

# Confirmation of pygmy sperm whale, *Kogia breviceps* from Palk Bay, India through Cytochrome Oxidase<sup>1</sup> (COI) sequence

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## ABSTRACT

The identity of a stranded cetacean from the Palk Bay has been confirmed as a pygmy sperm whale (*Kogia breviceps*) by the partial sequencing of mitochondrial gene cytochrome oxidase subunit-I (COI). The specimen was unambiguously discriminated from the COI sequence of *Kogia sima* by matching exactly with the sequence of *K. breviceps*. Stranding events of these pygmy whales are considered to be uncommon. The sequence developed for *K. breviceps* is the first of its kind from Indian waters.

**KEYWORDS:** COI; MARINE MAMMAL STRANDING; PALK BAY; PYGMY SPERM WHALE

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## INTRODUCTION

Pygmy sperm whale (*Kogia breviceps*, de Blainville 1838) and dwarf sperm whale (*Kogia sima*, Owen 1866) are the only extant members of the family Kogiidae (Caldwell and Caldwell, 1989; McAlpine, 2009; Waring *et al.*, 2004; Jefferson *et al.*, 2015; Collareta *et al.*, 2019). They inhabit offshore tropical, subtropical and temperate waters. Whereas *K. breviceps* is frequently reported from both tropical and warm temperate waters, *K. sima* generally restricts its presence to tropical or subtropical waters (Caldwell and Caldwell, 1989; McAlpine, 2009; Jefferson *et al.*, 2015). Stranding reports and sighting surveys are important sources of information regarding the distribution and biology of uncommonly sighted *Kogia* species (Beasley *et al.*, 2013; Bonato *et al.*, 2016). Due to the morphological similarities, it is extremely difficult to differentiate the two species during sighting events (Willis and Baird, 1998; Baird, 2005). The distribution of *K. breviceps* from Indian waters is poorly understood (Molur *et al.*, 1998; Kumaran, 2002; Jeyabaskaran and Vivekanandan, 2013) due to the challenges in field identification (Chantrapornsyl *et al.*, 1991). Most of the early reports are in the form of historical notes (Jerdon, 1867; De Silva, 1987) and are based on morphological examinations during stranding, sightings and fishery interactions which often compromise accurate species identification (Kumaran, 2002). The morphological similarity between pygmy and dwarf sperm whales often results in the misidentification of the species in the field. Hence, molecular techniques and the examination of body parts such as skulls are valuable tools in identifying the closely related taxon (Chivers *et al.*, 2005; Jefferson *et al.*, 2015).

A total of 26 marine mammal species are documented from Indian seas (Kamalakaran and Nameer, 2019) and information on cetacean strandings has been recorded since 1852 along the coastal waters of India. The present paper confirms the identification of a stranded specimen of pygmy sperm whale *K. breviceps* by partial sequencing of its mitochondrial gene COI, which is a first of its kind attempt from Indian waters. In addition, stranding events of *Kogia* whales for the last ninety-three years (1926 to 2019) from Indian waters are presented.

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## MATERIALS AND METHODS

The carcass of a freshly stranded *K. breviceps* was found in Sambai Village, Ramanathapuram District (9° 41' 47.137"N, 78° 58' 25.341"E) in the Palk Bay region on 25 May 2019 (Fig. 1). The photographs and morphometric measurements of the specimen were compared to identify the species following the identification keys of Jefferson *et al.* (2015). The data collection protocol for stranded marine mammals was followed (Geraci, 2005). A portion of muscle was dissected from fin, fluke and dorsal body and preserved in 95% ethanol for molecular analysis.

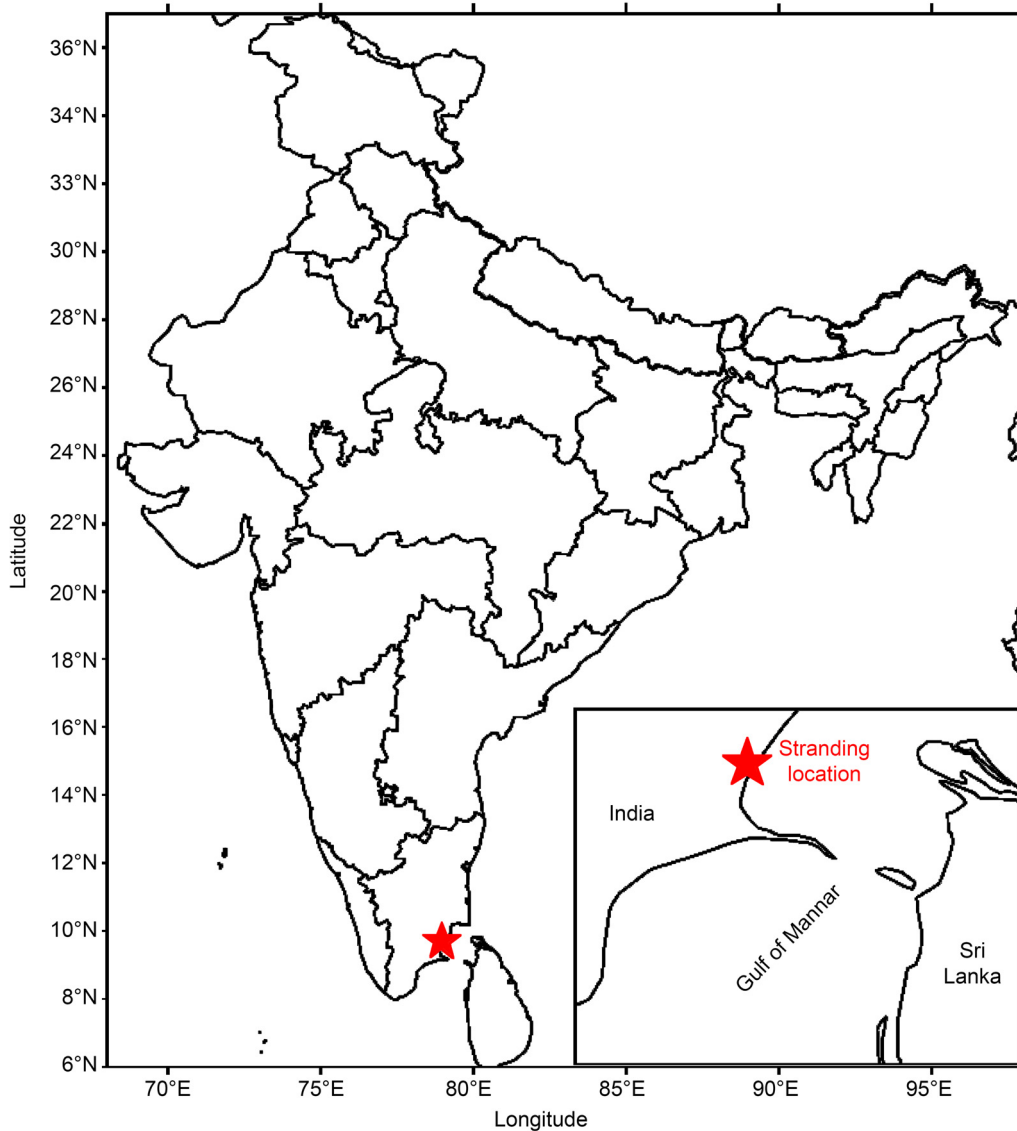


Fig. 1. Map of Bay of Bengal showing the stranding location (red star) of *Kogia breviceps*, de Blainville 1838.

The genomic DNA was extracted from the tissue by using the DNeasy blood and tissue genomic kit (Qiagen) following the manufacturer's protocols. A fragment of the mitochondrial gene cytochrome oxidase subunit I (COI) was amplified using universal LCO1ea (5'-tcggccattttacctatgttcata-3') and HBCUem (5'-ggtggccgaagaatcagaata-3') (Alfonsi *et al.*, 2013). PCR reactions were performed in 25µl, containing 50ng of DNA, 2.5mM MgCl<sub>2</sub> and 0.3mM of each primer. Amplification reaction consisted of an initial denaturation step at 95°C for 10 mins, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 53°C for 30 seconds and extension at 72°C for 1 min. The final extension was at 72°C for 10 mins. PCR products were subject to electrophoresis and visualised on 1.2% agarose gels containing ethidium bromide.

Sequences of COI gene of *K. breviceps* (EU496307.1, KY542084.1, AJ554055.1) and its closely related *K. sima* (EU496308) were extracted from the GenBank database and aligned using Bio-Edit sequence alignment editor version 5.0.9 (Hall, 1999) for genetic analysis. Maximum Likelihood Phylogenetic analyses were performed using MEGA version 7 (Kumar *et al.*, 2016). Kimura 2-parameter divergences were calculated with Maximum Parsimony criterion using the MEGA version 10 (Kumar *et al.*, 2018). Branch support was assessed using 1,000 bootstrap replicates, following Felsenstein (1985). To understand the stranding events of the genus *Kogia* along the Gulf of Mannar and in Palk Bay, both published and unpublished information for the period between 1926 and 2019 were compiled (Table 1).

Table 1  
Stranding records of pygmy sperm whale *Kogia breviceps* (de Blainville, 1838) from Indian waters.

S. No	Period	Details	References
1.	Feb. 1925	Trivandrum coast, Kerala. 2 Nos (1 adult – 10 feet and 1 calf)	Pillay (1926)
2.	May 1985–Dec. 1988	Gulf of Mannar (west coast of Sri Lanka, 1 sighted)	Ilangakoon (1997)
3.	1987	Waltair coast, Andhra (1 female specimen reported)	Mohan and Mohan (1987)
4.	08.07.1988	Port Blair, Andaman (2 individuals, 1 adult and one calf)	Chantrapornsyl <i>et al.</i> (1991)
5.	05.03.2010	Cuddalore (Silver Beach), Tamil Nadu	Ravi and Murugan (2010)

## RESULTS AND DISCUSSION

The morphological characteristics (Table 2) of the stranded specimen matched well with that of *K. breviceps* (Fig. 2). The species is distinguished by its ‘porpoise-like’ head appearance, blunt and large compared to its body size, and its small but strongly falcate dorsal fin, well back on the body (Wall, 1851; Ross, 1979; Wynne and Schwartz, 1999). The dorsal fin of *K. breviceps* is small in size compared with that of *K. sima* (Handley, 1966; Roest, 1970; Bloodworth and Odell, 2008). Unlike *K. sima*, the present specimen was without maxillary teeth and had more mandibular teeth. However, due to ambiguous external morphology, identification remains difficult especially for younger animals. The Maximum Likelihood phylogenetic tree reconstruction based on the CO1 sequences places *K. breviceps* as a separate sister clade of *K. sima* with high bootstrap value, confirming the whale specimen stranded at Sambai village of Palk Bay on 25 May 2019 as *K. breviceps*. The inter species COI

Table 2  
Morphometric measurements of the present stranded specimen (*K. breviceps*) at Palk Bay, India.

Measurements	GoM (cm) present study	Ravi and Murugan (2010)
Total body length	315	235
Approx. body weight	350	300
Max. flipper length	53	25
Max. flipper width	17	–
Fluke length	20	35
Fluke width	69	–
Standard length	210	200
Head to dorsal fin base	166	128
Head to flipper base	72	–
Head to eye	50	25
Eye diameter	38	8
Flipper base	20	–
Dorsal fin base	34	–
Dorsal fin height	28	25
Fluke side length	60	–
Peduncle girth	45	–
Anal pore to peduncle	60	50
Lower jaw length	24	–
Gap	44	–
Snout height	28	20
Max. body girth	177	65
Blow hole to head	40	22



Fig. 2. Stranded specimen of *Kogia breviceps* (a) Fresh specimen washed ashore, (b) head with injuries due to probable ship or fishing vessel strike, (c) ventral side with many injuries probably due to entanglement in fishing rope/net, (d) Fishing net entangled on the fluke.

sequences based K2P genetic distance between *K. breviceps* and *K. sima* is 10%. Although several publications (e.g. Jerdon, 1867; Molur *et al.*, 1998) indicated the presence of *Kogia* species from Indian waters, much doubt has existed about their identification (Moses, 1947; Pillay, 1926; De Silva, 1987) with very few studies conducted on molecular sequences. Our results based on a partial mitochondrial COI sequence along with classical taxonomy provide a reliable species identification tool for pygmy sperm whale.

There have been no visual sightings of live pygmy sperm whales reported from Indian waters to date. Examination of the carcass of the stranded whale revealed that it was probably fresh as the body was undecomposed and organs were intact. Presence of injuries on the ventral side of the body might have been caused by fishing vessels or gear entanglement. There were no unusual weather or oceanographic events observed in the Palk Bay region during the period of the stranding event. From historic cetacean stranding data, it was observed that there were no earlier reports available about *K. breviceps* stranding in Palk Bay.

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