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Short Note

Molecular Identification of a Southern Elephant Seal (*Mirounga leonina*) from the Nayarit Coast, Mexico

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The movement of some pinniped species from tropical regions and the Southern Hemisphere to northern areas has increased since the 1990s (Johnson, 1990; Reeves et al., 2002; Alava & Carvajal, 2005; Avila et al., 2014; Alava & Aurioles-Gamboa, 2017; Páez-Rosas et al., 2017; Quintana-Rizzo et al., 2017), which is also true for the southern elephant seal (*Mirounga leonina*; SES). Adult and subadult males move over distances of thousands of kilometers in the Antarctic Ocean; during the non-breeding season, some individuals make long migrations for foraging activities (Hindell & McMahon, 2000; Fabiani et al., 2003; Lewis & Campagna, 2005; Campagna et al., 2006; Lewis et al., 2006; Reisinger & Bester, 2010; Jefferson et al., 2015). Also, some vagrant individuals have been documented in different parts of the world, traveling from their breeding and feeding areas to places such as Australia (Mills et al., 1977), South Africa (Oosthuizen et al., 1988), New Zealand (Taylor & Taylor, 1989), Brazil (Lodi & Siciliano, 1989; De Moura et al., 2010), Oman (Johnson, 1990), the Galapagos Islands (Vargas et al., 2004), Ecuador (Alava & Carvajal, 2005; Páez-Rosas et al., 2018), Chile and the Juan Fernández Islands (Sepúlveda et al., 2007; Acevedo et al., 2016), the Gulf of Panama (Redwood & Félix, 2018), Colombia (Avila et al., 2019), and Mexico (Elorriaga-Verplancken et al., 2020).

Sightings of *M. leonina* in the South Pacific occur from Cabo de Hornos, Chile, up to Ecuador (Acevedo et al., 2016) and in the Easter Islands, Chile (Hucke-Gaete et al., 2014). Avila et al. (2020) published the first sightings of an SES north of the equator on the Pacific coast of Colombia—a young male was documented in January and February 2018, and a group of three subadults were sighted in December 2019 and in January 2020. Their presence in Colombia had not been documented before, and it may have been due to foraging or to the impact of environmental changes in the Southern Ocean (Avila et al., 2020). Herein, we report the second occurrence in Mexico of an SES subadult male, the first observed on the Nayarit coast in western Mexico. It is possible that the SES reported in this short note could be one of the individuals observed in Colombia (Figure 1).

On 5 July 2020, the Stranding Network of Bahía de Banderas-Puerto Vallarta received a report about a large pinniped at San Francisco Beach in the State of Nayarit (20.9042670 N, -105.4145900 W; Figure 2). At first, this individual was identified as a northern elephant seal (*Mirounga angustirostris*; NES) as this is the only elephant seal species historically distributed in Mexican waters and on the shores of the Pacific (Arias-Del Razo et al., 2017). The NES inhabits several islands of the northeastern Pacific in Mexico along the western coasts of the Baja California Peninsula, including Archipelagos of Islas Coronados, Isla Guadalupe, Isla Cedros,

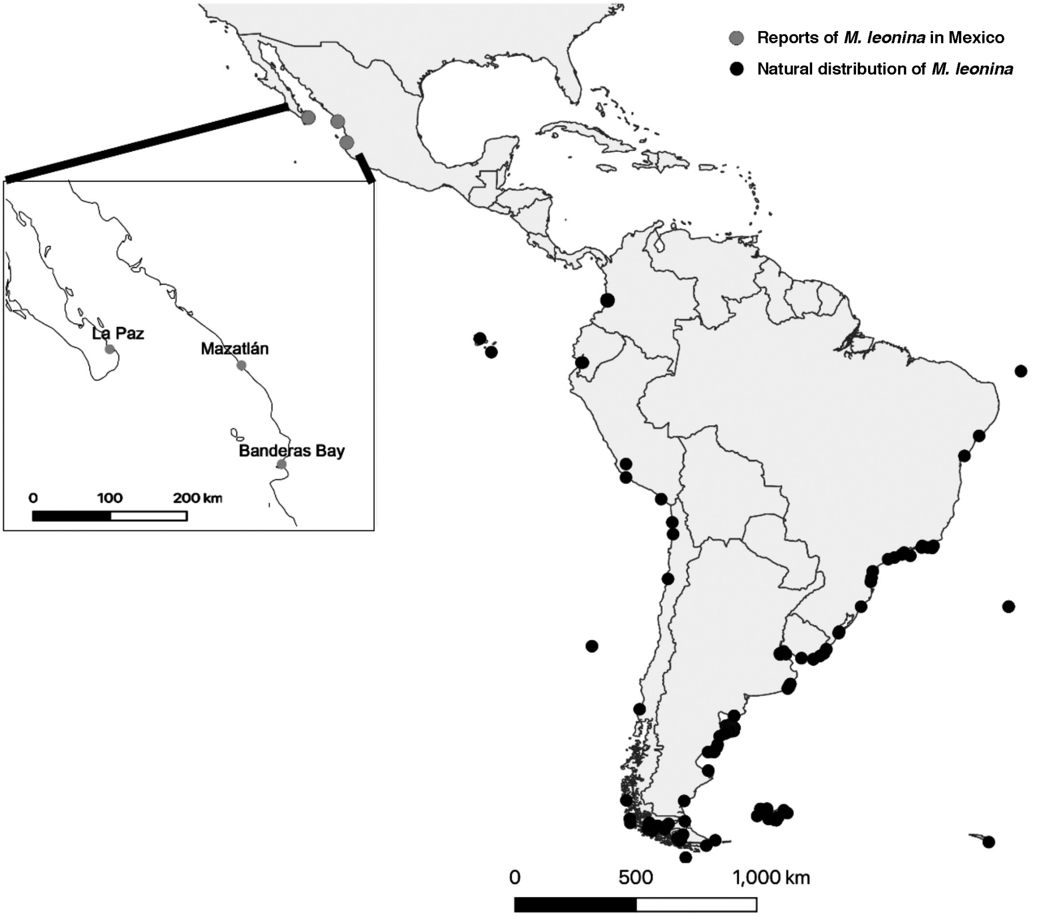

Figure 1. Southern elephant seal (*Mirounga leonina*) sighting localities. Black dots correspond to records published by Lewis & Campagna (2005). Gray dots represent sightings of *M. leonina* individuals that have crossed to the Northern Hemisphere. The locations in the Colombian area were published by Avila et al. (2019). The northernmost sighting corresponds to the observation of Elorriaga et al. (2020). In this short note, we present two new sightings of the same individual in two different localities along the western coast of the Mexican Pacific.

and the San Benito Archipelago (Elorriaga-Verplancken et al., 2015). NESs perform long foraging migrations after molting, traveling from Mexican islands to the Gulf of Alaska, covering 8,000 km, during which they dive up to 400 m to feed on deep sea fish (Gallo-Reynoso, 2005; Maxwell et al., 2011; Robinson et al., 2012). Still, different authors have reported their occasional presence in the Gulf of California (Aurioles et al., 1993; Vidal et al., 1993; Mesnick et al., 1998; Gallo-Reynoso et al., 2010).

Some members of the stranding network attending the event, as well as local and international specialists, shared opinions about the identity of this pinniped (3.87 m body length) as potentially being an SES (Figure 3). It was suggested that this seal might be a class 1 subadult, ~5 y of age, following Galimberti & Boitani (1999) and Sanvito et al. (2007). Given the morphological similarity between both elephant seal species, the taxonomic identification of this individual was based on external morphological characteristics such as the size and shape of the proboscis, which is different in both species (i.e., larger in NESs than in SESs; Reeves et al., 1992). Furthermore, genetic analysis was used to confirm the species (Figure 4).

On 6 July, the SES returned to the sea but came back to the beach at 1700 h (20.9113610 N, -105.4113760 W) and 800 m north from the previous location. The safety perimeter was moved and installed around the animal each time it returned to the beach. The seal was resting, flipping sand

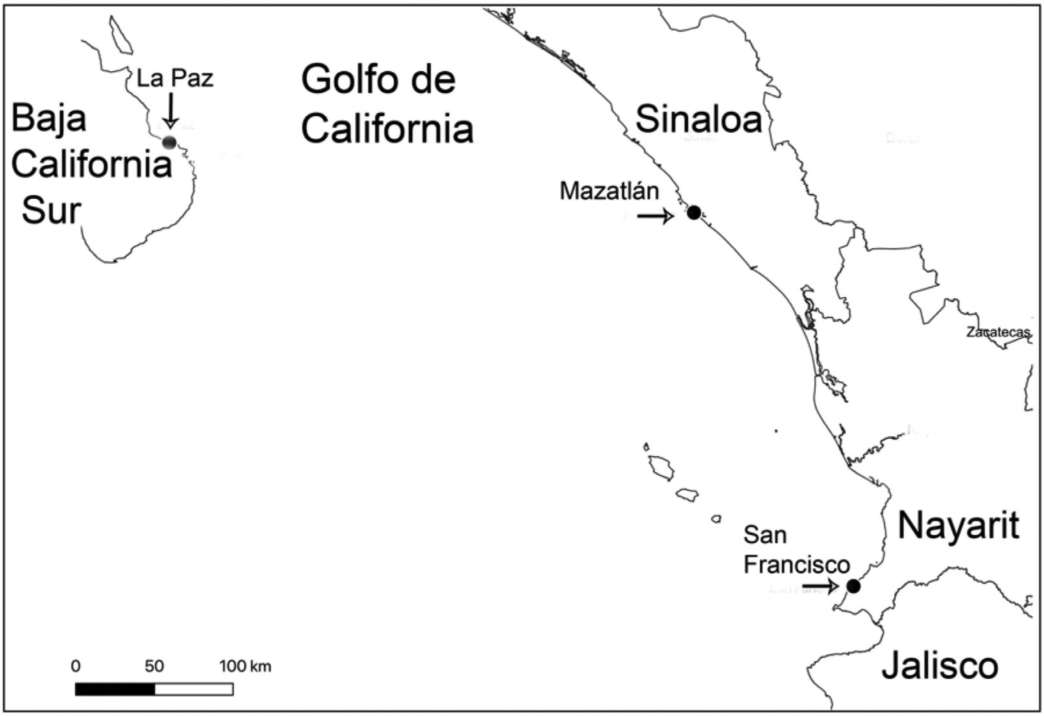

Figure 2. Locations of sightings in Mexico

Figure 3. Subadult male southern elephant seal observed on 5 July 2020 on San Francisco Beach, Nayarit, Mexico (southern Gulf of California). The individual is starting to moult. (*Photo credit:* L. González-Hernández)

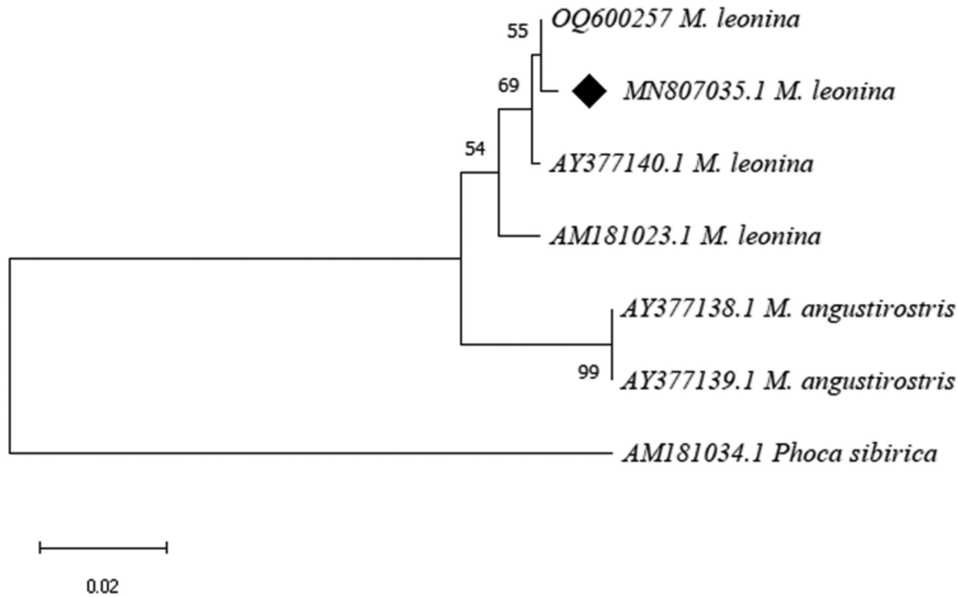

Figure 4. Neighbor-Joining tree of 355-bp mtDNA *cox1* from *Mirounga* sp. sequences (♦Elorriaga-Verplancken et al., 2020). The percentage of trees in which the associated taxa clustered together (10,000 replicates) is shown next to the branches. *Phoca sibirica* was used as an outgroup.

over itself while on the beach; it completed several short-term swims in the sea between rests on the beach. Laws (1956) and White & Odell (1971) suggest sand flipping is used to dissipate heat, which can offer warmth on cold, windy days or mitigate skin irritation during molting. The latter explanation is likely since this seal was shedding its fur. The ambient temperature in July in San Francisco, Nayarit, was between 23º to 27ºC, which is much warmer than their typical habitat in Patagonia (3º to 15ºC). This huge temperature difference may explain the sand flipping behavior of this seal.

On 9 July, the seal remained on the beach, flipping sand on its back. From this point on, the number of heart beats per minute (HPM) and respirations per minute (RPM) were recorded opportunistically. The HPM was estimated by looking and counting heartbeats in the chest/heart area when the animal was laying on its side. As this animal was low on body fat, the HPM was easy to observe. Respirations were easily detected at a safe distance from the animal by using binoculars—for this day, it averaged 60. RPM was estimated by counting the number of times the animal opened and closed its nostrils to breathe—on this day, we counted 6 to 7 RPM.

On 10 July, the seal moved ~60 m south from its last position and continued flipping wet sand over its body and staying close to the surf zone. At ~1100 h, its respirations decreased to 4 RPM and its heart rate reduced to a range of 53 to 58 HPM. Fat loss became visually more evident; this subadult seemed weak, with molting evident as large patches of fur and skin hung around its neck. Elephant seals molt once a year (Laws, 1956; Le Boeuf & Laws, 1994). On 11 July, the seal did not enter the sea.

On 13 July, at approximately 1600 h, the seal departed to the sea and did not return to the beach. It was observed again on 15 July at Punta Monterrey (20.939327 N, -105.386107 W), ~4 km from its last location. On 16 July, the seal was observed to enter the sea in the morning, travel offshore, and head north.

On 20 July, at ~1200 h, this SES was reported on a beach at Isla de Piedra (23.175651 N, -106.384605 W) in Mazatlán, Sinaloa (Figure 2). The molting process was advanced, and new gray-dark fur was evident. On the beach, the animal rested and flipped sand on itself. At ~1730 h, it returned to the sea. This was the last known sighting for this individual.

To confirm the SES's identity, a tissue sample was obtained from shed skin for genetic analysis;

the sample was obtained when the seal was at San Francisco Beach, Nayarit. The collected skin was preserved in a 2 mL Eppendorf tube with 70% ethanol and stored at 4°C until processed. Total DNA was extracted via the cetyltrimethyl ammonium bromide method (CTAB) (Wagner et al., 1987). A 910-bp fragment of the mtDNA cytochrome oxidase subunit I (*cox1*) was amplified with specific primers designed in this study (*cox1MiF:5'-GTGCCCCTGACATAGCATTT-3'*; *cox1MiR:5'-TGCTCAGGTGTCATCGAGAG-3'*) from the consensus sequence of *Mirounga* sp. MN807035.1 (Elorriaga-Verplancken et al., 2020) and AY377138.1 (Davis et al., 2004) using the 'Primer3Plus' package (Untergasser et al., 2007). The polymerase chain reaction (PCR) protocol for a 12.5 µl final volume was 7.23 µl nuclease-free water, 0.75 µl MgCl, 0.66 µl each dNTP, 2.5 µl Buffer 10x, 0.13 µl each primer, 0.1 µl Taq DNA Polymerase Promega, and 1 µl (58.9 ng/mL) isolated DNA which was partially degraded. Amplification conditions were as follows: 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 59°C, 1 min at 72°C, and a final extension for 5 min at 72°C. PCR product (3 µl) mixed with 1 µL dye was performed on 2% agarose gel dyed with RedGel and checked for integrity. The remaining 25 µl PCR product was purified using the commercial kit Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA), and the final product was sent to Macrogen Inc. (Seoul, Korea) for Sanger sequencing.

The forward and reverse sequences were manually edited for quality control and assembled by MUSCLE using *Geneious Prime*®, Version 2020.2.4 (Kearse et al., 2012) to obtain the consensus amplicon. The taxonomic identity was analyzed using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990), which is available on the website of the National Center for Biotechnology Information (NCBI; https://blast.ncbi.nlm.nih.gov/Blast.cgi; *M. leonina* link: https://www.ncbi.nlm.nih.gov/nuccore/OQ600257). Partial mtDNA *cox1* sequences of *Mirounga* sp. from the GenBank database were downloaded, and multiple alignment was performed by applying the MUSCLE algorithm through *MEGA*, Version 10.0.5 (Tamura et al., 2013), including sequences of the elephant seal of this study (GenBank ID OQ600257), three from *M. leonina*, two from *M. angustirostris*, and one from *Phoca sibirica* as the outgroup (Table 1). A Neighbor-Joining (N-J) tree was constructed (Figure 4) with the Kimura 2-parameters model (10,000 bootstraps) in *MEGAX* software (Kumar et al., 2018).

We analyzed a partial mtDNA *cox1* sequence (910 bp) from the elephant seal (*Mirounga* sp.), and the BLAST analysis showed a high percent identity with the DNA sequence of *M. leonina* (query cover 100 and 99.56% identity) and a low percent identity for *M. angustirostris* (query cover 100 and 96.59% identity) sequence. The N-J tree (Figure 4) displayed a clear topology where the sequences from the NES represent a separate clade from the SES. The analysis placed the sample of *Mirounga* sp. from this study within the clade of *M. leonina* sequences, corroborating the morphological identity of this specimen. Moreover, the analysis also assigned this sequence in the same cluster of the *M. leonina* specimen previously reported on the Mexican coast by Elorriaga-Verplancken et al. (2020).

The presence of *M. leonina* individuals in the Northern Hemisphere is interesting, especially because this is the second individual observed in less than a year; the first one was registered by Elorriaga-Verplancken et al. (2020). The probable distance traveled by these individuals suggests

Table 1. Partial mtDNA *cox1* sequences of *Mirounga* sp. and *Phoca sibirica* from the GenBank database, included for multiple alignment

GenBank ID#	Species	Geographical origin
MN807035.1	*M. leonina*	Beach La Ribera, Baja California Sur, Mexico (Elorriaga-Verplancken et al., 2020)
AY377140.1	*M. leonina*	Heard Island, Australia
AY377138.1	*M. angustirostris*	California, USA (recovery center)
AY377139.1	*M. angustirostris*	Unknown (Davis et al., 2004)
AM181023.1	*M. leonina*	Unknown
AM181034.1	*Phoca sibirica*	Unknown (Arnason et al., 2006)

that they might come from the same geographical area. Thus, future research regarding population identity will shed light on whether their presence corresponds to changes in movement patterns related to environmental factors such as global warming or to some other cause. It is important to emphasize the need for teamwork between international research groups to monitor events in which vagrant individuals are found far away from their normal geographical range.

We agree with Elorriaga-Verplancken et al. (2020) that SESs provide an important contribution to our knowledge of pinniped movements over long distances (e.g., from one hemisphere to another). Efforts should be made to work with other researchers on tagging and monitoring individuals of this species to better understand their long-range movements.

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