



Report on genetic identification of unknown whale tissue sample T15991 MA Millar 1/11/2023

The tissue and blubber sample (T15991), suspected to be a pygmy sperm whale (*Kogia breviceps*) or dwarf sperm whale (*Kogia sima*) was collected from circus beach Walpole (20/9/2023) and received from Kelly Waples 23/10/2023.

DNA was extracted from the tissue sample using a TNES extraction method. The Displacement Loop primers Wada-D-Loop Forward/Wada-D-Loop Reverse (Region 1) were used to amplify non-coding mitochondrial D-Loop DNA using a Shaw hot start PCR program. This region produced a strong single PCR product. The PCR product was sequenced using the same Forward and Reverse primers at the Western Australian State Agricultural Biotechnology Centre (SABC) Murdoch University. Sequence data was edited, Forward and Reverse sequences aligned and a consensus sequences obtained using the Geneious sequence alignment editor.

The sequenced region produced a consensus alignment sequence read of 963bp. This sequence region was queried using the blastn suite (megablast) for highly similar sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The queried sequence was significantly aligned to 102 sequences of *Kogia*, a genus of toothed whales. There were 66 significant alignments to the pygmy sperm whale *Kogia breviceps*, and 36 significant alignments with *Kogia sima* (Fig 1), the dwarf sperm whale, which was synonymous with the pygmy sperm whale until 1998. Of the sequences producing significant alignment, the top 34 with the highest Maximum Scores, highest Total Scores, highest Query Coverages, lowest E values, and highest Percent Identities were from *K. breviceps* (Fig 2).

Organism	Blast Name	Score	Number of Hits	Description
<u>Kogia</u>	whales & dolphins		<u>102</u>	
 Kogia breviceps 	whales & dolphins	1681	<u>66</u>	Kogia breviceps hits
. <u>Kogia sima</u>	whales & dolphins	1443	<u>36</u>	Kogia sima hits

Figure 1. Number of significant alignments (Hits) for tissue sample T15991.







Figure 2. Sequences producing significant alignments for tissue sample T15991.





Results were also visualised as a Fast Minimum Evolution distance tree (Fig 3).

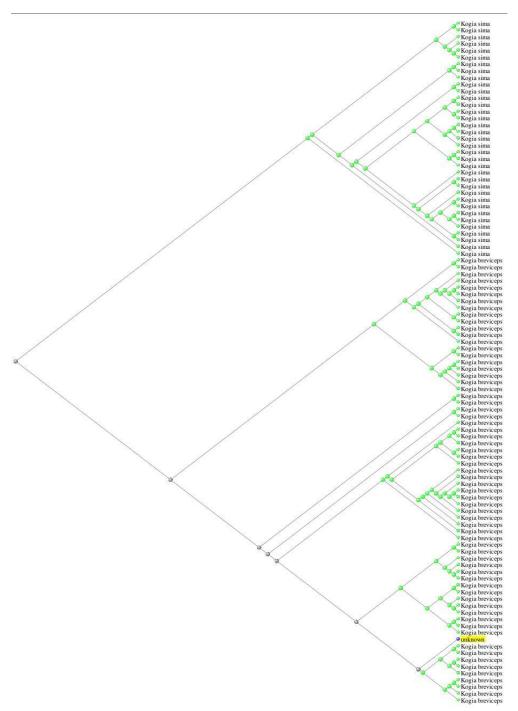


Figure 3. Yellow indicates the queried (sample) region. Green are GenBank sequence accessions with significant sequence alignment.

Conclusion





T15991 has the greatest sequence alignment with and is identified as a pygmy sperm whale *K. breviceps*.