GOVERNMENT OF
WESTERN AUSTRALIA

Department of **Biodiversity,**
Conservation and Attractions

Biodiversity and
Conservation Science

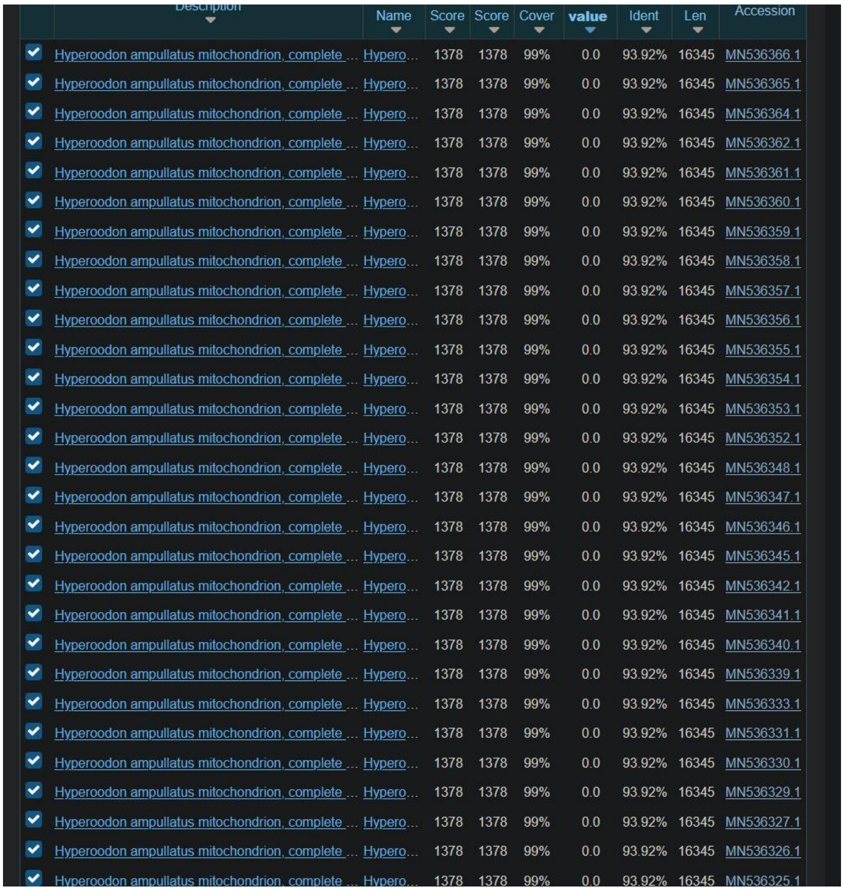
Report on genetic identification of Whale tissue sample T16874 (T00298).
MA Millar, K Ottewell, 18/06/2024

The sample from a suspected Baleen whale carcass was collected from Ningaloo Marine Park, at Hunter's Beach on the west side of northwest Cape, in the Jurabi coastal park and received from Kelly Waples May 2024.

DNA was extracted from the tissue sample using a TNES extraction method. The Displacement D-Loop primers Wada-D-Loop Forward/Wada-D-Loop Reverse were used to PCR non-coding mitochondrial D-Loop DNA using a Shaw hot start PCR program. PCR products were sequenced twice using the same Forward and Reverse primers at different concentrations, at the Western Australian State Agricultural Biotechnology Centre (SABC), Murdoch University, and sequence data for each region was aligned and edited using the Geneious Sequence Alignment Editor.

The sequenced region produced two (*a* and *b*) contiguous alignment sequence reads of 916bp and 894bp respectively. Both sequence reads were queried using the blastn suite (megablast) for highly similar sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and gave identical results. Results for the longer read (*a*) are shown.

Database matching – Genbank*:* Of the top 100 sequences producing significant alignments 100% were from *Hyperoodon ampullatus*, the Northern Bottlenose Whale (Fig 1). Sequence coverage was high with many whole genome accessions of *H. ampullatus* present in the GenBank database, E values were 0 and maximum identity was 93.92%.



	Description	Name	Score	Score	Cover	value	Ident	Len	Accession
☑	Hyperoodon ampullatus mitochondrion, complete ...	Hypero...	1378	1378	99%	0.0	93.92%	16345	MN536366.1
☑	Hyperoodon ampullatus mitochondrion, complete ...	Hypero...	1378	1378	99%	0.0	93.92%	16345	MN536365.1
☑	Hyperoodon ampullatus mitochondrion, complete ...	Hypero...	1378	1378	99%	0.0	93.92%	16345	MN536364.1
☑	Hyperoodon ampullatus mitochondrion, complete ...	Hypero...	1378	1378	99%	0.0	93.92%	16345	MN536362.1
☑	Hyperoodon ampullatus mitochondrion, complete ...	Hypero...	1378	1378	99%	0.0	93.92%	16345	MN536361.1
☑	Hyperoodon ampullatus mitochondrion, complete ...	Hypero...	1378	1378	99%	0.0	93.92%	16345	MN536360.1
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☑	Hyperoodon ampullatus mitochondrion, complete ...	Hypero...	1378	1378	99%	0.0	93.92%	16345	MN536354.1
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☑	Hyperoodon ampullatus mitochondrion, complete ...	Hypero...	1378	1378	99%	0.0	93.92%	16345	MN536325.1

Figure 1. Sequences producing significant alignments for tissue sample T16874.

The queried sequence was significantly aligned to 100 accessions of *H. ampullatus* and no others (Fig 2.).

100 sequences selected

Organism	Blast Name	Score	Number of Hits	Description
Hyperoodon ampullatus	whales & dolphins	1378	100	Hyperoodon ampullatus hits

Figure 2. Number of significant alignments (Hits) for tissue sample T16874.

T16874 has the greatest sequence alignment on GenBank with *H. ampullatus*, the Northern Bottlenose Whale. This species is endemic to the North Atlantic Ocean however, with populations found only in the deep cold subarctic waters of the Davis Strait, the Labrador Sea, the Greenland Sea and the Barents Sea, ranging as far south as Newfoundland and Nova Scotia (Fig 3). The Northern Bottlenose Whale is a species of beaked whale in the ziphiid family, being one of only two members of the genus Hyperoodon.

Figure 3. *Range of H. ampullatus*. IUCN redlist.org.

The sister species, *Hyperoodon planiforms*, the Southern Bottlenose Whale, lives in Antarctic waters of over 1000m and has a circumpolar distribution in the Southern Ocean. It is found from the Antarctic coast to the tip of South Africa, the North Island of New Zealand and off the southern parts of Brazil (Fig4).

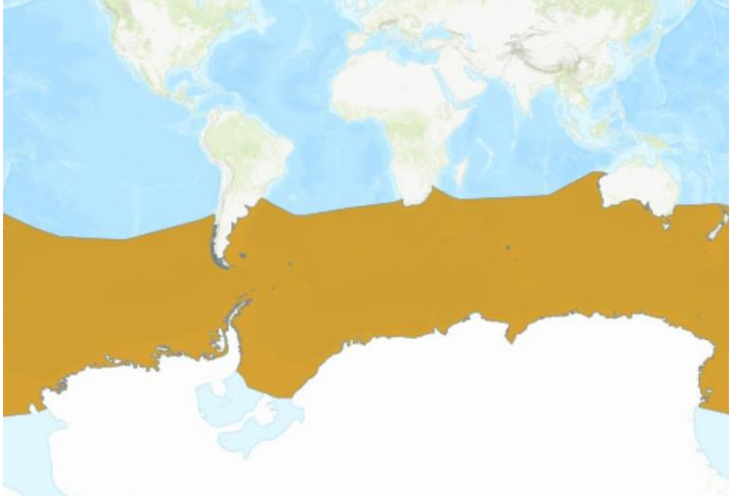
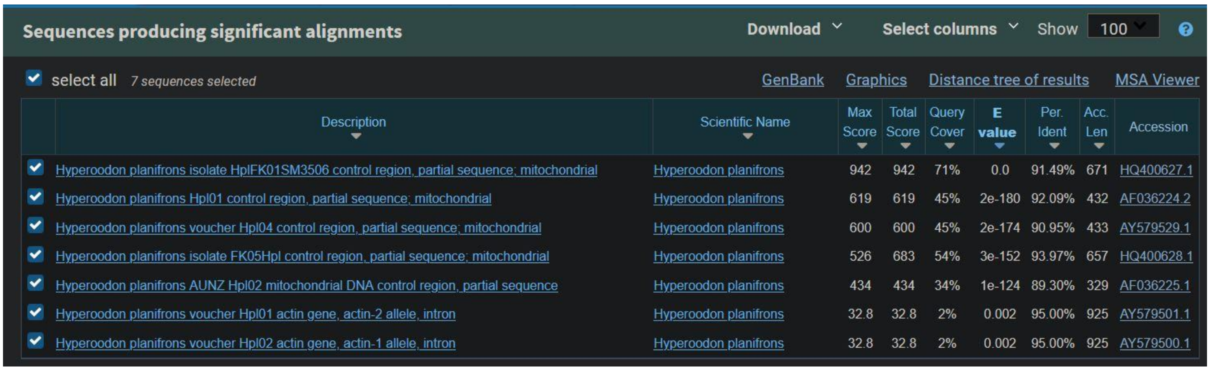


Figure 4. Range of *H. planiforms*. IUCN redlist.org.

A megablast of sequence *a*, for highly similar sequences limited to *H. planiforms* produced seven significant alignments (Fig 5). E values for sequence *a* and the seven *H. planiforms* accessions ranged from 0.002 to 0, with identity ranging from 89.30% to 95.00%. These high E values and lower percent identity values are reflective of the degree of sequence coverage with the queried sequence *a* and *H. planiforms* accessions present in the GenBank database. Sequence *a* did have an E value of 0 with the largest accession of *H. planiforms* available on GenBank (71% coverage with sequence *a*). The E value is the number of hits one can "expect" to see by chance e.g. an E value of 0 assigned to an alignment means that in a database of the same size one expects to see 0 matches with a similar score, or higher, simply by chance. Percentage coverage was low however, as *H. planiforms* accessions represent only partial sequences of mitochondrial control regions or the actin gene.



Sequences producing significant alignments

☑ select all *7 sequences selected*

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Hyperoodon planifrons isolate HplFK01SM3506 control region, partial sequence; mitochondrial	Hyperoodon planifrons	942	942	71%	0.0	91.49%	671	HQ400627.1
☑	Hyperoodon planifrons Hpl01 control region, partial sequence; mitochondrial	Hyperoodon planifrons	619	619	45%	2e-180	92.09%	432	AF036224.2
☑	Hyperoodon planifrons voucher Hpl04 control region, partial sequence; mitochondrial	Hyperoodon planifrons	600	600	45%	2e-174	90.95%	433	AY579529.1
☑	Hyperoodon planifrons isolate FK05Hpl control region, partial sequence; mitochondrial	Hyperoodon planifrons	526	683	54%	3e-152	93.97%	657	HQ400628.1
☑	Hyperoodon planifrons AUNZ Hpl02 mitochondrial DNA control region, partial sequence	Hyperoodon planifrons	434	434	34%	1e-124	89.30%	329	AF036225.1
☑	Hyperoodon planifrons voucher Hpl01 actin gene, actin-2 allele, intron	Hyperoodon planifrons	32.8	32.8	2%	0.002	95.00%	925	AY579501.1
☑	Hyperoodon planifrons voucher Hpl02 actin gene, actin-1 allele, intron	Hyperoodon planifrons	32.8	32.8	2%	0.002	95.00%	925	AY579500.1

Figure 5. Number of significant alignments (Hits) with *Hyperoodon planiforms* for tissue sample T16874.

There are also further species of beaked whales in the same clade as *H. planifrons*; including a number of *Mesoplodon* species and *Tasmacetus shepherdi*. We conducted a number of further megablast searches for the query sequence *a* on Genbank, with filters set to confine searches to accessions from beaked whale species with a distribution potentially covering Exmouth, Western Australia. Results (not shown) were inconclusive, with E values equalling 0 for many accessions from most closely related species. The degree of coverage is taken into consideration in the calculation of the E value, and accession lengths available on Genbank for closely related species additional to *H. planiforms* were limited (i.e. 259 bp for *T. shepherdi*), biasing results. Divergence among the beaked whales is also known to be shallow. Ultimately, we felt the results provided by Genbank were unclear.

Database matching – Witness for the Whales: Due to the limitations of the available Genbank accessions, we employed Witness for the Whales https://dna-surveillance.auckland.ac.nz/page/whales/title, a free online service for the phylogenetic identification of cetacean species, to generate and align whale sequences using the query sequence *a* and derive phylogenetic relationships. This database uses a highly curated set of reference nucleotide sequences, obtained from species specialists, from a comprehensive and representative range of cetaceans.

We used the Zippiidae reference sequence dataset from Witness for the Whales to perform a cluster analysis comparison of the mitochondrial D-Loop DNA sequence *a*. The resulting dendrogram shows the position of the queried sequence, clustering with *T. shepherdi*, Shepherd's Beaked Whale, and provides a clear species identification (Fig6.).

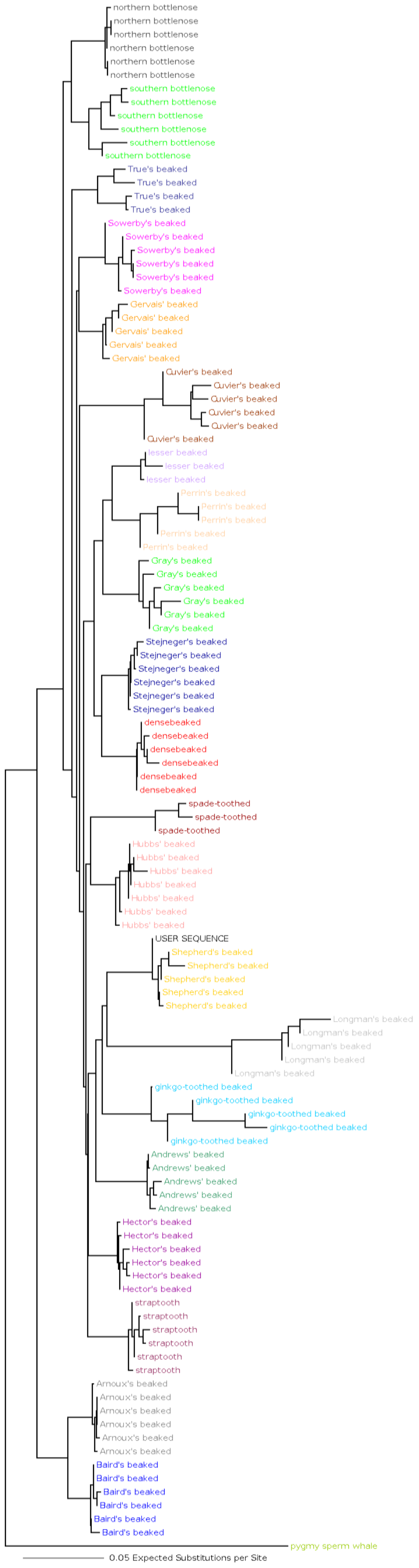


Figure 6. Dendogram showing the position of the queried sequence *a* of sample T16874 ("USER SEQUENCE") in relation to other Ziiphidae in the Witness for the Whales database based on mtDNA control region sequences.

Taken together, these results suggest it most likely that sample T16874 was from *T. shepherdi*, Shepherd's Beaked Whale. This is a little-known known cetacean. Although it has a widely published distribution in the Southern mid-latitudes of around 33°-50° S (Fig 7.), this is based only on two at sea sightings and stranding locations. The northern most recording of the species from a stranding has more recently been recorded from Shark Bay, Western Australia (~26° S) in 2008. It has been supposed that the species undertakes northward movement in summer approaching continental seas, so a distribution encompassing Exmouth (~22° S) is not unreasonable. This would represent a significant range expansion for the species.

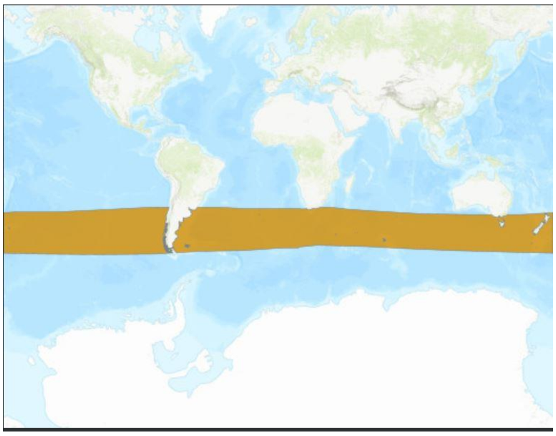


Figure 7. Range of *T. shepherdi*. IUCN redlist.org.

Conclusion

Given the limitations to the megablast results from Genbank and the clear phylogeny obtained from the Witness for the Whales database, as well as the potential geographic distribution of *T. shepherdi*, it seems most likely that sample T16874 was from a *T. shepherdi*, Shepherd's Beaked Whale.