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Molecular identification of a fin whale stranded in Veracruz, southern Gulf of Mexico

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Abstract

Baleen whale strandings are rare in the southern Gulf of Mexico and can provide valuable molecular information where species are infrequently documented. We analyzed tissue from a highly decomposed whale stranded on the central coast of Veracruz, Mexico, by amplifying and bidirectionally sequencing the mitochondrial DNA control region. A total of four chromatogram reads were generated: one forward and one reverse read for each of the two overlapping amplicons (5′ and 3′). Accordingly, nucleotide positions located within the overlap between fragments were independently covered by up to four reads, whereas positions outside the overlap were supported by the corresponding forward and reverse reads. A nucleotide BLAST search identified the specimen as a fin whale (*Balaenoptera physalus*), showing 99% identity to the mitochondrial reference sequence MF409243.1. Three nucleotide substitutions relative to that reference occurred at positions 15499 (A>G), 15650 (T>C), and 15754 (C>T). The three nucleotide substitutions were each supported by high-quality base calls and by all independent reads covering the corresponding position. In a maximum-likelihood analysis, the Veracruz sequence clustered within a clade containing sampled North Atlantic fin whale control-region sequences. The specimen's estimated length (~11 m) was consistent with a young individual, although decomposition prevented definitive age classification. The mitochondrial result indicates affinity with sampled North Atlantic diversity but does not establish geographic origin or stock identity. These results provide molecular confirmation of a rare fin whale occurrence in the southern Gulf of Mexico and demonstrate that highly decomposed stranding material can retain sufficient good quality mitochondrial DNA for reliable species identification. Systematic tissue preservation and archiving during stranding responses can therefore improve verified occurrence records in poorly documented regions. This case underscores the scientific value of collecting and preserving stranded specimen samples from vulnerable species that are exposed to heavy maritime traffic, artisanal fisheries, and ongoing oceanographic variability in poorly documented regions of the Gulf of Mexico.

Keywords:
Degraded tissue, mitochondrial control region, taxonomy

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Introduction

Fin whales (*Balaenoptera physalus*) are widely distributed across temperate and subpolar oceans. Still, they are rare in the Gulf of Mexico relative to adjacent North Atlantic regions and are generally regarded as extralimital in low-latitude Gulf waters (Jefferson &

Schiro, 1997; Würsig et al., 2000; Edwards et al., 2015; Ramírez-León et al., 2020). A substantial tropical and near-equatorial hiatus in the distribution of this species has been documented in the post-whaling era (Edwards et al., 2015), highlighting the paucity of records in the offshore waters of the Gulf of Mexico (Jefferson & Schiro, 1997; Würsig, 2017).

That context is particularly important for the state of Veracruz; for instance, Ortega-Argueta et al. (2005) compiled 21 strandings spanning 745 km of shoreline and only found a single balaenopterid record, represented by a 1965 photograph from Alvarado that could not be resolved to species. The authors concluded that baleen whale strandings are rare in the southwestern Gulf of Mexico and that the historical regional record remains sparse compared with that of the Mexican Pacific and the Gulf of California.

More recently, Ramírez-León's (2020) synthesis for the southern Gulf of Mexico waters showed that cetacean records are concentrated on the continental shelf and inner slope, and habitat-suitability analyses identified the Tamaulipas–Veracruz slope as one of four sectors expected to support elevated cetacean diversity, largely in association with depth structure, minimum sea-surface temperature, and productivity-linked shelf-break processes. However, this regional synthesis also found only two fin-whale strandings in the entire Mexican exclusive economic zone, one in Yucatán (February 2016 by Díaz-Gamboa et al., 2022) and one in southern Veracruz (January 2017), confirming that large whales and the fin whale in particular remain rare stranding records for the southern Gulf of Mexico (Ramírez-León, 2020).

Thus, in this study we used decomposed tissue from a baleen whale stranded on the central coast of Veracruz to test whether degraded DNA retains sufficient mtDNA data to confirm species identity. We then interpreted the result in a regional context, considering the rarity of balaenopterid strandings in Veracruz (Ortega-Argueta et al., 2005; Ramírez-León, 2020), the scarcity of fin-whale records in the Gulf of Mexico (Jefferson & Schiro, 1997; Edwards et al., 2015; Ramírez-León et al., 2020), and the conservation relevance of documenting rare large-whale occurrences in a region where maritime traffic, fisheries, and oceanographic variability intersect.

Materials and Methods

Sample provenance

The material available for molecular analysis consisted of a frozen transverse tissue block from a stranded whale from the central coast of Veracruz, Mexico. On 11 February 2025, federal authorities from the Federal Attorney's Office for Environmental Protection (PROFEPA in Spanish) in the state of Veracruz received a report of a marine mammal stranding (Sample ID: 250211NAULABMMARB1) on the coast of Maracaibo Beach, in the municipality of Nautla (20°12'16.0" N, 96°45'00.1" W; Figs 1 and 2). Based on the presence of baleen plates, we identified the specimen as a mysticete, with an estimated total length of 11 m. The remains were in an advanced state of decomposition (code 4 according to Geraci & Lounsbury, 2005) and had missing parts; this prevented reliable identification of the species, the age or growth stage of the individual, or determination of an apparent cause of death.

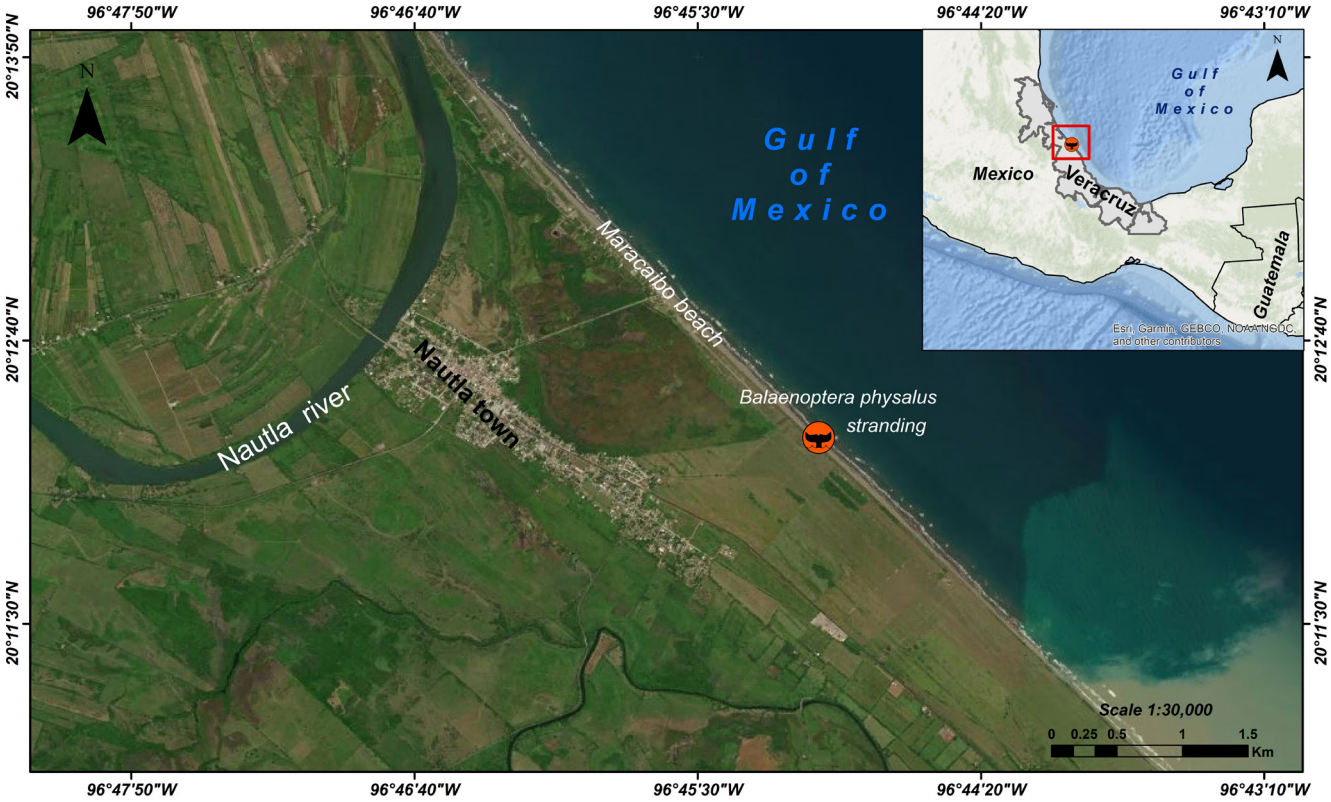


Figure 1. Stranding site of the fin whale *Balaenoptera physalus* individual in Maracaibo Beach, municipality of Nautla, Veracruz, Mexico.



Figure 2. Remains of the specimen of fin whale (*Balaenoptera physalus*) stranded near Playa Maracaibo in Nautla, Veracruz, Mexico.

Tissue samples (0.6 kg of skin, blubber, and muscle from the ventral region) were collected with the support and direct intervention of the federal authorities. Samples were placed in a properly labeled and sealed *Ziploc*® bag to prevent contamination and subsequently transported to the Laboratorio de Mamíferos Marinos (LabMMar) at Universidad Veracruzana under refrigerated conditions in a standard ice cooler. The authorities decided the process of final disposal of the specimen; therefore, civil protection personnel carried it out, by using excavation machinery at the same coordinates where the stranding was observed. Once in the laboratory, the tissue was rinsed with sterile Milli-Q water and divided into two equal portions. One part of the tissue was immediately stored at -70 °C, and the other part was processed as it arrived and cut into small pieces with a sterile scalpel and mechanically macerated in a mortar for DNA extraction.

DNA extraction and quality assessment

Macerated tissue was subdivided into six aliquots of approximately 50 mg each. DNA was extracted in duplicate using three different protocols, with a final elution volume of 100 µl per sample. Tubes 1 and 2 were processed using an organic-solvent extraction method based on phenol-chloroform-isoamyl alcohol (25:24:1), following overnight lysis (16 h) in buffer containing 0.1 M NaCl, 25 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 0.5% SDS, and 10 µl of proteinase K (10 mg/ml). Tubes 3 and 4 were extracted using the QIAamp DNA Blood Mini Kit (Qiagen), whereas tubes 5 and 6 were processed with the High Pure PCR Template Preparation Kit (Roche), following the manufacturers' instructions. DNA concentration and purity were determined using a NanoDrop One spectrophotometer (Thermo Scientific). DNA integrity was evaluated by electrophoresis on a 0.8% agarose gel, loading 250 ng of DNA per lane.

PCR amplification of the mtDNA control region

Two overlapping fragments spanning the mitochondrial control region (5' and 3') were amplified following the protocol described by Rosel and Wilcox (2014). GoTaq® G2 Colorless Master Mix, 2X (Promega) was used for PCR amplification. This premixed solution contains GoTaq® G2 DNA Polymerase in 2X Colorless GoTaq® G2 Reaction Buffer (pH 8.5), 400 µM each dATP, dGTP, dCTP and dTTP, and 3 mM MgCl-. The 5' fragment was amplified in a reaction containing 1x Master Mix, 0.2 µM each of primers L15824 (5'-CCTCACTCCTCCCTAAGACT-3'; Rosel et al., 1999) and H16498 (5'-CCTGAAGTAAGAACCAGATG-3'; Rosel et al., 1994), 200 ng of template DNA, and nuclease-free water to a final volume of 25 µl. The 25-µl reaction contained 12.5 µl of 2× GoTaq® G2 Colorless Master Mix. The DNA obtained showed a high degree of degradation due to the advanced state of tissue decomposition. Consequently, a higher concentration than that used by Rosel and Wilcox was required, particularly for the larger amplicon. Initially, we attempted to use DNA concentrations between 25 and 100 ng, resulting in barely visible bands for the 5' fragment and no visible bands for the 3' fragment. Both cases improved when the amount of DNA was increased, thereby providing a better opportunity for the primers to hybridize with the degraded whale DNA fragments. The 3' fragment was amplified in a reaction containing 1x Master Mix, 0.2 µM each of primers L16061 (5'-GCTATGTATTATTGTGCATTC-3'; Tolley & Rosel, 2006) and H00651 (5'-AAGGCTAGGACCAAACCT-3'; Kocher et al., 1989), 400 ng of template DNA, and nuclease-free water to a final volume of 25 µl. The 25-µl reaction contained 12.5 µl of 2× GoTaq® G2 Colorless Master Mix.

The thermocycling program for the 5' fragment consisted of an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min. For the 3' fragment, thermocycling consisted of an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. A negative control without template DNA was included in each PCR set.

PCR products were visualized by electrophoresis on a 2% agarose gel to verify the presence of single amplicons. Subsequently, 18 µl of each PCR product was purified using the Wizard® PCR Preps DNA Purification System (Promega) according to the manufacturer's instructions and resuspended in 50 µl of Milli-Q water.

Sequencing and sequence interpretation

Purified PCR products from both amplicons (5′ and 3′ fragments) were adjusted to 50 ng/µl and subjected to bidirectional Sanger sequencing by capillary electrophoresis using an Applied Biosystems 3500 Genetic Analyzer at the Molecular Biology Unit, Institute of Cellular Physiology, Universidad Nacional Autónoma de Mexico (UNAM). Forward and reverse chromatograms were inspected manually to verify sequence quality.

The resulting .ab1 files were analyzed using SnapGene V8.2.2, and low-quality bases at the ends of the sequences were trimmed. Each sequence was first edited independently, and the forward and reverse sequences were aligned to create a consensus sequence and then queried against the Nucleotide Basic Local Alignment Search Tool (BLAST) database to determine taxonomic identity (Zhang et al., 2000). The consensus sequence and comparative sequences were aligned with Clustal Omega (https://europepmc.org/article/MED/38597606).

Sequence alignments were visualized and inspected for genetic variants using Jalview (Waterhouse et al., 2009). The positions of identified variants within the mitochondrial genome and the amplified control region were visualized using Proksee (Grant et al., 2023). The complete control-region sequence was aligned with 56 fin whale sequences from Archer et al. (2013), representing the North Atlantic (NAtl, n = 13) and Southern Hemisphere (SHem, n = 43) populations, and one humpback whale (*Megaptera novaeangliae*) sequence (GenBank accession NC_006927.1) as the outgroup (see Supplementary Material 1, S1-S3, Table S1). The HKY85+G+I nucleotide-substitution model was selected by Smart Model Selection (SMS) in PhyML 3.0 (Guindon et al., 2010) according to the Bayesian Information Criterion (BIC) among the candidate substitution models evaluated by SMS (Lefort et al., 2017). Phylogenetic analysis was performed on the ATGC bioinformatics platform (https://website.atgc-montpellier.fr/). The tree was visualized and edited in Figtree 1.4.4.

Results

Table 1. Concentration and purity of genomic DNA extracted from whale tissue using different extraction methods.

Sample	Extraction method	Concentration (ng/µl)	A260/A280	A260/A230
Tube 1	Organic solvents	54.8	1.81	1.30
Tube 2	Organnic solvents	47.5	1.91	1.62
Tube 3	QIAamp DNA Blood Mini	14.6	2.28	0.59
Tube 4	Kit (Qiagen)	21.8	2.26	0.77
Tube 5	High Pure PCR Template Preparation	3.4	2.28	0.30
Tube 6	Kit (Roche)	3.8	2.34	0.38

DNA concentration and purity measurements are summarized in Table 1. The highest DNA concentrations were obtained using the organic-solvent extraction protocol. In contrast, both commercial kit-based protocols yielded lower DNA concentrations. Despite differences in concentration and purity, all extracts showed extensive degradation when evaluated by agarose gel electrophoresis (Fig. 3A). This degradation was likely associated with post-mortem DNA decay during the interval between the whale's death and sample collection, as well as prolonged exposure to environmental conditions prior to preservation.

DNA extracted using the organic-solvent protocol was selected for downstream PCR analyses. Amplification of the mitochondrial control region yielded products of the expected sizes: 538 base-pairs (bp) for the 5′ fragment and 874 bp for the 3′ fragment. (Fig. 3B).

High-quality bidirectional sequences were obtained from both the 5′ and 3′ amplicons, with read-level average Phred scores ranging from 54 to 58 (Supplementary Material 1 Fig. S1). Alignment of the individual sequences using Nucleotide BLAST identified the sample as *B. physalus* (GenBank accession MF409243.1), with 99% mtDNA control region sequence identity and only three nucleotide differences relative to the reference sequence.

The 5′ fragment aligned directly to positions 15388–15925 which correspond to the control region of the reference mitochondrial genome (MF409243.1). In contrast, the 3′ fragment spanned the junction between positions 16401 and 1, reflecting the circular organization of the mitochondrial genome and preventing its representation as a single continuous alignment against the linearized reference sequence in BLAST. Multiple sequence alignment against MF409243.1 enabled accurate placement of this fragment between positions 15623–16401 and 1–95. Together, the four bidirectional reads covered and reconstructed a 1109 bp consensus sequence encompassing the complete mitochondrial control region of *B. physalus* and adjacent flanking regions.

Electropherograms showed three nucleotide substitutions identified relative to the reference sequence (MF409243.1). All independent reads covering each position showed concordant base calls, with site-specific Phred quality scores supporting the substitutions. A total of four chromatogram reads were generated, comprising forward and reverse reads for each amplicon. Depending on whether a nucleotide position occurred within the overlap between amplicons, each substitution was therefore independently covered by either two or four reads. The specimen differed from the reference sequence at positions 15499 (A > G), 15650 (T > C), and 15754 (C > T) within the mitochondrial control region (Fig. 4A–C). Taken together, the DNA extraction, PCR amplification, sequencing and sequence alignment results provide robust molecular evidence supporting the identification of the stranded whale as *B. physalus.*

In the maximum-likelihood tree based on a 1,056-bp alignment of control-region sequences from 57 fin whales, the Veracruz sequence clustered within the clade containing the sampled North Atlantic fin whale control-region sequences (Fig. 5). The Veracruz consensus sequence (Bphy_Ver) was deposited in GenBank under accession number PZ723631.

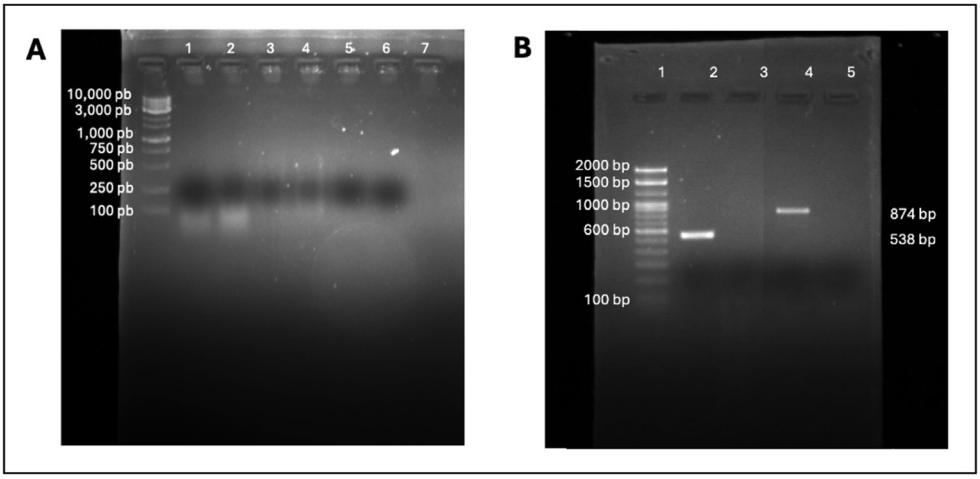


Figure 3. DNA integrity and PCR amplification of mitochondrial control region fragments. A) Electrophoresis on a 0.8% agarose gel. A total of 250 ng of genomic DNA was loaded into each well. Lane 1: molecular weight marker (1 kb Plus DNA Ladder). Lanes 1–2: DNA extracted using the organic solvent method. Lanes 3–4: DNA extracted using the organic solvent method followed by purification with the QIAamp DNA Blood Mini Kit (Qiagen). Lanes 5–6: DNA extracted using the organic solvent method followed by purification with the High Pure PCR Template Preparation Kit (Roche). Lane 7: negative PCR control; B) Electrophoresis on a 2% agarose gel showing PCR amplification products. Lane 1: molecular weight marker (100 bp DNA Ladder). Lane 2: amplification of the 5'region (538 bp). Lane 3: negative PCR control. Lane 4: amplification of the 3'region (874 bp). Lane 5: negative PCR control.

Discussion

Our contribution provides molecular confirmation that a rare balaenopterid stranding on the Veracruz coast was a fin whale. Historically, baleen whale stranding records in Veracruz are scarce; to the best of our knowledge, aside from two humpback whale strandings and the record from 2017 in the Ramírez-León (2020) compilation, there are no other stranding events of balaenopterids in the state. In this sense, the present case strengthens the regional record not by increasing the number of baleen whale strandings per se, but by converting a rare southern Gulf event into a species-level, genetically supported record. Although decomposition and missing body parts prevented precise morphometric assessment, the estimated body length (~11 m) is consistent with a young individual and falls within historical length ranges reported for fin whale calves (Ichihara, 1966). Suckling fin whale calves are generally below approximately 13.4 m, although body size varies among populations. The Veracruz individual is therefore most conservatively interpreted as a probable calf or young juvenile. A similarly small stranded fin whale from the Mediterranean was recently classified as juvenile at 13.5 m, further supporting the interpretation that the Veracruz specimen had not reached adult size (Ayas & Çiftçi, 2024).

Despite the high-diversity of cetaceans in the Gulf of Mexico waters (Ramírez-León, 2020), the southwestern area has been an under-documented region compared with other Mexican marine areas (Ortega-Argueta et al., 2005). Large whales should be expected near the continental slope and shelf-break ecotone (Díaz-Gamboa et al., 2022), where upwelling and current interactions elevate productivity and prey aggregation; yet records of fin whales are rare in these waters (Jefferson & Schiro, 1997; Edwards et al., 2015; Würsig, 2017).

The February timing, small estimated body size, and mitochondrial affinity of the Veracruz specimen provide a plausible biological context for its occurrence in the Gulf. Fin whales are primarily temperate-to-polar but exhibit complex seasonal movements, and low-latitude winter occurrence is part of the species' broader migratory repertoire; distinct calving grounds remain poorly defined for several populations. Fin whales also show considerable individual variation in migration, including partial migration and movements between otherwise differentiated regions (Geijer & Jones, 2016; Gauffier et al., 2020). Therefore, the small number of Gulf records should not necessarily be interpreted as unrelated anomalous occurrences. Together with previous records from Yucatán and Veracruz, the present case is consistent with rare or intermittent entry of fin whales into the Gulf, potentially involving young individuals during the winter period. Testing this hypothesis will require additional genetically verified records, acoustic detections, photo-identification, or telemetry.

The placement of the Veracruz haplotype within the clade containing sampled North Atlantic control-region sequences is biologically plausible but should be interpreted as mitochondrial affinity rather than stock assignment. North Atlantic fin whales exhibit population structure, yet movements and gene flow occur among nominal management regions, and individual movements can cross major oceanographic boundaries (Bérubé et al., 1998; Archer et al., 2019; Cabrera et al., 2019; Gauffier et al., 2020). For instance, North Atlantic fin whales seasonally enter the western Mediterranean despite genetic differentiation between Atlantic and resident Mediterranean populations, illustrating that occasional movement into semi-enclosed lower-latitude basins does not necessarily imply a distinct local stock. The Veracruz sequence is therefore compatible with a North Atlantic maternal lineage entering the Gulf, but confirmation would require longer mitochondrial sequences and, preferably, nuclear or genome-wide markers from additional Gulf specimens.

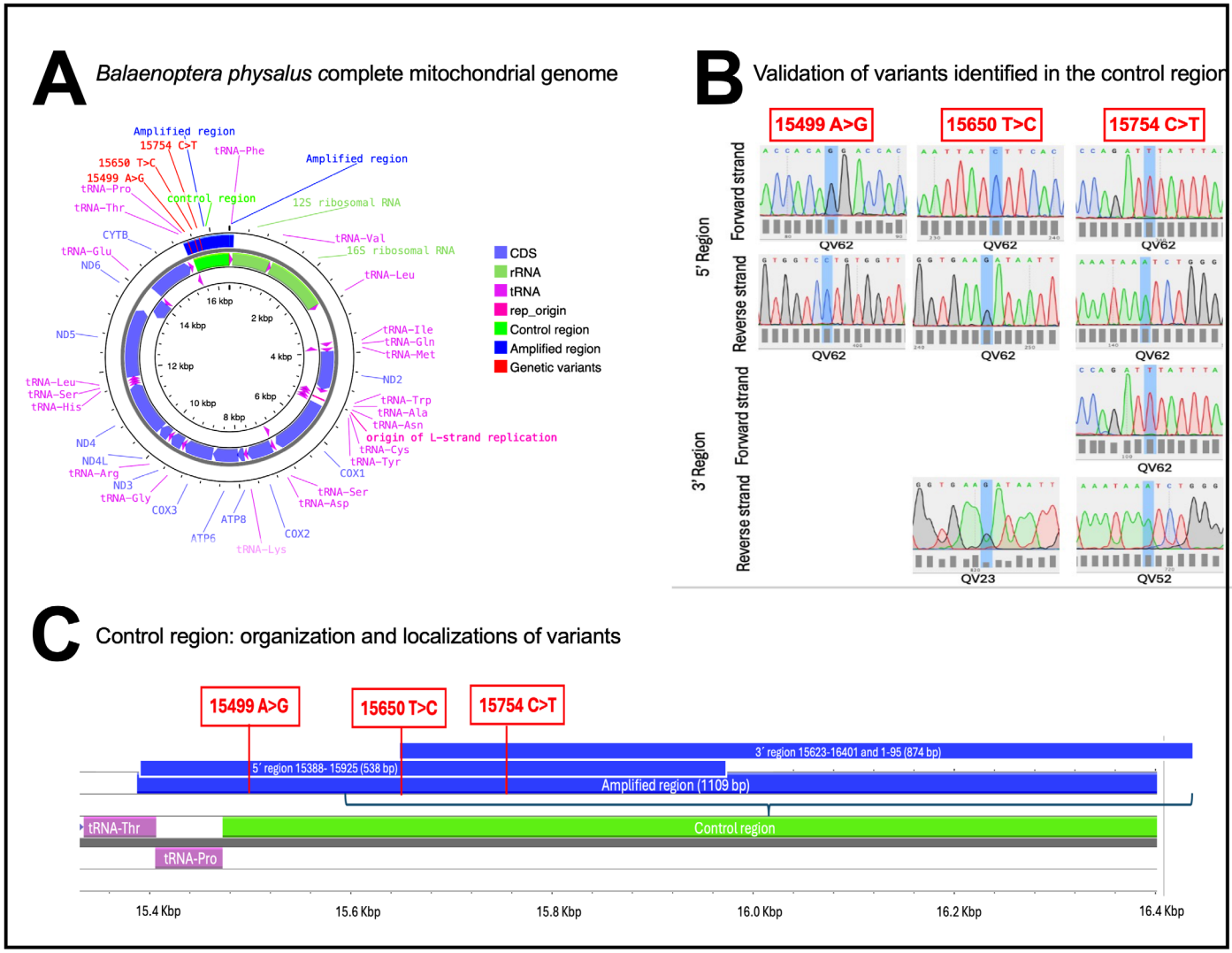


Figure 4. Identification and localization of genetic variants in the mitochondrial control region of the fin whale *Balaenoptera physalus*. A) Circular representation of the mitochondrial genome showing the control region and the amplified fragments analyzed in this study. The positions of the identified genetic variants are indicated. B) Electropherograms showing the nucleotide substitutions relative to reference sequence MF409243.1: 15499 A > G, 15650 T > C, and 15754 C > T. C) Linear representation of the amplified control region indicating the location of the identified variants relative to the 5′ and 3′ fragments.

The latter also illustrates that mysticete occurrence within the Gulf of Mexico can represent very different biogeographic processes. For instance, Rice's whales (*Balaenoptera ricei*) constitute a genetically distinct Gulf lineage with a highly restricted distribution (Rosel et al., 2021), whereas fin whales, humpback whales, and several other large cetaceans occur only rarely and are generally regarded as occasional migrants or extralimital visitors. Thus, molecular identification is especially valuable because superficially similar rare Gulf records may represent either resident evolutionary lineages or transient individuals connected to populations outside this basin.

The broader conservation relevance of this record lies in the interaction between rare whale occurrence and human pressures in the southern Gulf. Ortega-Argueta et al. (2005) documented that cetacean strandings in Veracruz occur along a coast used intensively by artisanal fisheries and that gillnets and longlines are widespread in the region. Although fin whales are not typical coastal residents there, any large whale entering neritic waters where fixed or drifting gear is common may face an increased risk of entanglement. That concern is consistent with the wider western North Atlantic picture where this source of mortality is under-detected (NOAA-NMFS, 2024). Vessel collision is an established mortality source for fin whales, but the decomposed state of this specimen precluded diagnosis of cause of death. Consequently, local vessel traffic should be regarded as relevant background risk rather than an explanation for this stranding (Laist et al., 2001; National Marine Fisheries Service, 2024). Likewise, regional productivity and shelf-break processes may influence prey availability and the occurrence of large cetaceans in the southern Gulf (Davis et al., 1998; Ramírez-León, 2020). However, no evidence from this specimen directly implicates entanglement, vessel collision, prey conditions, or oceanographic variability in the stranding. These factors should therefore be regarded as regional context rather than explanations for this event.

Viewed together, these lines of evidence argue that rare records should not be dismissed as mere curiosities. For a species that is uncommon in the Gulf, every genetically confirmed occurrence contributes to three linked tasks: refining the regional record, improving specimen archives, and identifying where emerging risk may go unnoticed. The immediate lesson from the present

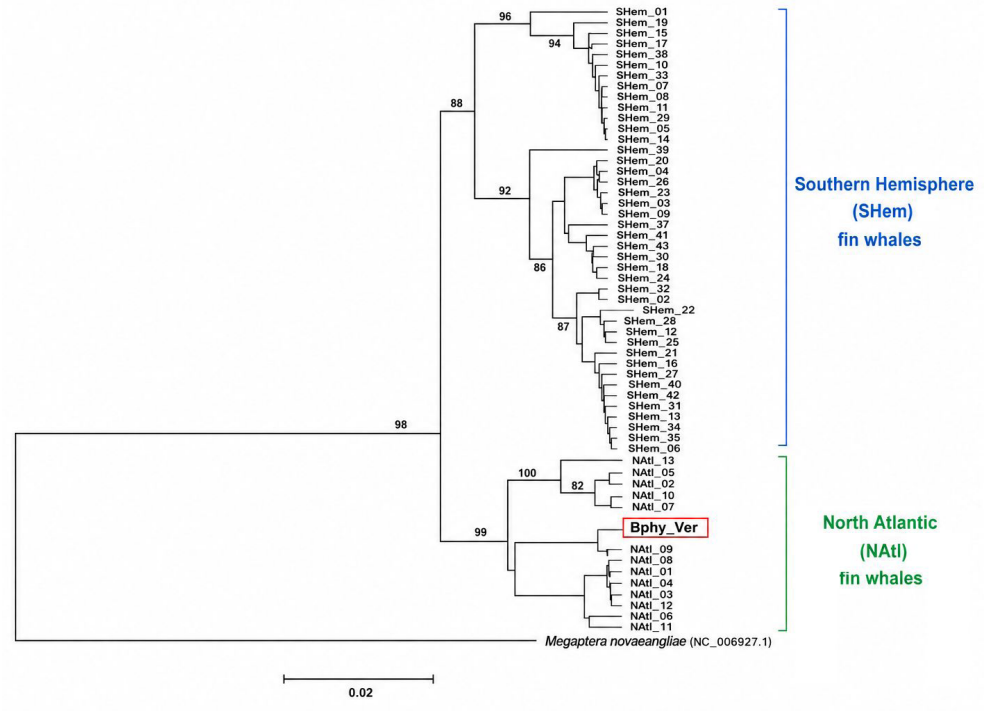


Figure 5. Maximum likelihood tree of control region sequences from North Atlantic, Southern Hemisphere, and Veracruz individuals. Bootstrap values (≥ 70%) are shown at internal nodes. Blue and green brackets identify Southern Hemisphere (SHem) and North Atlantic (NAtl) comparative sequences, respectively; the Veracruz specimen (Bphy_Ver) is highlighted in red. Humpback whale (*Megaptera novaeangliae*) sequence (NC_006927.1) was used as the outgroup.

case is methodological. The present case highlights the value of molecular approaches for taxonomic identification when conventional morphological assessment is uncertain. Despite extensive DNA degradation, testing multiple extraction protocols enabled recovery of amplifiable mtDNA and produced a 1,109-bp consensus sequence spanning the complete mitochondrial control region and adjacent flanking regions, which provided robust molecular evidence supporting the identification of the stranded specimen as *B. physalus.*

These findings demonstrate that highly decomposed tissue samples can still provide reliable taxonomic information despite substantial DNA degradation. When combined with appropriate extraction, amplification, and sequencing strategies, such samples may remain valuable for molecular species identification. Such approaches may be particularly valuable for documenting uncommon strandings and improving the taxonomic accuracy of biological collections and stranding records.

The larger lesson is strategic: unusual strandings along the Veracruz coast should trigger not only a standard necropsy response, but also rapid molecular preservation, archiving of chain-of-custody metadata, and integration of the event into broader assessments of maritime traffic, fisheries interactions, and environmental variability in the southern Gulf of Mexico.

Conclusions

The sequence showed 99% identity to a fin whale mitochondrial reference genome and contained three nucleotide substitutions relative to that reference. In the comparative analysis, the Veracruz haplotype clustered within the clade containing the sampled North Atlantic fin whale control-region sequences; however, the single mitochondrial marker does not support definitive stock or geographic-origin assignment. The study confirms the specimen as a fin whale and demonstrates the value of preserving degraded tissue for molecular identification when morphology is inconclusive. Together with the few previously verified Gulf records, the February occurrence of this small individual is consistent with rare or intermittent entry of fin whales into the southern Gulf, but additional records are required to determine whether such occurrences represent a recurrent seasonal pattern.

Acknowledgments

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Supplementary material

Supplementary Material 1- Figures S1-S3 showing sequence quality, alignment and identification of *B. physalus*, and Table S1 shows Genebank accession numbers for sequences used in the maximum likelihood tree.