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A record of the Porbeagle, *Lamna nasus*, in coastal waters of Buenos Aires (Argentina) confirmed by DNA barcoding

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Abstract: A record of a juvenile Porbeagle, *Lamna nasus*, in the coastal waters of Argentina is confirmed by DNA barcoding, since the specimen lacked some external diagnostic features. We discuss the occurrence of this species in the coastal waters of Argentina, its molecular affinity with conspecifics from a broad range of the species, and relevant conservation issues.

Keywords: *Lamna nasus*, Porbeagle, shark, Argentina, Coastal waters, DNA barcode, Conservation.

On 29 May 2009, a juvenile male shark was found dead at Chapadmalal beach (Buenos Aires, Argentina) (38°11'46''S, 57°40'19''W). Surface water temperature during this week ranged from 13.6 to 13.8°C. Unfortunately, the specimen lacked the head, but some external features identified it as belonging to the family Lamnidae. Moreover, its general body shape, the position of the first dorsal fin, a double caudal keel, and the white colour of the free rear tip of the first dorsal fin, suggested a

tentative identification as *Lamna nasus* (Bonnaterre, 1788). The specimen (without the head) measured 105 cm, corresponding to a total length of about 130 cm (Figure 1).

In order to confirm the tentative morphological identification, a sample of muscle tissue was excised from the specimen for genetic analysis and preserved in 96% ethanol at -20°C. DNA extraction, polymerase chain reaction (PCR) and sequencing of cytochrome oxidase subunit I (COI) gene were performed following standard DNA barcoding methods [1] and a 652-basepair amplicon for the 5' region of the mitochondrial COI gene was bidirectionally sequenced (Genbank accession number HM421926).

Using the library of sequences available on BOLD (Barcode of Life Data Systems), the closest matches to our DNA sequence were obtained. In addition, the relative placement of our sequence in the lineage cluster, or Barcode Index Number (BIN) assigned by BOLD, was assessed. The BIN system is “an online framework that clusters barcode sequences algorithmically, generating a web page for each cluster. Since clusters show high concordance with species, BINs can be used to verify species identifications as well as document diversity when taxonomic information is lacking” (see boldsystems.org and Ratnasingham and Hebert [2] for further details). In addition, the publically available COI sequences of *Lamna* spp. were downloaded and compared with the sequence from our specimen. Sequence divergences were calculated using the Kimura two parameter (K2P) distance model [3], and a Neighbor-joining (NJ) tree of K2P distances were created to provide graphic representations of divergence between specimens, using the software MEGA v5.0 [4]. The NJ tree was bootstrapped 500 times to provide percentage bootstrap values for branch points. Finally, we examined the COI sequences of the public records of *Lamna nasus* for diagnostic characters using MEGA v5.0.

When compared to the BOLD “Species Level Barcode Records” database, using the BOLD ID-engine (which includes every COI Barcode record with a species-level

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Figure 1. Specimen of *Lamna nasus* found at Chapadmalal beach (Argentina).

identification and a minimum sequence length of 500bp), our specimen (UNMDP 1511/FARG 664-09) matched *Lamna nasus* with a similarity range of 98.62% - 100% ($n = 99$). The database contains specimens collected all over the world, including both the southern (SH) and northern hemisphere (NH). All records fall into the same BIN, BOLD:AAA3577 (if there were different BINs within a set of specimens, putatively of the same species, it would indicate that specimens may represent different cryptic species, or there is unusually large genetic variation within a species, or, most commonly, there are misidentifications in the database).

The average K2P genetic distance between public records of *Lamna nasus* ($n = 85$) was 0.84% and the maximum intraspecific distance was 1.71%. The distance to the nearest neighbor was 5.47%, which occupied a BIN with 18 members identified as *Lamna ditropis* (BOLD: AAC0516). Significant structuring in COI sequences was detected in our analysis of *Lamna nasus* using public

records. Three main clusters of closely related haplotypes were found, one exclusively containing specimens from the NH, and other two with specimens only from the SH (Fig. 2). No haplotypes are shared between specimens from the northern and southern hemispheres. The two SH clusters both contain specimens from South Africa, New Zealand, Chile and Argentina. Our specimen shares its haplotype with one of the two SH clusters, i.e. only with specimens from the SH. Our specimen UNMDP 1511 showed much lower K2P genetic distances (GD) from specimens collected in the SH that belong to the same cluster (GD = 0-0.15%) than from those of the NH cluster (GD = 0.84-1.40%), or the second SH cluster (GD = 0.93-1.23%). In addition, the structuring of COI sequences was also indicated by single-nucleotide diagnostic characters: each of the three groups contain unique diagnostic characters (Table 1).

The Porbeagle inhabits coastal and oceanic waters in temperate and cold-temperate waters worldwide. It is most abundant on the continental offshore fishing banks but is

Table 1. Nucleotide position for each diagnostic character in public records of *Lamna nasus* from Northern (NH) and Southern (SH) hemisphere.

Groups/Nucleotide position	#4	#25	#79	#85	#169	#313	#385	#625
SH ₁	G	A	G	C	G	A	A	A
SH ₂	G	A	A	T	A	G	G	G
NH	C	G	G	T	G	G	G	G

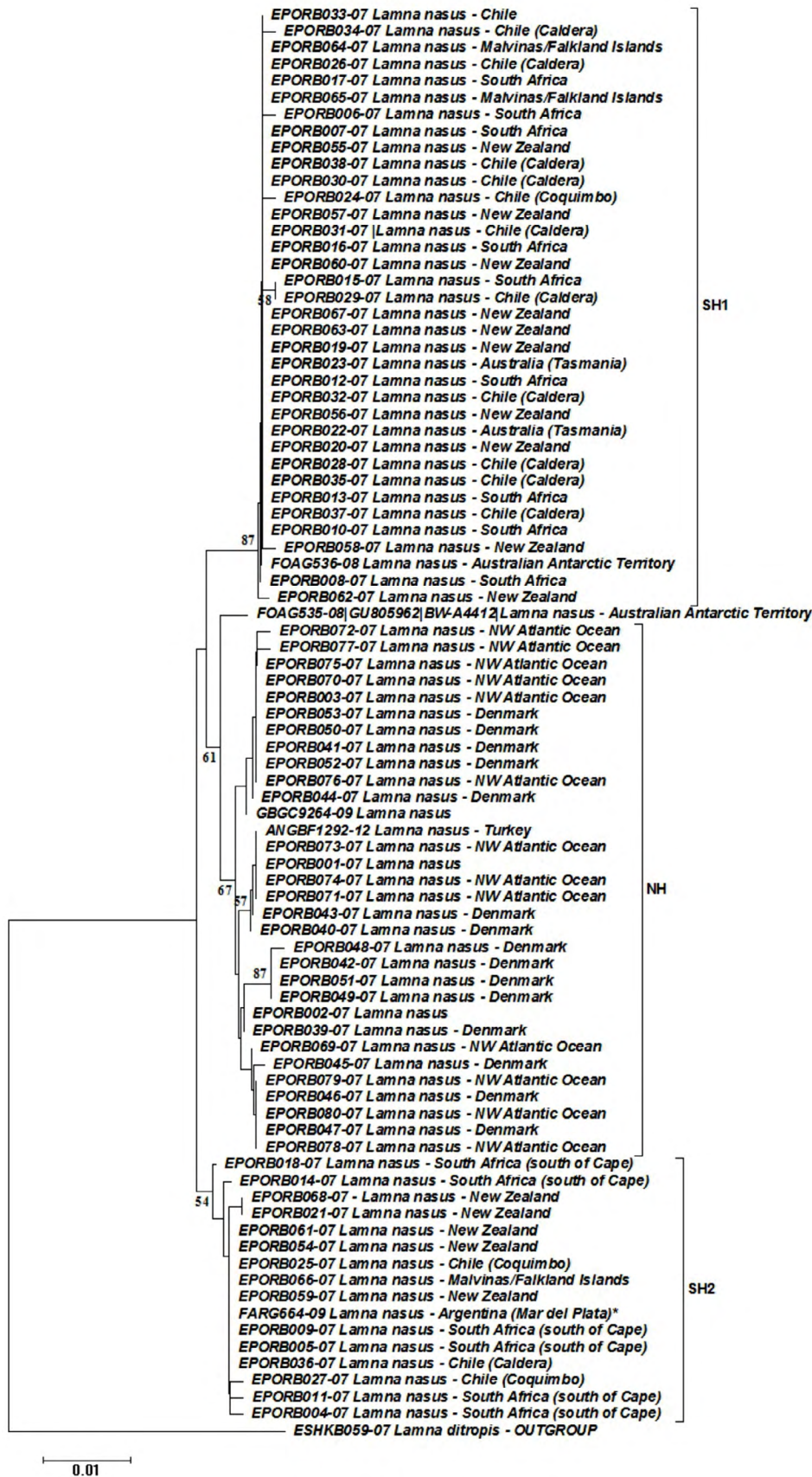


Figure 2. Neighbor-joining tree based on K2P distances of public barcode records of *Lamna nasus* from BOLD. Number at nodes represent bootstrap values, (only values greater than 50 are given). SH = southern hemisphere, NH = northern hemisphere.

also found far from land in ocean basins and occasionally inshore. It has centers of distribution in the temperate North Atlantic and in a circumglobal band of temperate water in the southern Atlantic, southern Indian, southern Pacific, and Antarctic Oceans [5]. The presence of this shark in shallow waters is rare. Previous records of *Lamna nasus* in Argentina, except that of Lucifora and Menni [6], are from open waters deeper than 90 m and comprise mature specimens (from 190 to 230 TL) [7-10]. The coastal record of Lucifora and Menni [6] was in summer and consisted of a juvenile female. According to Jensen *et al.* [11] male Porbeagles mature between 160 and 190 cm fork length (FL) (50% maturity length (L50) equals approximately 174 cm FL; 50% maturity age (A50) approximately 8 years), while females mature between 205 and 230 cm (L50 approximately 217 cm FL; A50 approximately 13 years). All offshore reports of *Lamna nasus* in Argentina correspond to mature specimens (from 190 to 230 cm TL). Porbeagles are a common catch in Uruguayan longline fisheries off the Río de la Plata [12].

The Porbeagle is included in appendix II of CITES and its status at this time is Vulnerable [13]. The species is caught in longline fisheries in both the North Atlantic and the Southern Hemisphere. In the Northwest Atlantic, the Porbeagle population has been depleted to about 10% of its original abundance [14]. Information on the status of the southern population is lacking; however, the apparent slower growth of southern Porbeagles as compared to the North Atlantic population suggests an even lower resiliency to fishing pressure than the northern population [15].

This report is a case study of the utility of DNA barcoding for specimen identification, but also an example of a phylogenetic application, in this case, the genetic separation of northern and southern hemisphere populations. It also exemplifies the role of molecular analysis in contemporary systematic studies. The library of DNA sequences is constantly growing, with data from all over the world, facilitating the identification of organisms when external morphology makes difficult a correct assignment to species (e.g. part of animals, certain stages of development), and also providing supplementary phylogenetic data and even accelerating the discovery of new species.

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Conflict of interest: Authors declare nothing to disclose.

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