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Stranded humpback whale (*Megaptera novaeangliae*) (Cetacea: Balaenopteridae) in Paraná River Delta, Buenos Aires Province, Argentina. Comments on the occurrence of marine mammals in the La Plata River Basin

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Abstract. The humpback whale (*Megaptera novaeangliae*) is distributed among most oceans and seas of the globe (except Mediterranean Sea). These whales migrate from feeding regions in the Antarctic waters to breeding areas in tropical and subtropical seas. Here we report the stranding of a female young humpback whale, which was founded dead in the vicinity of the Talavera Island, in the Paraná River Delta, Buenos Aires Province, Argentina. From the analysis of mitochondrial cytochrome c oxidase subunit I gene sequences, two novel haplotypes were found, totalizing four haplotypes described for the species. In the La Plata River Basin this species was found only twice at the end of the XIX century. Thus, the new finding constitutes an important addition to the list of cetaceans that occurs in Uruguay, Paraná and La Plata Rivers.

Key-Words. *Megaptera novaeangliae;* Humpback whale; Delta del Río Paraná; Haplotypes; Argentina.

INTRODUCTION

The humpback whale (*Megaptera novaeangliae*) is a misticete cetacean that is distributed among most oceans and seas of the globe, with the exception of the Mediterranean Sea (Clapham & Mead, 1999; Clapham, 2002). These whales migrate from the feeding regions in the Antarctic Waters to breeding areas in tropical and subtropical seas (*e.g.,* Mackintosh, 1942; Dawbin, 1966; Clapham & Mead, 1999; Clapham, 2002). In the Southern Hemisphere humpback whales have seven feedings areas (Dawbin, 1966; Clapham & Mead, 1999; Clapham, 2002; IWC, 2005). One of them, The Breeding Stock A, is distributed along the Southwestern Atlantic Ocean and constitutes one of the least known breeding groups (Andriolo *et al.,* 2010). This stock breeds at the Brazilian coasts, at the Abrolhos Bank, where approximately 3,500 individuals were censed (Engel, 1996; Martins *et al.,* 2001; Zerbini *et al.,* 2006, 2011; Andriolo *et al.,* 2010). Genetic studies have the potential to provide data allowing to descipher long-term patterns of affiliation, breeding, and dispersal, as well as to understand important topicas on social and geographical structures of populations. In spite to that, detailed genetic studies of Humpback whales in the Southwestern

Atlantic are still wanting, and the report of any evidence is urgently needed.

In the Argentine Sea the records are scarce, and this is probably because humpback whales migrate across very deep seas and avoid the large continental platform of Argentina. In this way, the strandings of the species are scarce and mostly correspond to isolated individuals (see Angeletti *et al.,* 2014). Strandings are reported from Buenos Aires Province, Chubut Province, Tierra del Fuego Province and Islas Malvinas (*e.g.,* Lichter & Hooper, 1984; Bastida & Rodríguez, 2009; Angeletti *et al.,* 2014).

The aim of the present paper is to report the stranding of a female young humpback whale, which was found dead in vicinity of the Talavera Island, in the Paraná River Delta, Buenos Aires Province, Argentina. Further, we explore the genetic identification of the species and the historical revision of the occurrence of marine mammals in the La Plata River basin.

MATERIALS AND METHODS

Stranding

The 21 of July of 2012 a large cetacean swimming at the Paraná River was reported by local people of Zárate town. Some days later, presumably the same individual was observed in San Pedro locality, and on July 25th, the cetacean was found dead at the proximities of Talavera Island (33°56′00,75″S; 58°58′02,82″W). The Talavera Island is located at the 4th section of the Delta del Paraná, at Zárate Township, emplaced at northeastern Buenos Aires Province (Fig. 1). This area is lined by a large number of watercourses, the most important being the Paraná River, that ends in the La Plata River and conforms a wide delta.

Genetic analyses

Tissue samples from the individual found in Talavera Island were collected (FHNA, hereafter). Additionally, we analyzed a sample from another individual found in Punta Alta, southern Buenos Aires province, Argentina (FHNB, hereafter). This specimen consist on skin samples and epizooic barnacles of a male specimen collected at 18/11/2011 and housed under the collection number CFA-MA-13095. This specimen was reported and its finding published in detail by Angeletti *et al.* (2014). Total DNA was extracted using a proteinase K digestion, extraction of proteins with phenol-chloroform method and alcohol precipitation of DNA (Sambrook *et al.,* 1989). A fragment of 711 bp from the mitochondrial cytochrome c oxidase subunit I gene (COI) was amplified by polymerase chain reaction (PCR) using primers FHNF 5′ ATT CTC AAC CAA CCA CAA AG y FHNR 5′ GTG AAA TTA TTC CGA AGC CA, specially designed for this study. Final concentrations used in PCR reaction volumes of 50 ul were: 5 µg/ml template DNA, Buffer 1X (Promega), 0.2 mM dNTPs, 0.2 µM each primer, 1.5 mM de $MgCl_2$ y 1.25 units of GoTaq polymerase (Promega). PCR cycling profile consisted of an initial denaturation at 94°C for 2 min, followed by thirty-five cycles of denaturation at 94°C for 1 min, anneling at 50°C for 1 min, and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were purified using a commercial kit (AccuPrep PCR Purification Kit, Bioneer) and sequenced in both direction using an ABI 337 Automated DNA Prism Sequencer (Applied Biosystems, Inc.). Sequences were aligned using CLUSTALX 2.0.11 (Larkin *et al.,* 2007) and compared with those previously published for the species (Genbank: NC006927, GQ353284, GQ353285, GQ353286, FJ590425, EU139285, EU139286, EU496287) (Table 1). From the 711 bp amplified, a 593 bp consensus region containing most variation was examined. Haplotypes were verified using DnaSP v5.10.01 (Librado & Rozas, 2009). To study patterns of geographical distribution and relationships among haplotypes, we implemented a Median-Joining

Table 1. Cytochrome c oxidase subunit I gene sequences analyzed and its geographic location.

GenBank Accession number	Geographic Location
AP006470	Antartic
EU139285	Northwestern USA
EU139286	Northwestern USA
EU496287	Northwestern USA
FJ590425	Unknown
GQ353284	USA
GQ353285	USA
GQ353286	USA
KY001615 (FHNB)	This study/Argentina
KY001616 (FHNA)	This study/Argentina
NC006927	Antarctic

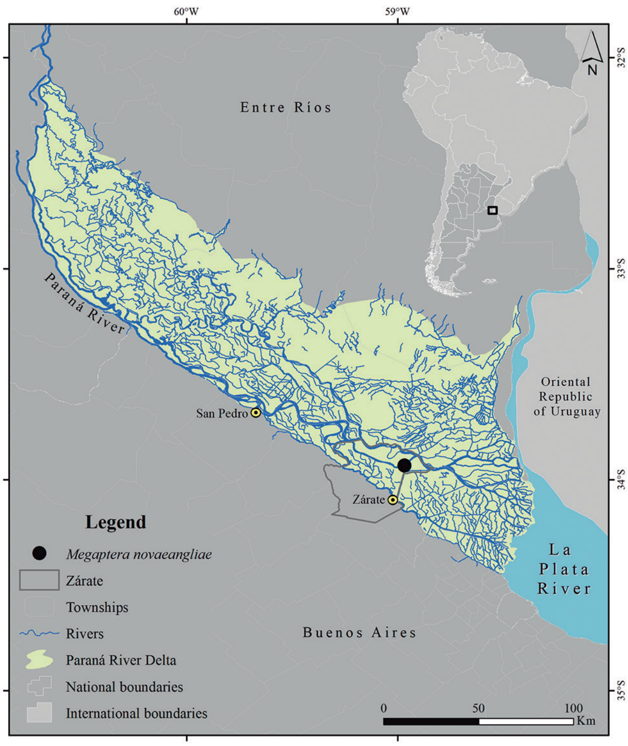

Figure 1. Paraná River delta map were *Megaptera novaeangliae* (CFA-MA-13084) was found dead (exact location is indicated with a black dot).

network (Bandelt *et al.,* 1999) in PopART 1.7 (Leigh & Bryant, 2015).

RESULTS

The stranded specimen was found floating in the watercourse and resting on its right side. With the help of a backhoe and a crawler the individual was aground in the island coast to proceed with its preparation. Although its body surface was in a good state of preservation, the inner organs and its musculature were badly decomposed and its thumb was inflated by organic gas. Therefore, details of inner anatomy were not available for study (Fig. 2).

The stranded individual showed a combination of characters that unambiguously identify it as belonging to *Megaptera novaeangliae:* pectoral fins notably enlarged, representing ⅓ of total body length, festooned margins of pectoral fins, entirely black dorsal coloration, and head and body with a midline row of bumps, among other features (Lichter & Hooper, 1984; Bastida & Rodríguez, 2009).

The skeleton, one eye and samples of fat, skin, and muscles were housed at the Mastozoology Collection of the Fundación de Historia Natural "Félix de Azara", Buenos Aires, Argentina (CFA-MA-13084) (Fig. 3).

The individual was 10.09 meters long and was classified as a juvenile, since the length of adult specimens range from 14 to 16 meters (Clapham & Mead, 1999). Furthermore, analysis of genitalia identified it as a female (Table 1).

Five deep transverse cuts (being approximately 5 cm wide and more than 30 cm depth) were found on the left lateral side of the head of the individual, which would correspond to the impact of the propeller of a large boat on the cetacean. This certainly constitutes the cause of death of the specimen.

As other individuals of this species, its body was covered by a large number of cirripedes, specially the protuberances of the head and the anterior margin of the pectoral fins. Cirripedes were determined as *Conchoderma auritum* (Cirripedia, Lepadidae), an epibionthic crustacean frequently found in *Megaptera novaeangliae* (Clarke, 1966; Zullo, 1979; Félix *et al.,* 2006; Angeletti *et al.,* 2014).

Figure 2. (A) CFA-MA-13084 specimen before start preparation. (B) Sampling extraction work.

Geographical distribution and relationships among haplotypes

From the analysis of the samples FHNA and FHNB, two novel haplotypes were found (GenBank accession numbers: KY001616 and KY001615, respectively) (Table 1).

Figure 3. *Megaptera novaeangliae* (CFA-MA-13084) mounted skeleton.

Table 2. Polymorphic sites within 11 cytochrome c oxidase subunit I gene sequences from *Megaptera novaeangliae.*

GenBank Accession number	Geographic Location
AP006467	Antartic
EU139285	Northwestern USA
EU139286	Northwestern USA
EU496287	Northwestern USA
FJ590425	Unknown
GQ353284	USA
GQ353285	USA
GQ353286	USA
KY001615 (FHNB)	This study/Argentina
KY001616 (FHNA)	This study/Argentina
NC006927	Antarctic

Note: For each haplotype, a dot indicates a nucleotide identical to the reference haplotype AP006467 at that given site.

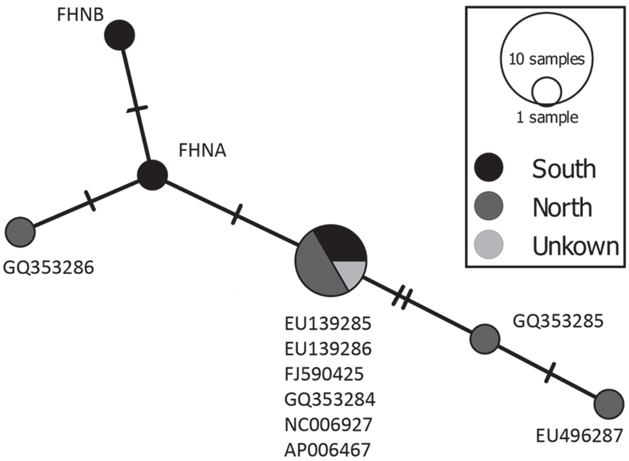

Figure 4. Median-joining network based on the cytochrome c oxidase subunit I mtDNA haplotypes of *Megaptera novaeangliae.* Haplotypes are represented with discs and colors that indicate geographical locations. Mutational steps are indicated with stripes.

Based on the our analysis, six sequences (EU139285-6, FJ590425, GQ353284, AP006467 and NC006927) conform a common haplotype (see Appendix 1). However, since a consensus region was analyzed, mutations present beyond the consensus region length were lost and those haplotypes were grouped together (Table 2). Additionally, no phylogeographic pattern was evident based on the network results (Fig. 4).

DISCUSSION

Occurrence of cetaceans in the La Plata River basin

Humpback whales migrate notable long distances that may overcome more than 8,000 km, being probably the longest distance achieved by any mammal species (Mackintosh, 1942). Humpback whales feed in summer and spring in cold regions and during winter and autumn migrate towards breeding areas in tropical and subtropical seas (Bastida & Rodríguez, 2009; Angeletti *et al.,* 2014). This whale is very scarce in Argentina, probably because it avoids the shallow waters of its continental platform. In fact, there has only been approximately 15 sights and strandings from 1866 to the date (see Table 3).

In the La Plata River Basin, the species has been found only twice at the end of the XIX century (Burmeister, 1867; Lahille, 1899a). Thus, this new finding constitutes an important addition to the list of cetaceans that occurs in Uruguay, Paraná and La Plata rivers.

Since the pioneering works of Burmeister at the second half of the XIX century, a large number of cetaceans were found penetrating freshwater courses of the La Plata Basin (Appendix 2). In fact, at least 19 cetacean species, and 4 pinnipeds (Carman, 2009) were found in these watercourses. These include the finding of a blue whale in the intersection between Luján and Paraná rivers by Burmeister (1872). Further, Burmeister (1867) and Lahille (1899a, b) mentioned fin whales in localities at the La Plata River coast. In addition, the holotype of the Antarctic Minke Whale, *Balaenoptera bonaerensis,* was found in the La Plata River, at shores of the Buenos Aires city (Burmeister, 1867; Zerbini & Castello, 2003). Minke whales are fairly common along the La Plata River coast, and the stranding of juveniles is not an uncommon fact (Marelli, 1918; Lichter & Hooper, 1984).

Table 3. Sights and strandings of Humpback whales from 1866 to the date in Argentina.

Locality	Sex	Age	Date	Observations
Buenos Aires coast				Gray, 1866
Island between Paraná Guazú and Paraná de las Palmas rivers, Buenos Aires province				Burmeister, 1866, 1867
Punta Indio, Buenos Aires province				Lahille, 1899b
Punta Indio, Buenos Aires province				Lahille, 1905
Beagle Channel, Tierra del Fuego province				Lahille, 1905
Patagonia and Islas Malvinas				Mörch, 1911
Islas Malvinas				Salvesen, 1914
Islas Malvinas				Carcelles, 1932
Islas Malvinas				Hamilton, 1952
Punta Ushuaia, Tierra del Fuego.	M	Juvenile		Angeletti *et al.,* 2014
Punta Tejada, Buenos Aires province	M	Juvenile	22 Julio 2011	Angeletti *et al.,* 2014
Delta del Paraná, Buenos Aires province	F	Juvenile		Present work
Ciudad Autónoma de Buenos Aires port			3-4 Agosto 2015	Present work, specimen observation aground
Mar del Tuyú, Buenos Aires province.			19 Julio 2016	Present work, observation of specimen aground

Considerable literature has been devoted to the potential causes of cetacean strandings (see Simmonds, 1997 for a review). Among the most important are the individuals that became lost during migrations, and specimens with some kind of illness, parasites or injuries (Martin *et al.,* 1990). These specimens are in a condition in which they cannot navigate or swim properly and thus accidentally comes ashore. The same may be applied to the occasional or accidental penetration of cetaceans in some freshwater courses (see Smith & Jefferson, 2002).

However, the record of cetaceans penetrating La Plata Basin watercourses (*e.g.,* Paraná, Uruguay, and La Plata rivers) is notably common (as explained above, at least 19 different species of cetaceans have been reported), and lost individuals are frequently found swimming in their waters. This pattern is not matched by other freshwater courses in the globe. The abnormal frequency of Cetaceans in these waters may have a special explanation. Some authors (Green, 1945 in Dudok van Heel, 1962) defended the idea that some cases of strandings may be due to whales that are attempting to follow ancient migration routes through areas that have been closed by changes in sea level or sediment deposition.

In this regard, the coastal plains and surrounding areas in the south of Entre Ríos Province, together with the Paraná Delta and La Plata River have been affected by the late Pleistocene-Holocene post-glacial marine transgression (Cavallotto *et al.,* 2005). The last evidence of this sea-level rise episode is shown as littoral deposits of mid-Holocene age, which contain concentrations rich in sea shells that are frequent in the La Plata Basin, including the La Plata River coast and southern ends of Paraná and Uruguay rivers (Guida & González, 1984; Fucks & De Francesco, 2000; Aguirre & Fucks, 2004; Martínez & Del Río, 2005). Thus, the La Plata River and the associated watercourses of southern Mesopotamia were neither an estuary nor a deltaic freshwater environment at that moment. Instead, between 6,000-6,500 and 3,000 years before present were part of a marine gulf (Cavallotto, 2002; Martínez & Del Río, 2005). Since around 3,000 years before present, there are no new records of marine influence in the area (Martínez & Del Río, 2005).

It is possible that such pre-La Plata River was an area that constituted at least a passageway for cetaceans during the Holocene. Thus, it is not improbable that cetaceans penetrating into the La Plata River are attempting to follow ancient marine routes that, since the Late Holocene to the present, are freshwater courses unsuitable for marine mammals. Although we recognize that this proposal rests on weak evidence, the unusually large number and diversity of cetaceans found in La Plata Basin may be indirect evidence sustaining this hypothesis.

Insights into *Megaptera novaeangliae* COI sequence analysis

Over more than a decade ago, COI region have been widely studied with the purpose to develop a "barcode" for species (*e.g.,* Arnason *et al.,* 2004; Amaral *et al.,* 2007). However, as occurs with any new genetic tool, the barcode method still presents some limitations, such as the low availability of intraspecific variation analyses in some species (*e.g.,* Frézal & Leblois, 2008; Mitchell, 2008). In this study, we have contributed to the knowledge of the genetic diversity of. *M. novaeangliae* with two novel COI sequences, extending the number of described sequences from one to three for the southern distribution range of the species, and from nine to eleven for its entire geographical distribution. The lack of a phylogeographic pattern, suggested by the network results, may be due to the low number of sequences previously described for the species and analyzed in this study. In order to develop a robust genetic tool, more analyses of COI sequences are urgently needed.

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APPENDIX 1

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APPENDIX 2

Cetaceans found penetrating freshwater courses of the La Plata Basin.
Abbreviations: MACN-Ma Colección de Mastozoología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"

Scientific name	Locality	Source
Balaenoptera physalus	San Fernando	Lahille (1899a)
Balaenoptera physalus	Quilmes	Burmeister (1867)
Balaenoptera physalus	Punta Carreta, Montevideo	Praderi (1980)
Balaenoptera musculus	Río Luján mouth	Burmeister (1872)
Balaenoptera musculus	Bahía de Samborombón	Burmeister (1866)
Balaenoptera musculus	Puerto Platero, Colonia	Praderi (1985)
Balaenoptera acutorostrata	La Plata River estuary	Castello en Lichter & Hooper (1984)
Balaenoptera acutorostrata	Uruguay coast	Praderi (1981)
Balaenoptera bonaerensis	Arroyo Medrano mouth	Burmeister (1867)
Balaenoptera acutorostrata	La Plata River	Marelli (1918)
Megaptera novaeangliae	Punta Indio	Lahille (1899a)
Megaptera novaeangliae	Isla Talavera	This work
Berardius arnuxii	Arroyo Pescado	Marelli (1920)
Ziphius cavirostris	La Plata River	Burmeister (1867)
Ziphius cavirostris	Uruguay coast	Praderi (1981)
Ziphius cavirostris	Arroyo Solís grande, Canelones	Praderi (1971)
Pontoporia blainvillei	Coast of Buenos Aires City	Burmeister (1869)
Pontoporia blainvillei	Punta Lara	Marelli (1951)
Kogia breviceps	Playa Ramírez, Montevideo	Vaz Ferreira & Praderi (1973)
Phocoena spinipinnis	La Plata River	Burmeister (1865)
Phocoena dioptrica	Quilmes	Lahille (1912)
Phocoena dioptrica	Santiago River	Bruch (1916)
Orcinus orca	La Plata River estuary	Castello in Lichter & Hooper (1984)
Orcinus orca	Bahía de Samborombón	Padorno in Lichter & Hooper (1984)
Pseudorca crassidens	Bahía de Samborombón	Burmeister (1868)
Pseudorca crassidens	Bahía de Samborombón	MACN-MA 20526
Stenella coeruleoalba	La Plata River estuary	Meyen (1833)
Tursiops truncatus	Punta Lara	Lichter& Hooper (1984)
Tursiops truncatus	Uruguay River, Gualeguaychú	Burmeister (1866)
Tursiops truncatus	Salto Grande dam	Castello in Lichter & Hooper (1984)
Tursiops truncatus	Punta Indio	Mermoz (1977)
Feresa attenuata	Punta Indio	MACN-MA 20472
Cephalorhynchus commersonii	Quilmes	MACN-MA? 4.421
Pontoporia blainvillei	Quilmes	MACN-Ma 49.215
Pontoporia blainvillei	Quilmes	MACN-Ma 49.217
Pontoporia blainvillei	Bahía de Samborombón	MACN-Ma 18109 and 18111 and 18115
Pontoporia blainvillei	Punta Indio	MACN-Ma 20507 and 20508 and 20514
Balaenoptera acutorostrata	Buenos Aires port	Lahille (1908)
Balaenoptera acutorostrata	San Isidro	MACN-Ma 25.176
Balaenoptera acutorostrata	San Isidro	MACN-Ma 17823
Balaenoptera borealis	Olivos	MACN-Ma 54.107
Balaenoptera acutorostrata	Olivos	MACN-Ma 20520
Balaenoptera acutorostrata	Buenos Aires port	MACN-Ma 20521

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