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Short Note

A Southern Elephant Seal (*Mirounga leonina*) in the Gulf of California: Genetic Confirmation of the Northernmost Record to Date

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The southern elephant seal (*Mirounga leonina*) (SES) presents an almost circumpolar distribution in the Southern Ocean, with a worldwide abundance of around 650,000 individuals (Scientific Committee for Antarctic Research – Expert Group on Seals [SCAR-EGS], 2008; International Union for Conservation of Nature [IUCN], 2015) distributed in four populations (Slade et al., 1998; IUCN, 2015): (1) in or close to Argentina, including the Valdes Peninsula and Falkland Islands, as well as the Magallanes and Chilean Antarctica region (Vargas, 2012); (2) in the Atlantic on South Georgia, the South Orkney Islands, the South Shetland Islands, and Bouvetøya and Gough Islands; (3) in the Indian Ocean on the Kerguelen Islands, the Crozet Islands, Heard Island, and the Prince Edward Islands; and (4) in the Pacific on Macquarie Island, Campbell Island, and Antipodes Island, near New Zealand. These distinct populations show different trends (IUCN, 2015). For instance, the Valdes Peninsula and Falkland Islands population has increased in recent years (Lewis et al., 1998; Galimberti et al., 2001). Most foraging areas are located between approximately 40° S and the Antarctic (Hindell & McMahon, 2000; IUCN, 2015).

The SES breeding season takes place in the austral spring (September to November), the molting season occurs in the austral summer (December to February), and immature individuals haul out in the austral winter (June to August) (Carrick & Ingham, 1962; Hindell & Burton, 1988). In the past two decades, the northern recurrence of pinniped species from tropical regions and the Southern Hemisphere has become more common than previously thought, and the SES is not the exception (e.g., Lodi & Siciliano, 1989; Johnson, 1990; De Moura et al., 2010; Alava & Aurioles-Gamboa, 2017; Páez-Rosas et al., 2018; Redwood & Félix, 2018).

Herein, we report the extralimital occurrence of one of these SESs, occurring at the end of the immature hauling-out season when it displayed a significant dispersal toward the Gulf of California, Mexico. On 4 September 2019, the report was made to the Marine Mammal Stranding Network of La Paz, Baja California Sur, by federal environmental authorities from the Procuraduría Federal de Protección al Ambiente (PROFEPA; Federal Bureau of Environmental Protection) and the Comisión Nacional de Áreas Naturales Protegidas (CONANP; National Commission for Protected Natural Areas) regarding the observation of an elephant seal on a beach in La Ribera (23° 36.03' N, 109° 34.55' W), Baja California Sur, Mexico, in the southern Gulf of California

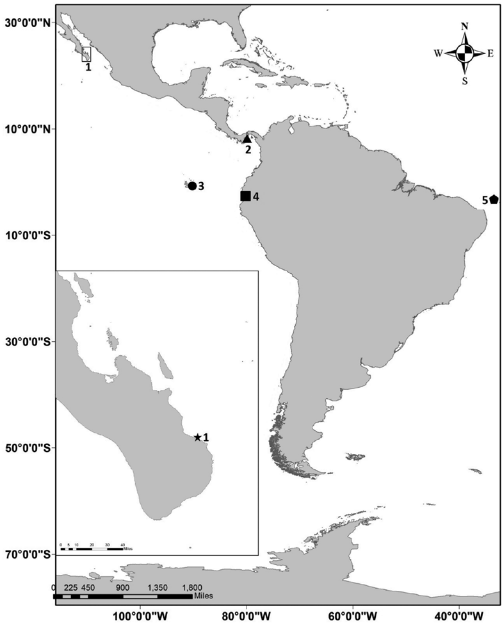

Figure 1. Location of the southern elephant seal (*Mirounga leonina*) that was recorded on La Ribera in Baja California Sur (southern Gulf of California), Mexico (1). The northernmost previous records of the species in the American continent are also shown: Taboga Island in the Gulf of Panama (2), Guayaquil, Ecuador (3), the Galapagos Islands (4), and Fernando de Noronha Archipelago, Brazil (5).

(Figure 1). This animal was first thought to be a northern elephant seal (*Mirounga angustirostris*) as this species inhabits the western region of the Baja California Peninsula (Elorriaga-Verplancken et al., 2015a; Arias-Del Razo et al., 2017). However, once the location could be reached and the animal was observed up close, it was identified and registered as a subadult male SES (code LP-Ml-040919) measuring *ca*. 3.5 m long and weighing *ca*. 2 tons (Figure 2). These estimations were made by a visual appreciation, and they should be considered as such.

The species identification was made based on a larger body size relative to subadult male northern elephant seals. Additional morphological traits were considered such as the bulkier neck relative to the head size, and the length and size of the proboscis, which was shorter and smaller with more forward facing nostrils than is typical of male northern elephant seals (Reeves et al., 2002; Páez-Rosas et al., 2018).

Individual LP-Ml-040919 showed an apparent good and robust body condition. Besides an expected normal resting behavior, the animal did not show signs of negative health, and, thus, it was not considered stranded. It also presented a large amount of barnacles on its body, especially on its hind flippers (Figure 3), suggesting a long stay at sea (Joseph et al., 1986). Samples of these barnacles were not obtained, and, hence, there was not a proper identification; however, they were similar to stalked barnacles (*Conchoderma auritum*), which have been recorded in northern elephant seals (Joseph et al., 1986) and sub-antarctic SES (Best, 1971). Moreover, this barnacle species has been recorded in the southern Gulf of California (Elorriaga-Verplancken et al., 2015b). Since there was not an accurate assessment in this regard, the identity of these barnacles on LP-Ml-040919 should be taken with caution.

While LP-Ml-040919 was sleeping, a small piece of hair and skin tissue that was loose on its body was sampled by hand using latex gloves and preserved in a 1.5-mL microcentrifuge tube with ethanol (70%) for genetic analysis. Individual LP-Ml-040919 stayed on the beach for 4 d (3 to 6 September) and then departed; it was not seen again.

To support the field identification, a section of the sample was cut and used for DNA extraction using a GF-1 tissue DNA extraction kit (Vivantis, Malaysia), following the manufacturer's specifications. Universal primers for COI (FishF2 and FishR2; Ward et al., 2005) and CytB (TGLU and CB2; Farías-Curtidor et al., 2017) were used to amplify fragments of specific mitochondrial DNA. PCR products were sequenced, and the COI and CytB sequences obtained were edited and aligned manually using the *GENEIOUS* premium software, Version 2019.2.1. (Kearse et al., 2012). The basic local alignment search tool (BLAST) was applied to each sequence to corroborate the specific identification using the NCBI-BLAST (*GENEIOUS*). The Similitude index calculated includes similitude percent and coverage values. These sequences were deposited at NCBI with the registration GenBank accession numbers. The sequences generated in the present study and sequences published from the *Mirounga* genus from GenBank (Table 1) were aligned with Clustal W (Thompson et al., 1994), with default values included in *MEGAX*, Version 10.0.5 (Kumar et al., 2016), in both genes (COI and CytB). Observed alignment was clean without gaps and was translated into amino acid sequences as an additional check of alignment. The relationships and similarity among species were assessed using the reconstruction of the Neighbor-Joining tree (NJ; bootstrap of 10,000) with the Kimura 2-parameters and genetic distances calculated with intra- and interspecific *Mirounga* genus. The gray seal (*Halichoerus grypus*) was added as an external group.

We analyzed 546 bp of the mitochondrial gene COI and 372 bp of CytB (partial gene). The

Figure 2. Subadult male southern elephant seal (LP-Ml-040919) in La Ribera (southern Gulf of California), recorded on 4 September 2019

Figure 3. Presence of barnacles on the hind flippers of LP-Ml-040919

Table 1. Sequences of the mitochondrial gene COI and CytB (partial gene) downloaded from NCBI that were included in this study for the reconstruction of the Neighbor-Joining tree and genetic distances analysis

	GenBank accession numbers	
Taxa	COI	CytB
Mirounga angustirostris	AY377138.1[a]	AY377325[a]
	AY377139.1[a]	AY424646[e]
Mirounga leonina	NC_008422.1[b]	NC_008422.1[b]
	AM181023.1[b]	AM181023.1[b]
	AY377140.1[a]	X82298[d]
		AY377326[a]
Halichoerus grypus (Outgroup)	GU167274[c]	GU167293[c]
This study LP-Ml-040919	MN807035	MN807449

Genes reported by [a]Davis et al. (2004), [b]Arnason et al. (2006), [c]Fulton & Strobeck (2010), [d]Arnason et al. (1995), and [e]Kinner et al. (not pub.).

BLAST analysis revealed high similarity with the SES (99.8% and E Value 0 – COI; 99.9% and E Value 0 – CytB). Topologies of the NJ tree of COI showed two main clades—one supported with a bootstrap of 71% included all sequences of the SES, downloaded from NCBI and the sequences of LP-Ml-040919; and the other clade included sequences of *M. angustirostris* from NCBI with a bootstrap of 99% (Figure 4a). Nucleotide divergence between these two clades was 2.5% (mean genetic distance between species obtained by Kimura 2-parameters), while the distance within the SES was 0.8%. The distance between these two clades and the outgroup was 19% (Table 2; Figure 4a). The NJ tree of CytB showed similar results relative to the COI gene, with two separate clades, including the SES sequences with a bootstrap of 72% and *M. angustirostris* with a bootstrap of 99% (Figure 4b). The genetic divergence obtained between the SES and *M. angustirostris* was 4.7% (mean genetic distance between species obtained by Kimura 2-parameters); the distance within the SES was 1.4%. The distance between the two clades and the outgroup was 13.7% (Table 3; Figure 4b). This genetic assessment confirmed the identification of this animal as a SES.

Prior to this sighting, the northernmost vagrant SES previously recorded worldwide was reported on 10 January 1989 in Sawqirah (18° 07' N, 56° 32' E) on the central-north coast of the Arabian Sea in the Sultanate of Oman (Johnson, 1990); and the northernmost SES in the Pacific Ocean was recorded on 31 December 2016 on Taboga Island in the Gulf of Panama (8° 47' N, 79° 33.6' W; Redwood & Félix, 2018), some 4,200 km south of our record at La Ribera. Individual LP-Ml-040919 could have moved *ca*. 9,000 to 10,000 km from the nearest probable origin in the Southern Ocean in the region that corresponds to Chile and Argentina (Figure 1). This probable distance traveled by LP-Ml-040919 is similar to that of a tagged female gray whale (*Eschrichtius robustus*) that traveled *ca*. 11,200 km from Sakhalin Island, Russia, to Cabo San Lucas, Mexico, in 2011 and was reported as the longest documented mammal migration to date (Mate et al., 2015). Both are remarkable distances; however, the difference between these two cases may be that, while other gray whales from the western North Pacific are able to migrate to the eastern North Pacific (Weller et al., 2012), the unprecedented event surrounding LP-Ml-040919 is, or should be, relatively isolated. However, the more common presence of northern elephant seals in the western region of the Baja California Peninsula could complicate correct identifications in the future when inexperienced observers are involved.

Other unusual SES sightings have been reported as far north as the Fernando de Noronha Archipelago, Brazil, at 3° 51' S (Lodi & Siciliano, 1989; De Moura et al., 2010), the central coast of Chile and the Juan Fernandez Islands (Acevedo et al., 2016; Sepúlveda et al., 2017), Ecuador (Alava & Carvajal, 2005; Páez-Rosas et al., 2018), the Galapagos Islands (Vargas & Steinfurth, 2004), Colombia (Avila et al., 2019; J. J. Alava, pers. comm., Institute for the Oceans and Fisheries, University of British Columbia, 17 November 2019), South Africa (Oosthuizen

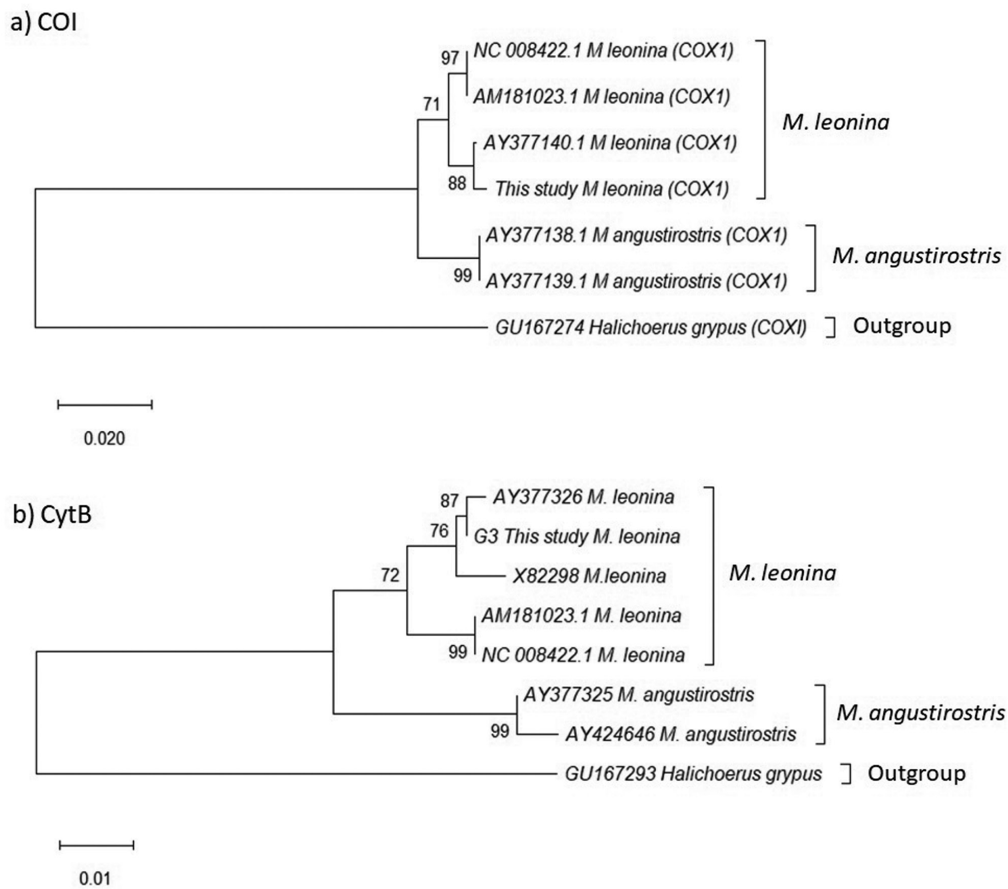

Figure 4. Neighbor-Joining tree using COI and CytB sequences of the *Mirounga* species, obtained from GenBank and this study. Number shown at the tree branches indicate bootstrap values (> 70%) based on 10,000 replicates.

Table 2. Matrix of Kimura-2 parameters' genetic distances of *Mirounga* species (%), based on the COI gene

COI gene	AY377138.1	AY377139.1	NC_008422.1	AY377140.1	AM181023.1	This study	GU167274
AY377138.1_*M. angustirostris*	--	0	0.59	0.73	0.59	0.74	2.28
AY377139.1_*M. angustirostris*	0.00	--	0.59	0.73	0.59	0.74	2.28
NC_008422.1_*M. leonina*	2.05	2.05	--	0.39	0.00	0.46	2.30
AY377140.1_*M. leonina*	3.01	3.01	0.92	--	0.39	0.25	2.23
AM181023.1_*M. leonina*	2.05	2.05	0.00	0.92	--	0.46	2.30
This study_*M. leonina*	3.01	3.01	1.30	0.37	1.30	--	2.22
GU167274_ *Halichoerus grypus* (outgroup)	19.17	19.17	19.21	18.94	19.21	18.90	--

Table 3. Matrix of Kimura-2 parameters' genetic distances of *Mirounga* species (%), based on the CytB gene

CytB gene	AY377325	AY424646	AY377326	AM181023.1	X82298	NC_008422.1	This study	GU167293
AY377325_*M. angustirostris*	--	0.37	1.14	1.03	1.22	1.03	1.11	2.13
AY424646_*M. angustirostris*	0.54	--	1.21	1.11	1.29	1.11	1.18	2.17
AY377326_*M. leonina*	4.79	5.37	--	0.72	0.52	0.72	0.26	2.06
AM181023.1_ *M. leonina*	3.91	4.49	1.92	--	0.80	0.00	0.66	2.11
X82298_*M. leonina*	5.39	5.97	1.09	2.48	--	0.80	0.44	2.00
NC_008422.1_ *M. leonina*	3.91	4.49	1.92	0.00	2.48	--	0.66	2.11
This study_*M. leonina*	4.50	5.08	0.27	1.64	0.81	1.64	--	2.03
GU167293_ *Halichoerus grypus* (outgroup)	13.58	14.24	13.24	13.58	12.55	13.58	12.90	--

et al., 1988), Australia (Mills et al., 1977), and New Zealand (Taylor & Taylor, 1989).

Some of the sightings in the Pacific Ocean, including those in the Gulf of Panama (Redwood & Félix, 2018) and Ecuador (Alava & Carvajal, 2005; Páez-Rosas et al., 2018), were associated with La Niña cold conditions at the end of 1998, 2016, and 2017. These conditions were suggested to provoke SESs to forage across abnormal expanded cool/nutrient-rich areas, increasing their coverage beyond their typical foraging grounds and leading them to extreme northern sites.

Certainly, those periods were related to negative sea surface temperature anomalies for the 3.4 region (El Niño Index or NOI = from -1.3 to -0.7; Páez-Rosas et al., 2018; National Oceanic and Atmospheric Administration [NOAA], 2019); however, prevailing conditions in weeks before and during the presence of LP-Ml-040919 in La Ribera were closer to slightly positive (NOI = 0.5 in July) or relatively neutral (NOI = 0.3 in August and 0.1 in September). For reference, NOI values for the 3.4 region were as high as 2.5 during the strong 2015-2016 El Niño. Because of these low or close to neutral NOI values during LP-Ml-040919's presence in La Ribera, coupled with the specimen's good body condition, temperature anomalies (negative or positive) can be ruled out as a predominant trigger of this unprecedented record. This extraordinary presence in the southern Gulf of California could be a consequence of the dispersal capacity of the species, which can extend to thousands of kilometers under normal conditions (Hindell & McMahon, 2000). Factors surrounding this event may be similar to those presented for the unexpected number of SESs along the Brazilian coast (45 individuals from 1958 to 2008) in terms of the movement patterns of this species, the lack of physical barriers, and currents moving northward (De Moura et al., 2010).

It is interesting that during this same time (6 September 2019), there was an important squid (unknown species) stranding in La Ribera. This could be indicative of its availability and relatively short distance from the coast at that time. Squids are an important dietary item for the SES (Bradshaw et al., 2003); thus, LP-Ml-040919 may have taken advantage of this resource, also approaching this coastal area and hauling out at La Ribera. However, this hypothesis should be considered with caution.

Even though our study period was not related to specific oceanographic anomalies, the accumulative effect of an increasing frequency of El Niño events in the Central Pacific in recent decades (Freund et al., 2019) cannot be ignored as a possible factor that has altered dispersal patterns of SES or other species that could potentially be part of its diet. Additionally, other long-term phenomena have been recorded regarding ocean warming and the global shoaling of hypoxic oxygen minimum zones and their relationship with the range expansion of the jumbo squid (*Dosidicus gigas*) from the Humboldt Current Ecosystem into the northeastern Pacific (Field et al., 2007; Stewart et al., 2014). This is an aspect that cannot be ignored when the extralimital dispersal of teutophagous predators is assessed.

This is the first SES record at such a northern latitude. Individual LP-Ml-040919 was confirmed based on both positive field identification and genetic assessment, providing an important contribution to our knowledge of pinnipeds, specifically the extralimital dispersal patterns of species from the Southern Hemisphere that are potentially capable of displacing into the Northern Hemisphere.

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