Short Note First Genetic Record of a Strap-Toothed Beaked Whale (Mesoplodon layardii) Stranding in Chile

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Short Note

First Genetic Record of a Strap-Toothed Beaked Whale (*Mesoplodon layardii*) Stranding in Chile

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Of all the cetacean families, the Ziphiidae are one of the most enigmatic (MacLeod, 2018). They are the second most diverse family after the Delphinidae (Dalebout et al., 2002). They dive very deep for prolonged periods, spending little time at the surface between dives; and they live predominantly offshore in oceanic waters (MacLeod & D'Amico, 2006), which makes studying them more difficult and explains why they are so poorly understood (MacLeod, 2018). Most of the studies regarding ziphiids have been carried out on stranded individuals (Rosario-Delestre et al., 1999; Dalebout et al., 2002). Certain key features of the Ziphiidae, such as the location and shape of male tusks (MacLeod, 1998, 2018; MacLeod & Herman, 2007; Pitman, 2018), facilitate species identification, particularly within the cryptic genus *Mesoplodon* (MacLeod, 2018); however, similarities among species and poor knowledge of body color patterns can lead to misidentification, especially in the case of female and juvenile specimens as body color patterns are to some extent unknown and are similar between females and juveniles (Pitman, 2018). In general, a molecular genetic analysis is considered the most reliable method for species identification (Kim et al., 2019).

The strap-toothed beaked whale (STW; *Mesoplodon layardii*) is classified in the International Union for Conservation of Nature's (IUCN) *Red List* as "Data Deficient" (Taylor et al., 2008). STW strandings have been registered in many countries: Argentina, Australia, Brazil, the Falkland Islands, French Southern Territories, Heard Island and McDonald Islands, Namibia, New Zealand, South Africa, South Shetland Islands, and Uruguay (Taylor et al., 2008). Genetic sequencing has only been carried out on some of these strandings: only 16 partial sequences (including ours) from the Control Region gene of mitochondrial DNA could be obtained from GenBank, mainly from the Falkland Islands (eight) and New Zealand (four) (Dalebout et al., 1998, 2004; Otley et al., 2012).

Most STW strandings in Chile have been found in the southernmost area of the country (Venegas & Sielfeld, 1978; Sielfeld, 1979; Gibbons et al., 2000; International Whaling Commission [IWC], 2007; Figure 1), but these records contain several inconsistencies and data gaps. Reviewing the information available for these strandings, records 1 and 2 (Figure 1) could correspond to the same animal as they were registered in the same year and similar locations; however, given the lack of bibliographic evidence for record 1, it is impossible to know the complete details. A mandibular symphysis appears in two 1978 records: one found on the eastern mouth of the Strait of Magellan (which would relate it to the tooth found in 1978), and the other found in Cabo del Espíritu Santo. The original documentation of these records are no longer available. The record of the northernmost stranding occurred on 22 May 2007 in Constitución; it remains stored in the Museo de Historia Natural e Histórico in San Antonio, but there is no record of genetic validation of its identification. According to the local newspaper, this specimen was male, but the official report registered it as female (IWC, 2007). Given the sparse evidence of STW in Chilean waters, more accounts and observations are needed to understand the range and habitat use of this species in this area. Herein, we document a recent STW stranding and provide the first genetic record of STW occurrence in Chilean Patagonia.

On 20 March 2019, near Caleta Tortel Bay (47° 50' 00" S, 73° 34' 00" W) in the Aysén region of Chile, a live STW was observed swimming with slow and aimless movements close to a little stone beach. Immediate rescue maneuvers began, performed by the Navy and personnel from the Chilean Fisheries and Aquaculture Service (SERNAPESCA) in Aysén. Several attempts to return the animal to the sea with zodiacs and scuba divers were made, but the animal repeatedly returned to the beach. While rescue maneuvers were being conducted, a second smaller individual of similar anatomical characteristics was sighted in the same area. This second individual was approached slowly in an inflatable boat to assess if it was injured, but it retreated to deeper waters. It remained in the area until the evening, after which it was not seen again. After 2 h of rescue attempts, the first STW died stranded on the beach (Figure 2).

This STW carcass was transported from the Caleta Tortel shore to the Colina Campus of the Universidad Andrés Bello School of Veterinary Medicine in Santiago for a full necropsy. The transport took 7 d in total, including a ferry ride. The carcass was moved in a freezer truck at -20°C. The necropsy was carried out 10 d after the stranding following protocols from the Stranding and Mass Mortality Committee of the Chilean Wildlife Veterinarian Association (Asociación de Médicos Veterinarios de Fauna Silvestre [AMEVEFAS], 2017) and the necropsy technique and tissue sampling from the European Cetacean Society (Barnett et al., 2018).

Skin samples were preserved for genetic analyses in a 100% ethanol solution at -20°C.

Record	Evidence	Year	Location	Reference
1	Skull	1968	Chabunco, Punta Arenas	Without reference
2	Photography	1968	17 km north of Punta Arenas	Venegas & Sielfeld, 1978
3	Right tooth (CE-4)	1978	Eastern exit of the Strait of Magellan	Venegas & Sielfeld, 1978
4	Mandibular symphysis	1978	Eastern exit of the Strait of Magellan	Venegas & Sielfeld, 1978
5	Skull (CE-25)	1979	Windhond Bay, Navarino Island	Sielfeld, 1979
6	Whole animal	2007	1 km north of Maule River, Constitución	IWC, 2007

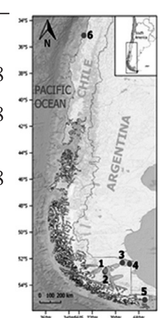

Figure 1. Location of all the stranding records of strap-toothed beaked whales (STWs; *Mesoplodon layardii*) in Chile reviewed in literature. The numbers in the figure correspond to those listed in the table. The GPS coordinates of the first five records are approximate according to the sites where the evidence was found. Only latitude was known for record 6, while longitude was estimated.

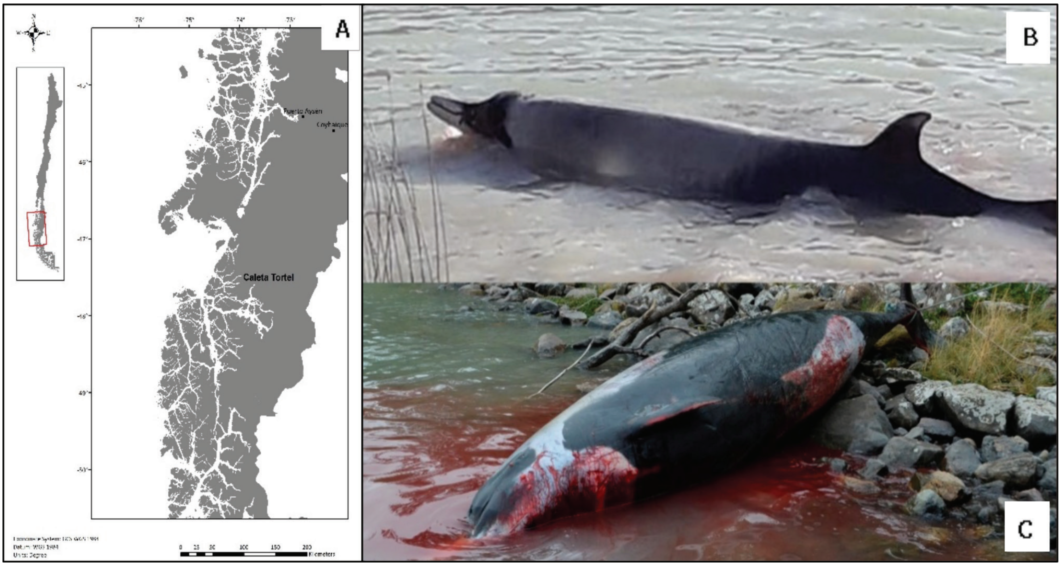

Figure 2. (A) Map of Caleta Tortel location; and photos of the stranded male STW in Caleta Tortel: (B) live animal and (C) color patterns observed on its ventral side.

DNA was extracted using the Phenol-Chloroform extraction method (modified from Sambrook et al., 1989; Aljanabi & Martinez, 1997). A 696 base pair fragment of the Control Region gene (GenBank Accession Number MN807948) was amplified by polymerase chain reaction (PCR) using light-strand primers tPro-whale Dlp-1.5 (5'-TCACCCAAAGCTGRAATTCTA-3'; Dalebout et al., 1998) and heavy-strand primers Dlp-8G (5'-GGAGTACTATGTCCTGTAACCA-3'; Dalebout et al., 2005). PCR products were observed in 1.5% agarose gel electrophoresis with SYBR Green® (Invitrogen, Carlsbad, CA, USA). PCR products were purified using a Wizard® clean-up kit (Promega, Madison, WI, USA) and sequenced using both PCR primers separately in an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were edited manually, and consensus sequences were blasted against the GenBank nucleotide data for species identification using BLAST (https://blast.ncbi.nlm.nih.gov/Blast).

To determine the phylogeography of the STW species in the Southern Hemisphere, the haplotype found in this beached STW specimen was compared to previously described haplotypes from the Falkland Islands and New Zealand strandings (Dalebout et al., 1998, 2004; Otley et al., 2012). A network of median-joining haplotypes of genealogical relationships was built using said sequences. This process provides information regarding the genetic structure of the species and its typical geographical distribution (Dalebout et al., 2004). Sequences were aligned using the Clustal W algorithm implemented in *Geneious*, Version 11 (https://www.geneious.com; Kearse et al., 2012). Once aligned, the median-joining haplotype network analysis was carried out with *Network*, Version 4 software (Bandelt et al., 1999).

The results confirmed that the studied individual is an adult male *M. layardii*. As far as we can determine, this is the first specimen of this species confirmed by genetic analysis in Chile. The mitochondrial DNA Control Region gene analysis and its subsequent comparison with similar sequences in the GenBank database provided additional confirmation that the stranded animal was a STW (100% blast analysis query cover). Fifteen previously published sequences of the Control Region gene were obtained from this database (Australia = 2; the Falkland Islands = 8; New Zealand = 4; South Georgia = 1), with a size of 329 base pairs. The haplotype found in the Chilean ziphiid corresponded to the most common haplotype, which previously has been found in STWs in Australia, the Falkland Islands, New Zealand, and South Georgia (Figure 3).

The STW carcass condition was classified as a code 4 out of 5, and its decomposition state was between 2 and 3 out of 5 (Rowles et al., 2001). The exact weight could not be obtained, but given the characteristics of the crane used to move the carcass, it weighed at least 1,500 kg and was 5.21 m long (for morphometric details, see Table 1). The external examination showed several marks on the body surface with multiple lacerations in the mid-caudal area, ventral to the left pectoral fin, and in the

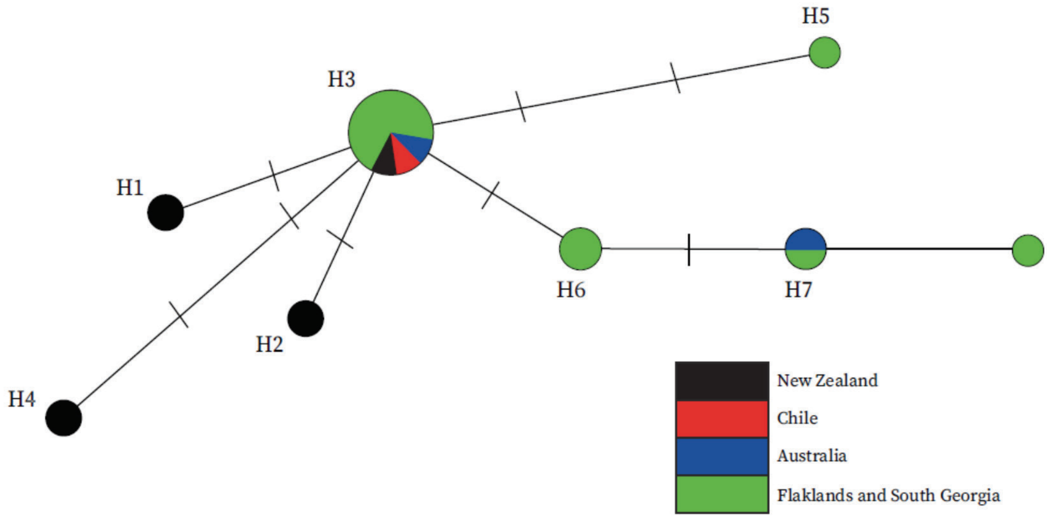

Figure 3. Network of median-joining haplotypes of genealogical relationships among the Control Region gene of mitochondrial DNA from STWs stranded in New Zealand, Chile, Australia, the Falkland Islands, and South Georgia. The length of the branches is proportional to the number of inferred mutational steps; this is indicated by a short gray line on the branches. The diameter of each circle is proportional to the number of individuals who share the same haplotype. The name of the haplotypes is indicated to the side of each circle.

Table 1. Morphometric measurements of the stranded strap-toothed beaked whale (STW; *Mesoplodon layardii*) near Caleta Tortel in March 2019

Characteristics measured	Length (m)	Characteristics measured	Length (m)
Total length	5.21	Tip of the snout to genital slit center	3.48
Length without fluke	4.85	Tip of the snout to anus	3.90
Tip of the snout to melon apex	0.39	Fluke midpoint to anus	1.25
Tip of the snout to oral commissure	0.44	Circumference of axillary region	2.86
Tip of the snout to center of an eye	0.84	Circumference in anterior insertion of dorsal fin	2.62
Tip of the snout to center of blowhole	0.77	Circumference in anal region	1.90
Tip of the snout to anterior insertion of pectoral fin	1.30	Dorsal fin height	0.32
Tip of the snout to posterior insertion of pectoral fin	3.26	Length of the left pectoral fin	0.50
Tip of the snout to tip of dorsal fin	3.65	Maximum width of left pectoral fin	0.19
Tip of the snout to umbilical zone	2.66	Fluke width	1.44

cervical area. Most of them were recent, most likely due to the stranding. A dorsoventral laceration was found in the rib zone measuring 28.9 cm long and 4.2 cm deep (Figure 4A). The fluke had a puncture wound (Figure 4B), and the penis was everted due to bloating of the inguinal area (Figure 4E). A hematoma was observed in the left thoracic area, 20 cm long and 4 cm wide (Figure 4C). From the caudal to the left eye, there were four tooth scars, possibly from a cookiecutter shark (*Isistius* sp.) bite; and small abrasions were observed on the external sides of the mouth. Two teeth were erupted, presenting a complete fracture at the base prior to death, caused by epibionts found anchored in the fracture of the left medial tooth (Figure 4G). The outer edges of the rostrum presented smooth bilateral indentations

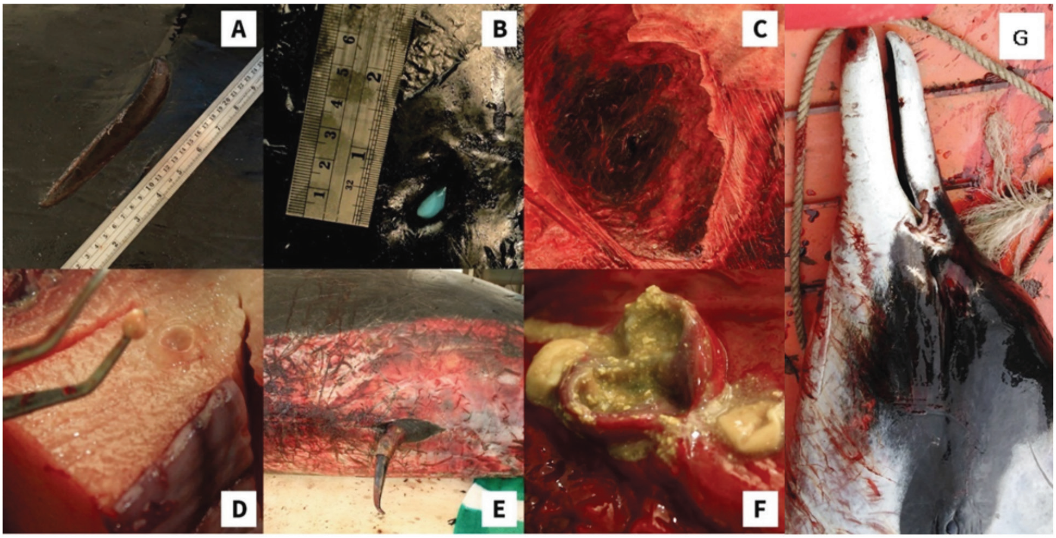

Figure 4. Main findings of the necropsy of the stranded STW near Caleta Tortel in 2019: (A) Large dorsoventral laceration in the rib zone, 4 cm deep; (B) perforating fluke lesion; (C) dorsal hematoma; (D) parasite cyst; (E) multiple lacerations in the ventral area and everted penis; (F) abscess near the bladder; and (G) detail of the head showing the epibionts in erupted teeth.

where the teeth crossed over, which was a sign of its adult age. Blubber thickness was thinner in the cranial area (7 cm) and thicker in the dorsal fin insertion area (7.5 cm). Multiple cysts (0.5 cm in diameter) of the cestode *Phyllobothrium* spp. were found in the adipose tissue near the caudal peduncle (Figure 4D).

Internal examination revealed abundant sero-sanguineous fluid in the thoracic cavity, congested lungs, and abundant liquid contained in the right pleura. These fluids were most likely caused by the defrosting process (Roe et al., 2012). The abdominal cavity presented two findings: (1) the main stomach had cephalopod beaks and fish otoliths and (2) a 2 cm abscess was located near the bladder (Figure 4F). Critical organs were weighed (Table 2), and additional analyses were performed, such as a cytology of the blowhole, blood, and spleen, which all presented normal bacteria associated with postmortem findings. The low definition of the x-rays performed may have been due to the size of the carcass or to a low accuracy of the kilovoltage used.

Table 2. Observed weights of various organs at necropsy of the stranded STW near Caleta Tortel in March 2019

Organs	Weight (kg)
Lungs with trachea	22.1
Heart	5.6
Stomach without content	5.7
Intestines	7.6
Liver	6.0

The cause of death of the studied specimen could not be determined given the general background of the event, the degree of tissue degradation, and the macroscopic findings. Processes such as autolysis and freezing/thawing impede an appropriate histological evaluation as they alter morphometry and usually mask the cause of death (Rowles et al., 2001; Roe et al., 2012). Strandings can occur due to a variety of causes. Natural causes include predation, diseases, parasites, injury, and biotoxins, while anthropogenic causes include entanglement, exposure to noise pollution (e.g., seismic tests, sonar, and shipping; MacLeod & D'Amico, 2006; Bernaldo de Quirós et al., 2019), vessel strikes, and harassment by tourists (Moore et al., 2018).

The necropsy revealed (unrelated to the stranding itself and the following decomposition) parasitism by *Phyllobothrium* spp. in the adipose tissue of the peduncle, an abscess, and a dorsal hematoma. The presence of *Phyllobothrium* spp. has been related elsewhere to deep pelagic foraging species that prey on squid (Loizaga de Castro et al., 2014; Santoro et al., 2018). This parasite rarely causes severe infections in small cetaceans (Sweeney & Ridgway, 1975). It has been suggested that marine mammals may be intermediate hosts (Aznar et al., 2007; Santoro et al., 2018). In the United States, a stranded pygmy sperm whale

(*Kogia breviceps*; cause of death unknown, but main lesions were renal) in Florida was infested with this cestode, but lesions were not associated with that parasitic infection either (Rousselet et al., 2019). Therefore, the infestation on the STW was not likely the cause of its death.

Cetacean strandings have provided critical insights into distribution, population structure, and phylogeny (Bilgmann et al., 2011). Live strandings are unusual and a great opportunity to obtain information on cryptic species, emphasizing the need for a more robust and methodical whale stranding network in Chile. Additionally, collaborative work, when facing a stranding of an understudied species in an isolated site, proved essential for collecting the most accurate and complete data possible for review.

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