

Short note

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New genetic diversity for endangered bottlenose dolphin subspecies along the Argentine coast

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Abstract: This study analyzed mtDNA sequences of two bottlenose dolphin subspecies found along the northern Patagonian coast, Argentina: the endangered *Tursiops truncatus gephyreus* and the data deficient *Tursiops truncatus truncatus*. Three haplotypes were recovered from nine samples. The most frequent haplotype represented the coastal morph, also two haplotypes showing falcate dorsal fin, a characteristic describing the oceanic morph. This finding suggests that both morphs may exist in sympatry in Argentina. Furthermore, sampling was extended beyond the species' previously known range, including individuals as far as seven hundred kilometers to the south. Therefore, new genetic data, despite being preliminary, hint at an unexpectedly higher genetic diversity of matrilineal lines than previously anticipated.

Keywords: Argentina; bottlenose dolphins; conservation; genetics; *Tursiops t. gephyreus*; *Tursiops t. truncatus*.

The bottlenose dolphin, *Tursiops truncatus*, is a cosmopolitan species found in all temperate and tropical marine environments (Wells and Scott 2018). In the North Atlantic, two different morphs are widely recognized, the coastal (or inshore) morph and the oceanic (or offshore) morph (Hoelzel et al. 1998; Tezanos-Pinto et al. 2009; Wells and Scott 2018). Coastal bottlenose dolphins are smaller, lighter gray, and form small fragmented populations, while oceanic dolphins are larger, darker, and form larger

groups. In the Southwestern Atlantic Ocean (SWA), a combination of morphology and genetics studies showed that coastal and oceanic morphs also exist and suggested that they may represent separate subspecies or even different species (Costa et al. 2015; Wickert et al. 2016; Fruet et al. 2017). Although the taxonomy of bottlenose dolphins in the area is not settled, the Society for Marine Mammalogy and the International Whaling Commission (SMM, Committee on Taxonomy 2018; IWC 2018) decided to accept the subspecies *Tursiops truncatus gephyreus* for coastal bottlenose dolphins, and *Tursiops truncatus truncatus* for oceanic bottlenose dolphins in the SWA as proposed by several authors (Costa et al. 2015; Fruet et al. 2017; Wickert et al. 2016). The presence of two morphs of *Tursiops truncatus* was proposed based on external morphology in Argentina. The coastal morph, *T. t. gephyreus*, has a triangular dorsal fin and lighter body coloration, and the oceanic morph, *T. t. truncatus*, has a falcate dorsal fin and darker body coloration. Currently, the species *Tursiops truncatus* is categorized as Vulnerable (VU) following criteria A2 by the condition “a” (direct observation) in the Red List of Argentina. Moreover, based on the subspecies recognized for this species in the SWA, the subspecies *T. t. gephyreus* is categorized as Endangered (EN); and *T. t. truncatus* is categorized as Data Deficient (DD) (Vermeulen et al. 2019a). Recently, the subspecies *T. t. gephyreus* was categorized as Vulnerable (VU) in The IUCN Red List of Threatened Species (Vermeulen et al. 2019b).

The distribution of bottlenose dolphins in coastal regions of the SWA appears to be discontinuous, ranging from the mouth of the Amazon River (Brazil) to northern Patagonia (Argentina), with probably extralimital records in Tierra del Fuego (Argentina) and the Falkland (Malvinas) Islands (Bastida et al. 2007; Goodall et al. 2011).

Recent studies using microsatellites and mtDNA analyses have shown remarkably low levels of genetic diversity and strong genetic differences among coastal populations along the SWA (Costa et al. 2015; Fruet et al. 2014). Moreover, two ESUs were proposed for bottlenose dolphins along SWA, an ESUs is a group of historically isolated populations with unique genealogical and adaptive legacy (Funk et al. 2012); one bottlenose ESU includes

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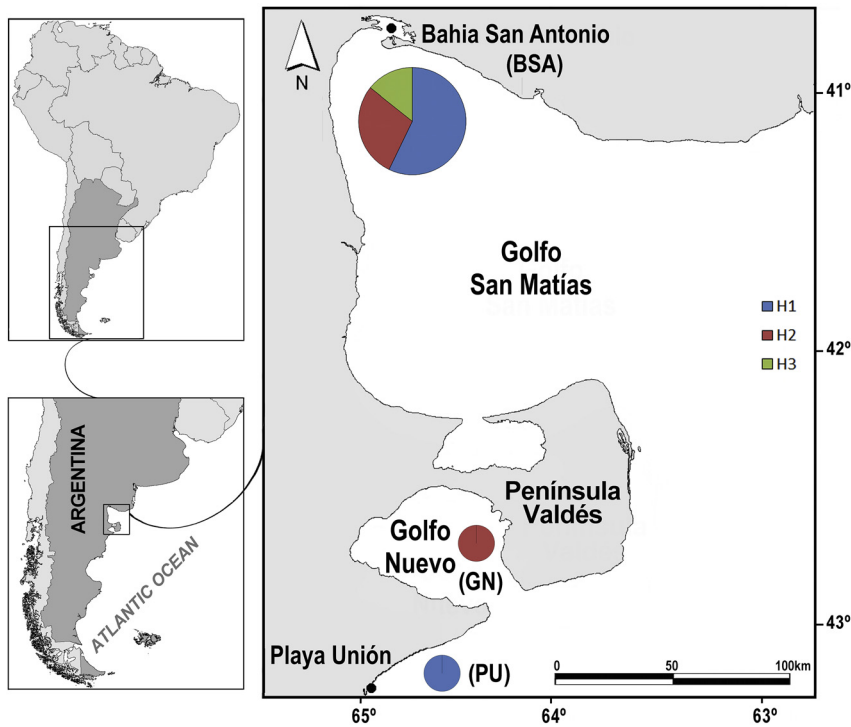


Figure 1: Study area, in the northern Patagonia for coastal bottlenose dolphins (*Tursiops* spp.), and the respective frequencies of mtDNA control region haplotypes (pie charts).

populations along southern Brazil and Uruguay; the other ESU considers bottlenose dolphins in Bahía San Antonio (BSA), Golfo San Matías, Argentina (Fruet et al. 2014). Later, Fruet et al. (2017) detected strong levels of structuring and contrasting genetic diversity between the oceanic and coastal bottlenose dolphin morphs along the SWA, with evidence of minimal connectivity between them, suggesting that bottlenose dolphins follow discrete evolutionary trajectories. Hence oceanic bottlenose dolphins could comprise a third ESU.

In addition, Vermeulen and Cammareri (2009) suggested the co-occurrence of both morphs in BSA (41°S), where one of the ESU proposed by Fruet et al. (2014) is found.

For the past decades, there has been a considerable reduction in sightings along the coast of Buenos Aires (38°S) and Chubut (42°S) (Bastida et al. 2007; Coscarella et al. 2012; Vermeulen and Bräger 2015), with certain areas where dolphins have almost completely disappeared (Vermeulen et al. 2017). Additionally, there are no new coastal areas where this species has increased substantially over time, which seems to falsify a hypothesis of potential changes in distribution. Abundance analysis on *T. t. geophysus* morph indicate that the population has reduced by at least 1.1% per year and the proportion of annual reproducing females lost had a significant impact on the decreasing population trend (Vermeulen and Bräger 2015). Moreover, if calf mortality is considered, the population shows an estimated reduction

of 2.2% per year and risk of local extinction within 72 years (Vermeulen and Bräger 2015).

Based on the information from Coscarella et al. (2012), the scenario for the oceanic morph, *T. t. truncatus*, is not much better. The only abundance estimates available for *Tursiops* in Chubut (42°S) is less than ~50 individuals (Coscarella et al. 2012). The sum of all available data shows an estimated abundance of <300 individuals (from both subspecies combined) for Argentina, possibly less, considering that animals sighted in BSA (41°S) were also observed in Golfo Nuevo (GN-42°S) and Playa Unión (PU-43°S) (Coscarella et al. 2016).

Genetic diversity data provides critical information for endangered bottlenose dolphin populations in Argentina, and decisions based on this information must be taken to conserve the species. The present study reports new mtDNA haplotypes of coastal and oceanic bottlenose dolphin morphs from northern Patagonia, Argentina. The objectives were to provide a new assessment of the genetic diversity for the local population and to expand the geographic area of BSA-ESU, previous described for the species in Argentina.

Samples were collected along seven hundred kilometers of coastline, during the period 1997–2014. A total of 10 samples were collected from stranded or bycaught animals, and through biopsy sampling. Sampling included eight samples from BSA (41°S): four stranded animals,

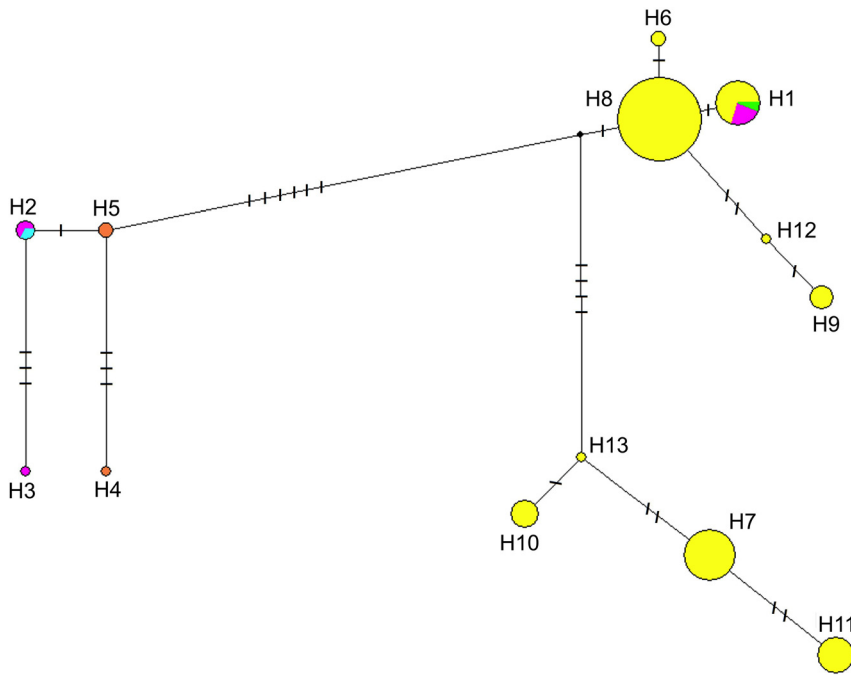


Figure 2: Median-joining network of mtDNA control region haplotypes in bottlenose dolphins (*Tursiops* spp.). The size of the circles is proportional to the total number of individuals belonging to each haplotype. Hatch marks indicate the number of mutational steps between haplotypes. Different colors represent the different sampling localities: pink: Golfo San Matias; green: Playa Unión; blue: Golfo Nuevo; yellow and orange show coastal and oceanic morphs from Fruet et al. (2017), respectively.

three that stranded alive and were returned to the sea (three animals showed falcate dorsal fin), and one from incidental catch; **onebiopsy** sample from GN (42°, falcate dorsal fin); and one sample from a stranded animal from PU (43°S, triangular dorsal fin) (Figure 1). All tissue samples were preserved in 20% dimethylsulfoxide saturated with sodium chloride.

DNA extraction was performed at the Barcode of Life reference Laboratory at CENPAT-CONICET (Ivanova et al. 2006). The mtDNA control region was amplified with the primers MTCRf (5'-TTCCCCGGTGTAACC) and MTCRr (5'-ATTTTCAGTGTCTTGCTTT) (Hoelzel et al. 1998) with the following PCR profile: 5 min at 95 °C; then 35 cycles of 1 min at 53 °C, 1:30 min at 72 °C; then a final extension at 72 °C for 8min. All PCRs (25 µL, final volume) contained: 1 µL of DNA template (~50 ng), 10 mM Tris-HCl (pH 8.4), 2 mM MgCl₂, 200 µM each dNTPs, 0.3 µM of each primer and 0.5–1-unit Taq polymerase. PCR products were purified with QIAGEN PCR purification columns and both strands were sequenced in an ABI3500 using BigDye Terminator v. 3.1 chemistry (Applied Biosystems).

Sequences were visually checked and manually corrected using CHROMAS (<http://www.technelysium.com.au/chromas.html>) and aligned with BioEdit (Hall 1999). Sequences were deposited in GenBank under accession numbers MT081195-MT081197.

Statistics such as nucleotide (π) and haplotype (h) diversities were computed using MEGA (Kumar et al. 2008). DnaSP (Librado and Rozas 2009) was used to compute Fu's

Fs (Fu 1997) neutrality test. Genealogical relationships among haplotypes were reconstructed using a median-joining network built with the program Network (Bandelt et al. 1999), including mtDNA sequences from the SWA available in GenBank (accession numbers: MF405801–MF405833).

A 457 bp consensus mtDNA control region was successfully amplified and sequenced in nine samples. One sample failed to amplify. A total of three haplotypes were defined by 12 variable sites. H1 was present in five animals, one from PU and four from BSA; H2 was found in three animals, one from GN and two from BSA; and H3 was a singleton from BSA (Figure 1). Sequences extracted from Fruet et al. (2014, 2017) were used to compare with the data. The haplotype network showed a reticular pattern, suggesting a relative stability for bottlenose dolphins in the area (Figure 2). The overall haplotype diversity (h) 0.727 ± 0.113 and the nucleotide diversity (π) 0.013 ± 0.00156 showed moderate levels of genetic diversity compared to other studies for the species in SWA (Table 1). Nonetheless, the small sample size has to be taken into consideration. Values for Fu's F_s (2.639, $p < 0.02$) were positive and statistically significant, indicating a deficiency of alleles as expected under a recent bottleneck or balancing selection (Fu 1997).

When comparing our data to those from Fruet et al. (2017), individuals with haplotype H1 had the triangular dorsal fin characteristic for the coastal morph. This haplotype was also shared by individuals from BSA and

Table 1: *Tursiops* sp.: sample size N (female:male), haplotype diversity (h) and nucleotide diversity (π) in the SWA Ocean.

Locality	N (f:m)	H	π	References
Northern Patagonia	9 (6:3)	0.727	0.013	Present study
FLN Southern Brazil–Uruguay ESU	8 (6:2)	0.7500	0.0045	Fruet et al. 2014
LGN Southern Brazil–Uruguay ESU	10 (2:8)	0	0	Fruet et al. 2014
NPL Southern Brazil–Uruguay ESU	19 (8:11)	0.5425	0.0067	Fruet et al. 2014
PLE Southern Brazil–Uruguay ESU	63 (38:25)	0.4808	0.0072	Fruet et al. 2014
SPL/URU Southern Brazil–Uruguay ESU	12 (5:7)	0.6484	0.0067	Fruet et al. 2014
Bahía San Antonio ESU	12 (2:10)	0	0	Fruet et al. 2014
Santa Catarina/Rio Grande Sul	34 (8:26)	0.715	0.00159	Costa et al. 2015
Southern Atlantic offshore ecotype	45 (20:25)	0.940	0.019	Fruet et al. 2017
Southern Atlantic inshore ecotype	124 (61:63)	0.702	0.009	Fruet et al. 2017

PU, therefore extending seven hundred kilometers south the BSA-ESU geographical range, and also representing the most frequent haplotype for the species in northern Patagonia. Additionally, haplotypes H2 and H3 are closely related to haplotypes found in dolphins that had falcate dorsal fin, typical of the oceanic morph (H4 and H5, Fruet et al. (2017)) (Figure 2). From photographic data, individual dolphins belonging to these two haplotypes had falcate dorsal fin. More importantly H2 was shared by individuals from BSA and GN, six hundred kilometers apart which extend the geographic range for the oceanic morph along the Patagonian coast. Finally, H3 is a singleton from BSA and is a novel haplotype for the species in the SWA.

Understanding population dynamics and evolutionary potential in species with conservation concerns should be a central issue for regional research programs. Genetic distinctiveness between coastal and oceanic morphs of bottlenose dolphins has been detected previously in the study area (Fruet et al. 2017), with both ecotypes showing exclusive haplotypes and microsatellite profiles. However, in Fruet et al. 's (2017) study, only one locality along the Argentina coast was considered and only one haplotype

was recovered for the species. In the present study, three haplotypes were recovered for the species along the coast, and two new haplotypes were more closely related to the oceanic than to the remaining coastal morphs defined by Fruet et al. (2017). Those were observed in three individuals from BSA and one from GN. All those individuals showed falcate dorsal fins. Moreover, the presence of those oceanic haplotypes in live animals sampled from the Argentine coast suggests that both morphs co-occur more frequently than expected. Fruet et al. (2017) did not discuss in detail the mixing of coastal and oceanic morphs in BSA–ESU, which became evident only since the genetic confirmation of three dispersers from the oceanic ESU living with coastal dolphins. In addition, their proposal was unclear about whether all oceanic bottlenose dolphins in the SWA would comprise another single ESU. If so, two ESUs would exist along the Argentine coast: BSA–ESU and part of the oceanic ESU. Moreover, the range for the BSA–ESU is probably larger than previously thought, possibly encompassing the coast as far south as PU, seven hundred kilometers south from BSA. On the other hand, the dolphin sampled in GN shared a haplotype with individuals sampled in BSA, six hundred kilometers away along the coast. This haplotype and the novel haplotype are closely related to the oceanic morph. Although information on their morphology is unavailable, individuals with these haplotypes may be additional dispersers with the oceanic morphotype or have oceanic ancestry. Those hypotheses may be further explored with microsatellite data. Nonetheless, the inclusion of relatively few additional samples has revealed new genetic diversity for the species in a region that has not yet been properly surveyed. Therefore, more sampling effort is needed in order to clarify the genetic diversity.

Bottlenose dolphins are negatively impacted by many activities, but the causes of decline remain incompletely known along the Argentine coast. During the 1980's a decline in the occurrence of bottlenose dolphins along Argentina and Uruguay was observed, and overfishing and habitat degradation of coastal environments could have been the cause (Bastida et al. 2007; Lázaro and Praderi 2000). The population of bottlenose dolphins in Chubut province has declined slowly for the last 40 years (Coscarella et al. 2012) while in BSA the population has showed a rapid decline (Vermeulen and Bräger 2015). The present study reports new maternal lines not sampled before, including haplotypes from two divergent genetic clusters. The current scenario for the species is a rapid population decline along the Argentine coast (Vermeulen and Bräger, 2015); small local populations interact with

multiple anthropogenic activities such as dolphin watching along BSA, industrial pollution in BSA estuary, fishery activity in PU, making the species more vulnerable. It is now evident that more information is required to reassess the population structure of bottlenose dolphins in Argentina. The information presented herein serves as an essential baseline for future conservation management protocols.

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