanger Species Res 43: 323-339.

JAKUBOWSKA J, MACIEJEWSKA A & PAWLOWSKI R. 2012. Comparison of three method of DNA extraction from human bones with different degrees of degradation. Int J Legal Med 126: 173-178.

JAYASANKAR P. 2020. Taxonomic identification of marine mammals - current research and approaches. Mar Fish Infor Serv T & E Ser 246.

KAMAL MM, BUTET NA, RAHAYU ES & HAKIM AA. 2021. Molecular Characteristic Identification of Partial 16S rRNA Genes of Sperm Whales (Physeter macrocephalus Linnaeus, 1758). Habitus Aquatica 2(1): 21-28. doi: 10.29244/HAJ.2.1.21.

KAPINSKA E & SZCZERKOWSKA Z. 2004. Personal identification based on nuclear DNA extracted from bones of deceased individuals. Probl Forensic Sci LX: 104-116.

KEARSE M ET AL. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647-1649.

KIMURA M. 1980. A simple method for estimating evolutionary rate of base substitutions through

comparative studies of nucleotide sequences. J Mol Evol 16: 111-120.

KITTEL P. 2001. Inventory of whaling objects on the Admiralty shores (King George Island, South Shetland Islands) in the years 1996-1998. Pol Polar Res 22: 45-70.

KRESTOFF ES, CREECY JP, LORD WD, HAYNIE ML, COYER JA & SAMPSOM K. 2021. Mitochondrial DNA Evaluation and Species Identification of Kemp's Ridley Sea Turtle (*Lepidochelys kempii*) Bones After a 3-Year Exposure to Submerged Marine and Terrestrial Environments. Front Mar Sci 742.

KUMAR S, STECHER G, LI M, KNYAZ C & TAMURA K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35: 1547-1549.

LINDQVIST C, PROBST A, MARTIN AR, WIIG Ø & BACHMANN L. 2009. Molecular species identification of historical whale remains from South Georgia. Mar Mammal Sci 25(1): 229-238.

MCBRIDE M, STOKKE OS, RENNER AHH, KRAFFT BJ, BERGSTAD OA, BIUW M, LOWTHER AD & STIANSEN JE. 2021. Antarctic krill *Euphausia superba*: spatial distribution, abundance, and management of fisheries in a changing climate. Mar Ecol Prog 668: 185-214.

MILINKOVITCH MC, MEYER A & POWELL JR. 1994. Phylogeny of all major groups of cetaceans based on DNA sequences from three mitochondrial genes. Mol Biol Evol 11(6): 939-948.

MITANI T, AKANE A, TOKIYASU T, YOSHIDA S, OKII Y & YOSHIDA M. 2009. Identification of animal species using the partial sequences in the mitochondrial 16S rRNA gene. Leg Med 11: 449-450.

NEI M & KUMAR S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

PALUMBI SR. 1996. Nucleic acids II: The polymerase chain reaction. In: Hillis M, Moritz C & Mable BK (Eds), Molecular Systematics, 2nd ed., Sinauer, p. 205-248.

PALUMBI SR, MARTIN A, ROMANO S, MCMILLAN WO, STICE L & GRABOWSKI G. 1991. The simple fool's guide to PCR, version 2 .O. University of Hawaii, Honolulu, p. 43.

PERRY WL, BASS WM, RIGGSBY WS & SIROTKIN K. 1988. The autodegradation of deoxyribonucleic acid (DNA) in human rib bone and its relationship to the time interval since death. J Forensic Sci 33: 144-153.

RAKUSA-SUSZCZEWSKI S. 1998. The past and present of King George Island (South Shetland Islands, Antarctica). Pol Polar Res 19: 249-252.

RAKUSA-SUSZCZEWSKI S & NĘDZAREK A. 2002. Whale bones and macroalgae as source of nutrients and cations in the nearshore geoecosystem of Admiralty Bay (King George Island, Antarctica). Pol J Ecol 50: 389-396.

RICE D. 1998. Marine mammals of the world: systematics and distribution. Allen Press, Lawrence, KS.

SAITOU N & NEI M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425.

SCHWARTZ TR, SCHWARTZ EA, MIESZERSKI L, MCNALLY L & KOBILINSKY L. 1991. Characterization of deoxyribonucleic acid (DNA) obtained from teeth subjected to various environmental-conditions. J Forensic Sci 36: 979-990.

SILVA ET AL. 2021. Integrating morphology and DNA barcoding to assess cetacean diversity in Brazil. Mammal Res 66: 349-369.

ŠIROVIĆ A, HILDEBRAND JA, WIGGINS SM, MCDONALD MA, MOORE SE & THIELE D. 2004. Seasonality of blue and fin whale calls and the influence of sea ice in the Western Antarctic Peninsula. Deep-Sea Res II 51: 2327-2344.

ŠIROVIĆ A, HILDEBRAND JA & WIGGINS SM. 2009. Blue and fin whale acoustic presence around Antarctica during 2003 and 2004. Mar Mamm Sci 25 1: 125-136.

SOLAZZO C, FITZHUGH W, KAPLAN S, POTTER C & DYER JM. 2017. Molecular markers in keratins from Mysticeti whales for species identification of baleen in museum and archaeological collections. PLOS ONE 12(8): e0183053.

SPELLER C ET AL. 2016. Barcoding the largest animals on Earth: ongoing challenges and molecular solutions in the taxonomic identification of ancient cetaceans. Phil Trans R Soc B 371(1702): 20150332.

STACEY E. 2022. Assessing the effects of whaling on the genetic diversity of north atlantic blue whales (*Balaenoptera musculus*). Saint Mary's University, Halifax, Nova Scotia, p. 4-7.

STAITI N, DI MARTINO D & SARAVO L. 2004. A novel approach in personal identification from tissue samples undergone different processes through STR typing. Forensic Sci Int 146S:S171-3.

SZABO VE. 2008. Monstrous fishes and the mead-dark sea: whaling in the medieval North Atlantic. Leiden, The Netherlands: Brill.

TAMURA K & NEI M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10: 512-526.

THOMAS PO, REEVES RR & BROWNEL RL JR. 2016. Status of the world's baleen whales. Mar Mamm Sci 32: 682-734.

TØNNESSEN J & JOHNSEN O. 1982. The history of modern whaling. C. Hurst & Company, Australian National University, Press London-Canbera.

VENCES M, THOMAS M, VAN DER MEIJDEN A, CHIARI Y & VIEITES DR. 2005. Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. Front Zool 2 1: 1-12.

VIGHI M, BORRELL A, JACKSON JA, CARROLL EL, PENNINO MG & AGUILAR A. 2020. The missing whales: relevance of "struck and lost" rates for the impact assessment of historical whaling in the southwestern Atlantic Ocean. ICES J Marine Sci 78(1): 14-24.

VIRICEL A & ROSEL PE. 2012. Evaluating the utility of cox1 for cetacean species identification. Mar Mammal Sci 28(1): 37-62.

WILLERSLEV E, HANSEN AJ & POINAR HN. 2004. Isolation of nucleic acids and cultures from fossil ice and permafrost. Trends Ecol Evol 19: 141-147.

SUPPLEMENTARY MATERIAL

Figure S1.
Table SI.

How to cite
ANJOS D, DONATO A, GOLDENBERG-BARBOSA R, CARVALHO EF & AMARAL CRL. 2024. Molecular identification of whales remains from the Keller Peninsula, Admiralty Bay, King George Island, Antarctica. An Acad Bras Cienc 96: e20240502. DOI 10.1590/0001-3765202420240502.

Manuscript received on May 15, 2024;
accepted for publication on August 2, 2024

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DA - Designed the study, worked on sampling, laboratory assays, analysis of samples and results, and wrote a preliminary version of the manuscript. AD - collaborated in the laboratory assays, analysis of samples, writing the manuscript and reviewing. RGB - collaborated in the laboratory assays, analysis of samples, writing the manuscript and reviewing. EFC - collaborated in the laboratory assays, analysis of samples, writing the manuscript and reviewing. CA - designed the study, worked on sampling, data interpretation and wrote a final version of the manuscript.