MOLECULAR DETERMINATION OF THE IDENTITY OF *MESOPLODON* Sp A.

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ABSTRACT

A beaked whale stranded in 2012 at Michoacan, Mexico, it was identified based on its body coloration as *Mesoplodon* sp. A. DNA was extracted from powder from a vertebral bone. A fragment of the mitochondrial DNA control region and two of the cytochrome b were sequenced. Only one partial sequence of control region was generated successfully, and this was tested with BLAST and DNA-Surveillance for molecular identification. With both tests, the sequence was identified as lesser beaked whale (*Mesoplodon peruvianus*). These results support the proposal of Pitman and Lynn (2001) and Pitman and Brownel (2012), and confirm that organisms with the coloration pattern referred as *Mesoplodon* sp. A, belong to *M. peruvianus*.

INTRODUCTION

Mesoplodon sp. A, was described from sightings and photographic registers by Pitman *et al*. 1987. According to coloration, they describe two forms: a black and white form and another type of coloration, with gray and brown shades. Both distributed in the eastern tropical Pacific.

The first form shows a characteristic white strip that starts in the posterior part of the head until the anterior region of the dorsal fin, this wraps the body until ventral region. The width and intensity color of the strip varies among individuals. The authors suggest that this variation manifests differences between gender and age. The white strip is distinctive for males, and the width and the intensity of the color increase with age (Pitman *et al.* 1987).

Since the initial description of *M*. sp. A, its identity has not been confirmed to a species level. Pitman and Lynn (2001) from morphological data (body length) and tooth location in adult males, suggest that *M*. sp. A is the same species than lesser beaked whale (*Mesoplodon peruvianus*).

The revision of the actual acknowledgment of the pigmy beaked suggests the consideration of the *M*. sp. A as a part of *M. peruvianus* (Pitman and Brownel 2012). Also, the authors recommend genetic analysis of adult males and the detailed description on the coloration of *M*. sp. A.

This paper presents the result of the comparisons between a fragment of the mitochondrial DNA control region of a mesoplodont recognized as *M.* sp. A, and databases of related species sequences. The study was conducted to identify molecularly the *M.* sp. A.

MATERIALS AND METHODS

Samples

Samples was collected from a stranded beaked whale in May 2012, at Playa Jardin Michoacan, Mexico (17° 55' 56.61" N y –102° 13' 31.35" W). The animal was identified as *Mesoplodon* sp. A. It was a male about 3.5m long. The complete body was photographed and buried *in situ* (Fig. 1). One of the fishermen who found and buried the stranded animal, when observed that the skeleton began to appear in the surface, due the strong rains during the hurricane season, collected a vertebra as souvenir. The vertebra was donated to us four months after the stranding. Under sterility precautions, samples powder from bone was obtained with a drill and stainless stell bur. DNA genomic was extracted each sample.

Fig. 1. A stranded *Mesoplodon* sp. A in May 2012, at Playa Jardin Michoacan, Mexico. (Photos by Luis A. Valdovinos)

Molecular analysis

The total genomic DNA extraction form approximately 300 mg of powder from bone using the GENECLEAN® Kit for Ancient DNA with a preincubation with Proteinase K (20mg/ml) at 65°C for 24 h. Extracted DNA was stored in 30 µl DNA–free Elution Solution at ~20°C until further use.

Using the PCR we try to amplify four fragments of the DNAmt. Two of the control region using the primers M13–Dlp1.5–L and Dlp4–H (Baker C. S., unpublished data, taken of Dalebout 2004), and Dlp10–L (Baker *et al.* 1993) and Dlp4–H. And two fragments of cytochrome B using the primers CB1–L and CB2–H (Palumbi 1996), and CYBMF–L and CYBMR–H (Dalebout 2002). Reactions were carried out in 55 µl, containing 50 mM $MgCl_2$, 2.5 mM dNTPs, 5x Buffer, 250 µg/ml of each oligo–nucleotide and one unit of Taq DNA polymerase. After an initial denaturation step at 94 °C for 3 minutes, a PCR amplification cycle of one minute at 94 °C, followed by one minute at 54 °C by the control region y 50 °C by cytochrome B and one minute at 72 °C was repeated 35 times. 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 and the sequencing was done in a commercial laboratory.

Molecular identification of the specie.

The sequences were edited using Mega 5.05 (Tamura et al. 2011) and were aligned using Clustal W (Higgins et al. 1994), finally were corrected manually.

Molecular identification of the specie was searched in two databases: GenBank (www.ncbi.nlm.nih.gov) (Benson *et al*. 2007) with BLAST (Basic Local Alignment Search Tool), and the database DNA–Surveillance (www.cebl. auckland.ac.nz: 9000) (Ross *et al.* 2003; Baker *et al.* 2003).

Were deployed phylogenetic trees in both analyses, which were generated using the neighbor–Joining method (Saitou and Nei 1987). The seeking in DNA–Surveillance was done with Cluster (Advanced) tool, with 1,000 bootstrap simulations.

RESULTS

A fragment of the DNAmt control region approximately 281 pb (*Mspa*) was successfully sequenced using primers Dlp10–L and Dlp4–H (Fig. 2). The molecular identification of the specie with BLAST, *MspA* sequence showed 100/99 % similarity with the sequence of lesser beaked (*Mesoplodon peruvianus*). Same results were obtained with DNA–Surveillance (bootstrap value 70) (Figs. 3 and 4).

1	GTACTATGTC	CGTATTGAAA	AAGAAATACC	CTACAGTACA	TTTACTGTAT
51	TAATAATACA	GACACACCCA	CCTAGGCGCT	AATATATAGC	GTCTCTCCAG
101	GAGTGTATGT	ATATATATGT	TATGTATAAC	TGTGCATTCA	TTTATTTTCA
151	CTACGGAGAG	TTAAAGCTCG	TAATTAATTT	TTTTAATTTT	ACATAAGTAC
201	ATAATTTGCA	TTATTCGTAC	ATGTGCCCGT	TCCATTAGAT	CACGAGCTTA
251	ATCACCATGC	CGCGTGAAAC	CAGCATCCCG	C	

Fig 2. Sequence of the PCR product (*MspA*) obtained from the amplification of the mitochondrial control region using the primers Dlp10–L y Dlp4–H.

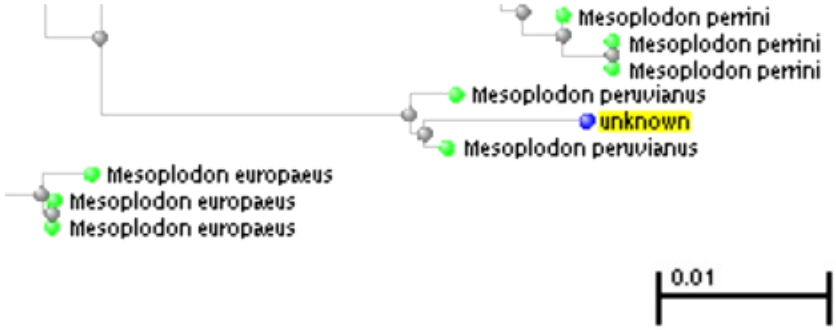

Figura 3: Neighbor-joining tree of mtDNA control region partial showing the similarity of the *MspA* sequence (given as unknown) to the reference sequences of the DNA Genbank database.

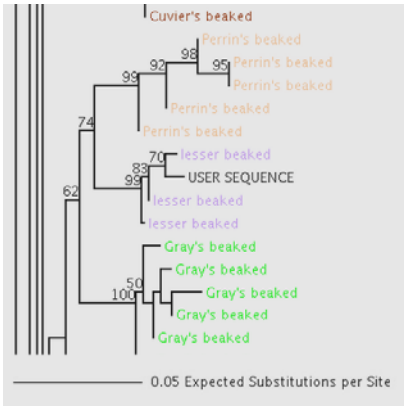

Fig 4: Neighbor–joining tree of mtDNA control region partial showing the similarity of the *MspA* sequence (given as USER SEQUENCE) to the reference sequences of the DNA–Surveillance database. The numbers are bootstrap values (1,000 simulations).

DISCUSSION

The distribution area and the coloration pattern of the beaked whale analyzed matches the ones described for *Mesoplodon* sp. A (Pitman *et al.* 1987). However, our genetic analysis indicates that the studied organism belongs to *M. peruvianus*. Phylogenetic trees show a definitive relationship between the problem sequence and the sequence available in databases, the similarity and bootstrap values confirm this result.

These results support the proposal of Pitman and Lynn (2001) and Pitman and Brownel (2012), and confirm that organisms with the coloration pattern referred as *Mesoplodon* sp. A, belong to *M. peruvianus*.

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