

Molecular confirmation of *Mesoplodon* sp. A as *M. peruvianus*

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An unidentified species of beaked whale, referred to as *Mesoplodon* species "A" (hereafter *Mesoplodon* sp. A), was first described by Pitman et al. (1987) from sightings in the Eastern Tropical Pacific Ocean. Individuals of this species displayed sexual dimorphism. Adult females and juveniles showed a homogeneous light white stripe and extensive white scars on most of the body (Pitman et al., 1987). Considering morphological data and tooth location in adult males, Pitman and Lynn (2001) suggested that *Mesoplodon* sp. A could be the lesser-beaked whale (*Mesoplodon peruvianus*), also known as the pygmy-beaked whale (Van Waerebeek et al., 2018). Pitman and Brownell Jr (2012) recommended that it would be helpful to confirm whether *Mesoplodon* sp. A is indeed *M. peruvianus*. To this end, they proposed using genetic analysis of adult males from biopsy or describing the color pattern of a freshly stranded adult male *M. peruvianus*. The present study aimed to identify *Mesoplodon* sp. A. Here, molecularly, we compared a fragment of the mitochondrial DNA (mtDNA) control of a stranded beaked whale recognized as *Mesoplodon* sp. A, with sequences from related species.

Keywords:

species identification, mitochondrial DNA, beaked whale, Mexico, Pacific Ocean

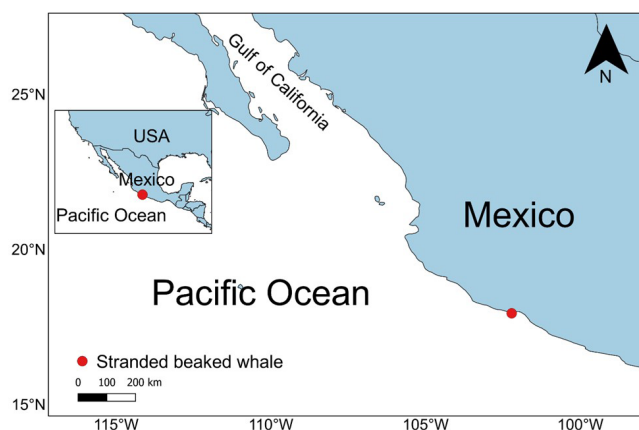


Figure 1. Location of the beaked whale *Mesoplodon* sp. stranded in Mexico in 2012.

Samples were collected from a freshly dead beaked whale stranded at Playa Jardin, Michoacán, Mexico (17°55'59" N, 102°13'40" W; Fig. 1) in May 2012. The specimen was an adult male based on the erupted tusk and scarring, with an approximate length of 3.5 meters (Fig. 2). The whale conformed to the color pattern and morphological characteristics described for *Mesoplodon* sp. A (Pitman et al., 1987; Pitman & Lynn, 2001), and so was recorded as such. A vertebra of this specimen was pulverized, under sterile conditions, using a drill and a stainless-steel bur and approximately 300 mg of powder was obtained. The genomic DNA was extracted using the GENECLEAN® Kit for Ancient DNA with a Proteinase K (20 mg/ml) preincubation at 65°C for 24 h. Extracted DNA was stored in 30 µl, free Elution Solution at ~20°C. Polymerase chain reaction (PCR) was conducted to amplify four fragments of mtDNA. Two fragments of the control region were amplified using the primers M13–Dlp1.5–L (Dalebout et al., 1998) and Dlp4–H (Baker, unpubl. data), and Dlp10–L (Baker et al., 1993) and Dlp4–H. The two fragments of cytochrome B were amplified using the primers CB1–L and CB2–H (Palumbi, 1996) and CYBMF–L and CYBMR–H (Dalebout et al., 2002). Reactions were carried out in 55 µl solutions containing 50 mM MgCl₂, 2.5 mM dNTPs, 5x Buffer, 250 µg/ml

ARTICLE INFO

Manuscript type: Note

Article History

Received: 17 March 2023

Received in revised form: 23 April 2023

Accepted: 23 April 2023

Available online: 10 August 2023

Handling Editor: Carolina Loch

Citation:

Urbán R., J., Viloría-Gómora, L., Antichi, S., & Valdovinos, L. A. (2023). Molecular confirmation of *Mesoplodon* sp. A as *M. peruvianus*. *Latin American Journal of Aquatic Mammals*, 18(2) <https://doi.org/10.5597/lajam00306>



Figure 2. *Mesoplodon* sp. A, adult male, stranded at beach Jardín Michoacán, Mexico in May 2012. Photo: Luis A. Valdovinos.

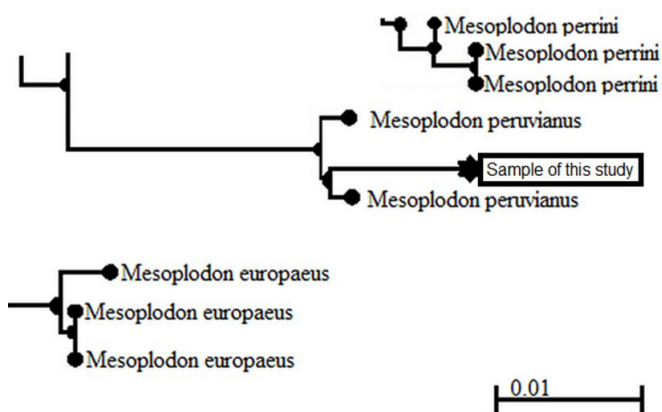


Figure 3. Partial neighbor-joining tree of mtDNA control region showing the similarity of the *Mesoplodon* sp. A sequence (sample from this study) to the reference sequences of the DNA GenBank database.

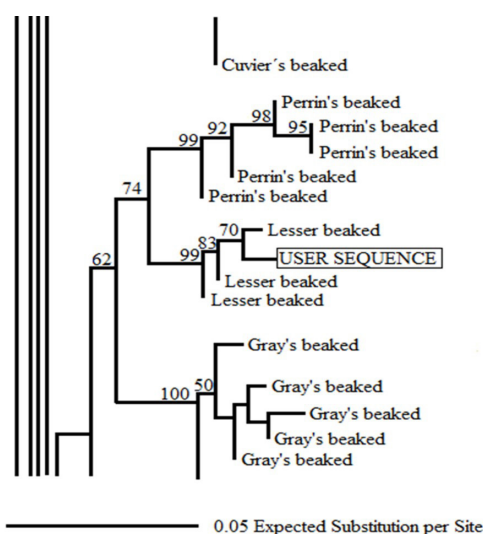


Figure 4. Partial neighbor-joining tree of mtDNA control region showing the similarity of the *Mesoplodon* sp. A sequence (given as USER SEQUENCE) to the DNA—Surveillance database reference sequences. The numbers are bootstrap values (1,000 simulations).

of each oligonucleotide and one unit of TaqDNA polymerase. The amplification was completed with a final extension step of seven minutes at 72°C. The fragments were purified with Wizard® SV gel and PCR clean-up system. The sequences were edited using Mega software (version 5.05) (Tamura et al., 2011) and aligned using ClustalW software (Thompson et al., 1994). The molecular identification of the species was carried out by comparing these reference sequences with those in GenBank databases (www.ncbi.nlm.nih.gov) (Benson et al., 2007) using BLAST (Basic Local Alignment Search Tool) algorithm, and DNA Surveillance (www.cebl.auckland.ac.nz:9000) (Baker et al., 2003; Ross et al., 2003) with Cluster (Advanced) tool, with 1,000 bootstrap simulations. Phylogenetic trees were generated using the Neighbor Joining method (Saitou & Nei, 1987).

A fragment of the mtDNA control region of approximately 281 pb (*MspA*) was successfully sequenced using primers Dlp10 L and Dlp4 H (GenBank access KF574044). During the molecular identification of the species with BLAST, the *MspA* sequence showed 100/99% similarity with the sequence of *M. peruvianus*. The same result was obtained with the DNA Surveillance database (bootstrap value 70) (Figs 3 and 4).

The core geographic range, the color pattern, and morphology of the stranded beaked whale from the present study matched the description of *Mesoplodon* sp. A by Pitman et al. (1987). The genetic analysis conducted in this study indicates that the studied specimen belongs to *M. peruvianus*. Phylogenetic trees show a definitive relationship between the sequence in question and sequences of *M. peruvianus* in available databases. This result was confirmed by sequence similarities and high bootstrap values. Our findings confirmed that *Mesoplodon* sp. A should be considered *M. peruvianus*, as Pitman and Lynn (2001) and Pitman and Brownell Jr (2012) had previously proposed.

Acknowledgments

We want to thank Fernando Elorriaga for providing the information about the stranding of this specimen, and Constanza Torres and Alejandro Torres for their help in obtaining the vertebra used in this study. Anton van Helden suggested important comments to the improvement of the manuscript. Samples were collected under the permit SEMARNAT SGPA/DGVS/01329/12.

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