Biol. Mar. Mediterr. (2025), 29 (1): 178-181 www.biologiamarinamediterranea.it

53° Congresso SIBM: Cetacei

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UNVEILING THE POPULATION STRUCTURE OF THE GENUS *KOGIA* (ODONTOCETES, CETACEA) IN THE CENTRAL EASTERN ATLANTIC OCEAN

INDAGINE SULLA STRUTTURA DI POPOLAZIONE DEL GENERE KOGIA *(ODONTOCETI, CETACEA) DELL'OCEANO ATLANTICO CENTRO-ORIENTALE*

Abstract – *Up to the last two decades, the species composing the family Kogiidae* – Kogia breviceps *and* Kogia sima *– were almost completely unknown. Thanks to the use of molecular techniques, few studies were able to reconstruct the population genetics of the two species worldwide. The populations inhabiting different ocean basins appear to be discrete. Yet, there is paucity of molecular data from specimens retrieved in the Central Eastern Atlantic and the Mediterranean Sea. In this study we analysed a sample set from the Canary Islands (n=17 Kb; n=1Ks), and a single specimen of Kogia sima stranded in Sicily (Italy) in 2005. All samples were sequenced for two mtDNA regions, consistently with previous molecular studies. Our preliminary data show that while* Kogia breviceps *samples from Canary Islands fall inside clades including also Western Atlantic sequences, for* Kogia sima*, the few show to be extremely distinct from Western Atlantic conspecifics.*

Keywords*: population, genetics,* Kogia*, mitochondrial DNA, species.*

Introduction - The Kogiidae family is composed by two different species of odontocetes: the pigmy sperm whale (*Kogia breviceps* Blainville, 1838) and the dwarf sperm whale (*Kogia sima* Owen, 1866), but until the early 2000s the available information was scarce.

The two species present many common features: their body shape is generally small, but robust, with a square head and left-displaced blowhole; both characterised by the spermaceti organ and "false gills" – their peculiar sign- posterior to the eyes.

Both species can be considered cosmopolites and observed near the continental shelf, eating mainly squids, cephalopods and small fishes (especially the dwarf sperm whale). They perform slow movements and low blows, while staying near the surface "logging" (the act of resting near the surface remaining still and facing all the same direction) or showing just the upper part of the head and a portion of the dorsal fin. If approached not too carefully, they tend to emit a red-brownish gastrointestinal fluid.

It isn't easy to distinguish between two species sharing so many anatomical traits and so elusive, therefore, nowadays we are considering mainly the dorsal fin (in the pygmy sperm whale it tends to be more prominent, while in *Kogia sima* is bigger and taller), and - even if both species don't present teeth in the upper jaw - the dental formula (between 20 and 32 in *Kogia breviceps* and between 14 and 26 in *Kogia sima*) is used

as a distinctive trait. Regarding dimensions, the Pygmy sperm whale is bigger (2.7-3.4m) then the Dwarf sperm whale (2.1-2.7m).
Due to the difficulties in observing these animals in the wild, most of the information we have come from stranded specimens.
These species are protected by the CITES under Annex II and by the Marine Mammal Act, since they are potentially threatened by marine debris, pollution, maritime traffic and sonars. In IUCN'S Red List, Pygmy sperm whale and Dwarf sperm whale are both considered as "Least Concern" globally, but the Pygmy sperm whale falls under "Data Deficient" if just Europe is taken into account. These data combined with the absence of other information about these species in the Central Eastern Atlantic and Mediterranean Sea confirm the gap of knowledge present in these areas.
With this study we aim to fill in these gaps in *Kogia*'s population structure and compare data with the ones already present in international molecular banks.

Materials and Methods - Nineteen tissue samples (18 skin samples coming from all the Canary Islands and 1 liver sample coming from Sicily, Italy) collected from animals stranded between 2002 and 2023 were analysed. Samples collected in the Canary Islands were preserved in ethanol 70%, the Italian one in DMSO (20% sat. in NaCl). First, all samples were extracted using a spinNAker Universal Genomic DNA mini kit (Euroclone) following manufacturer instructions. Before starting with PCR amplification, the primers found in literature (Chivers *et al*., 2006) targeting 2 specific regions of mitochondrial DNA, targetting the Control Region and Cytochrome B genes respectively, were redesigned in order to be more specific to the genus *Kogia*. PCR amplifications were performed in 25 µL solutions containing 5µL Reaction Buffer, 1 µL of Forward and Reverse Primer, 0.3 µL of Wonder Taq DNA polymerase, 1 µL of extracted DNA and 16.7 µL of Milli-Q H_20.
Tab. 1 shows the PCR conditions that were used: the *Kogia sima* tissue samples were highly degraded and performed less efficiently in the PCR amplification, we therefore selected less stringent PCR conditions.

Tab. 1 - PCR profiles used on *K. breviceps* and *K. sima* samples used for both markers.
Profili utilizzati per la PCR sui campioni di K. breviceps *e* K. sima *per entrambi i marcatori.*

Kogia breviceps		*Kogia sima*	
95° x 3'	denaturation	95° x 3'	denaturation
96°C x 30''		96°C x 30''	
52.5°C x 30''	annealing x40 cycles	47.5°C x 30''	annealing x40cycles
72°C x 30''		72°C x 45''	
72°C x 5'	extension	72°C x 5'	extension

Subsequently, all samples were purified using a QIAGEN DNA purification kit and sent for Sanger sequencing. For each individual/sample the two (CR and CytB) sequence fragments were merged in order to obtain a longer and thus more informative DNA sequence. The resulting 917bp sequence was used for phylogenetic reconstructions including existing North Atlantic *Kogia* spp. sequences.

Results - The sequences obtained were verified, aligned and compared with 22 (12 *Kogia breviceps* and 10 *Kogia sima*) entries already present on GenBank from three different areas of US East Coast (Chivers *et al*., 2006) defined as GOM (Gulf of Mexico), MAB (Mid-Atlantic Bight, from Massachusetts to North Carolina) and SAB (South-Atlantic Bight, from North Carolina to Upper Florida Keys). The complete list of the compared sequence and their origine is shown in Tab. 2 and Fig. 1.

Tab. 2 - Sample size and geographic origin of the 41 specimens analysed in this study.
Numero e provenienza geografica dei 41 campioni utilizzati in questo lavoro.

	CANARY ISLANDS	ITALY	WESTERN ATLANTIC
Kogia breviceps	17	0	12
Kogia sima	1	1	10

Fig. 1 - Map of sample's location in West Atlantic Ocean and Mediterranean Sea.
Mappa della localizzazione dei campioni provenienti dall'Atlantico Occidentale e dal Mar Mediterraneo.

Figs. 2 and 3 (a, b) show the maximum-likelihood trees obtained by comparing the combined sequences of the 27 *Kogia breviceps* and the 12 *Kogia sima* sequences analysed in this study, respectively. As for *Kogia breviceps*, the 12 samples collected along the US coast appear to be well distinct and creating different clades; Canary Islands samples clustered in different clades together with their Western Atlantic conspecifics. Two haplotypes coming from Canary Islands' samples cluster differently, but for both individuals the sequence presented many ambiguities, and thus they were removed from the tree shown in Fig. 2.

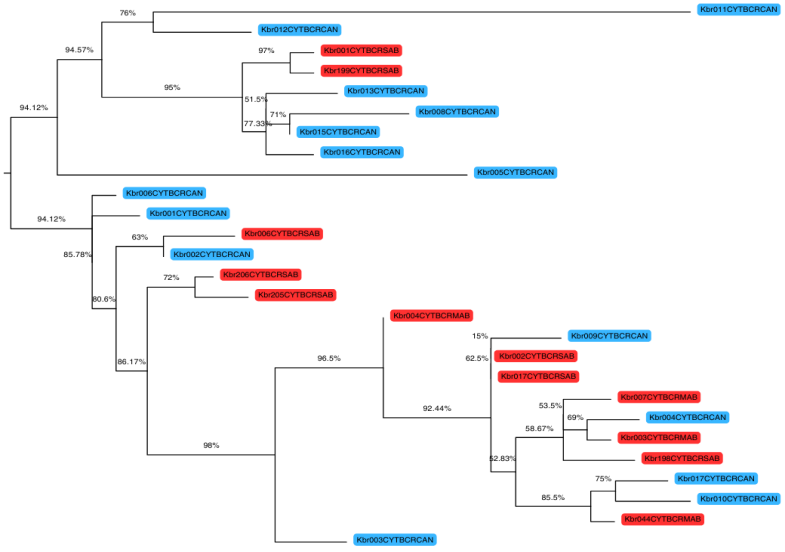

Fig. 2 –Maximum likelihood tree with 100 bootstrap replicates reconstructed with our data: the red circles signal US samples, blue circles Canary Islands samples.
Albero filogenetico con il metodo Maximum likelihood calcolato con 100 bootstrap ricostruito con i nostri dati: i campioni provenienti dagli US sono cerchiati in rosso, i campioni dalle Isole Canarie in azzurro.

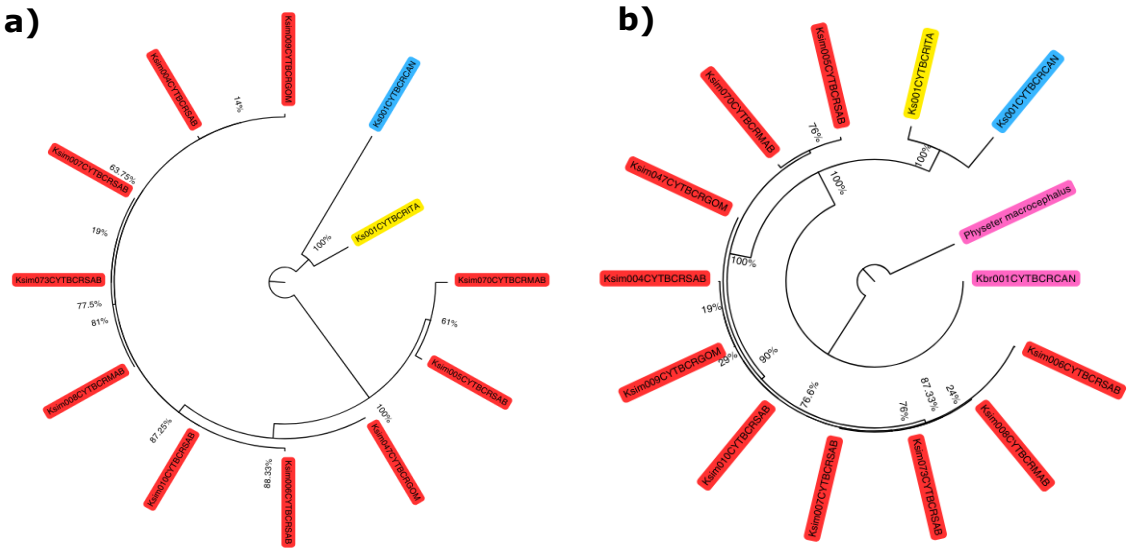

Fig. 3 - **a)** Maximum likelihood tree with 100 bootstrap replicates reconstructed with our data: red circles indicate US samples, blue circle Canary Islands sample, yellow circle Italian sample. **b)** ML tree with 100 bootstrap replicates, adding one random *Kogia breviceps* sample and Sperm whale (*Physeter macrocephalus*) mitochondrial DNA as outgroups (both in pink).
a) *Albero filogenetico con il metodo Maximum likelihood calcolato con 100 repliche bootstrap ricostruito con i nostri dati: i campioni provenienti dagli US sono cerchiati in rosso, quello dalle Isole Canarie in blu e il campione dall'Italia in azzurro.* **b)** *Albero filogenetico con il metodo Maximum likelihood calcolato con 100 repliche bootstrap, con in aggiunta un campione casuale di* Kogia breviceps *ed il DNA mitocondriale di un capodoglio (*Physeter macrocephalus*) come outgroup (entrambi in rosa in figura).*

Kogia sima's tree presented a different structure (Fig. 3a): Western Atlantic samples appear to be well distinct in comparison to those coming from Eastern Atlantic (Canary Island) and Mediterranean (Italy). Interestingly, reconstructing the same ML tree including also one *Kogia breviceps*' sequence from the Canarian sample set and the mitochondrial DNA of a Sperm whale (*Physeter macrocephalus*, Linnaeus, 1758) as an outgroup, the "eastern" sequences seem to be more similar to the pigmy sperm whale (*Kogia breviceps*) than to their western conspecific (Fig. 3b).

Conclusions - Looking at *Kogia breviceps'* phylogenetic tree, we could assume the presence of an occasional historical and on-going genetic flow in local subpopulation in the North-Atlantic (Chives *et al.,* 2006; Viricel *et al*., 2012), while the situation differs for *Kogia sima*, where we can observe a total isolation of the Eastern population from all the remaining Atlantic samples analysed. However, *Kogia sima*'s sample set considered for this preliminary phylogenetic analysis is too small to give us a final reliable conclusion. New samples of the same species coming from other areas of interest of the Eastern Atlantic Ocean (Portugal, France and United Kingdom) are going to be added into this project to get a clearer view on Kogia's population structure in Central Eastern Atlantic Ocean.

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