GOVERNMENT OF
WESTERN AUSTRALIA

Department of **Biodiversity, Conservation and Attractions**

Biodiversity and
Conservation Science

# Report on genetic identification of unknown whale tissue sample T15104

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The sample (T15104) suspected to be a short-finned pilot whale (*Globicephala*) or pygmy sperm whale (*Kogia*) was collected from Eighty Mile beach and received from Holly Raudino 17/04/2023.

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) and Delph-D-Loop Forward /Delph-H00034 Reverse (Region 2) were used to PCR non-coding mitochondrial D-Loop DNA using a Shaw hot start PCR program. Region 1 produced a strong single PCR product while Region 2 failed to amplify. 2 x PCR products of Region 1 were sequenced using the same Forward and Reverse primers at the Western Australian State Agricultural Biotechnology Centre (SABC) Murdoch University. Sequence data was edited and aligned for both Forward and Reverse sequences of Region 1 and consensus sequences obtained, with a final alignment and a contiguous consensus of both Forward and Reverse sequences combined obtained using BioEdit Sequence Alignment Editor.

Region 1 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).

Region 1 was significantly aligned to 106 sequences of *Kogia*, a genus of toothed whales. There were 100 significant alignments to the pygmy sperm whale *Kogia breviceps*, and 6 significant alignments with *Kogia sima* (Fig 1), the dwarf sperm whale, which was synonymous with the pygmy sperm whale until 1998. Alignments with the highest Maximum Scores, highest Total Scores, highest Query Coverages, lowest E values, and highest Percent Identities were all with sequences from *K. breviceps* or *K. sima* (Fig 2).

| Organism | Blast Name | Score | Number of Hits | Description |
|---|---|---|---|---|
| Kogia | whales & dolphins | | 106 | |
| . Kogia breviceps | whales & dolphins | 1652 | 100 | Kogia breviceps hits |
| . Kogia sima | whales & dolphins | 1424 | 6 | Kogia sima hits |

Figure 1. Number of significant alignments (Hits) for Region 1 of tissue sample T15104.

| | Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|---|
| ☑ | Kogia breviceps isolate 2014072602 tRNA-Pr… | Kogia br… | 1652 | 1652 | 98% | 0.0 | 97.90% | 990 | KY542109.1 |
| ☑ | Kogia breviceps mitochondrion, complete gen… | Kogia br… | 1628 | 1628 | 99% | 0.0 | 97.09% | 16406 | NC_005272. |
| ☑ | Kogia breviceps isolate 20120412 tRNA-Pro … | Kogia br… | 1607 | 1607 | 96% | 0.0 | 97.84% | 941 | KY542108.1 |
| ☑ | K.breviceps mitochondrial DNA control region | Kogia br… | 1591 | 1591 | 97% | 0.0 | 97.03% | 940 | X72201.1 |
| ☑ | Kogia sima mitochondrion, complete genome | Kogia si… | 1424 | 1424 | 99% | 0.0 | 93.28% | 16403 | NC_041300. |
| ☑ | Kogia breviceps mitochondrial DNA, D-loop,… | Kogia br… | 876 | 876 | 51% | 0.0 | 98.20% | 500 | AB571996.1 |
| ☑ | Kogia breviceps mitochondrial DNA, D-loop,… | Kogia br… | 859 | 859 | 51% | 0.0 | 97.60% | 499 | AB571995.1 |
| ☑ | Kogia breviceps haplotype Kb48 tRNA-Thr ge… | Kogia br… | 800 | 800 | 46% | 0.0 | 99.10% | 519 | JX403748.1 |
| ☑ | Kogia breviceps isolate KI024_dlp1.5 D-loop,… | Kogia br… | 798 | 798 | 50% | 0.0 | 95.88% | 485 | KC540696.1 |
| ☑ | Kogia breviceps haplotype Kb51 tRNA-Thr ge… | Kogia br… | 795 | 795 | 46% | 0.0 | 99.07% | 519 | JX403758.1 |
| ☑ | Kogia breviceps voucher IBRC 041801 tRNA-… | Kogia br… | 789 | 789 | 46% | 0.0 | 98.65% | 472 | KY983292.1 |
| ☑ | Kogia breviceps haplotype Kb63 tRNA-Thr ge… | Kogia br… | 789 | 789 | 46% | 0.0 | 98.65% | 519 | JX403775.1 |
| ☑ | Kogia breviceps haplotype Kb102HPL tRNA-… | Kogia br… | 785 | 785 | 46% | 0.0 | 98.42% | 519 | JX403752.1 |
| ☑ | Kogia breviceps haplotype Kb56 tRNA-Thr ge… | Kogia br… | 784 | 784 | 46% | 0.0 | 98.42% | 520 | JX403766.1 |
| ☑ | Kogia breviceps haplotype Kb33 tRNA-Thr ge… | Kogia br… | 784 | 784 | 46% | 0.0 | 98.42% | 519 | JX403745.1 |
| ☑ | Kogia breviceps haplotype Kb46 tRNA-Thr ge… | Kogia br… | 784 | 784 | 46% | 0.0 | 98.42% | 519 | JX403739.1 |
| ☑ | Kogia breviceps haplotype Kb57 tRNA-Thr ge… | Kogia br… | 778 | 778 | 46% | 0.0 | 98.19% | 519 | JX403769.1 |
| ☑ | Kogia breviceps haplotype Kb30 tRNA-Thr ge… | Kogia br… | 778 | 778 | 46% | 0.0 | 98.19% | 519 | JX403736.1 |
| ☑ | Kogia breviceps haplotype Kb65 tRNA-Thr ge… | Kogia br… | 773 | 773 | 46% | 0.0 | 97.97% | 519 | JX403777.1 |
| ☑ | Kogia breviceps haplotype Kb52 tRNA-Thr ge… | Kogia br… | 773 | 773 | 46% | 0.0 | 97.97% | 519 | JX403759.1 |
| ☑ | Kogia breviceps haplotype Kb32 tRNA-Thr ge… | Kogia br… | 773 | 773 | 46% | 0.0 | 97.97% | 519 | JX403747.1 |
| ☑ | Kogia breviceps haplotype Kb42 tRNA-Thr ge… | Kogia br… | 773 | 773 | 46% | 0.0 | 97.97% | 519 | JX403742.1 |
| ☑ | Kogia breviceps haplotype Kb43 tRNA-Thr ge… | Kogia br… | 767 | 767 | 46% | 0.0 | 97.74% | 519 | JX403760.1 |
| ☑ | Kogia breviceps haplotype Kb49 tRNA-Thr ge… | Kogia br… | 767 | 767 | 46% | 0.0 | 97.74% | 519 | JX403751.1 |
| ☑ | Kogia breviceps haplotype Kb100HPL tRNA-… | Kogia br… | 767 | 767 | 46% | 0.0 | 97.74% | 517 | JX403741.1 |
| ☑ | Kogia breviceps clone END2046 tRNA-Pro g… | Kogia br… | 761 | 761 | 46% | 0.0 | 96.90% | 452 | MZ401338.1 |
| ☑ | Kogia breviceps haplotype Kb55 tRNA-Thr ge… | Kogia br… | 761 | 761 | 46% | 0.0 | 97.52% | 520 | JX403764.1 |
| ☑ | Kogia breviceps haplotype Kb47 tRNA-Thr ge… | Kogia br… | 761 | 761 | 46% | 0.0 | 97.52% | 519 | JX403746.1 |
| ☑ | Kogia breviceps haplotype Kb31 tRNA-Thr ge… | Kogia br… | 761 | 761 | 46% | 0.0 | 97.52% | 519 | JX403737.1 |

Figure 2. Sequences producing significant alignments for Region 1 of tissue sample T15104.

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

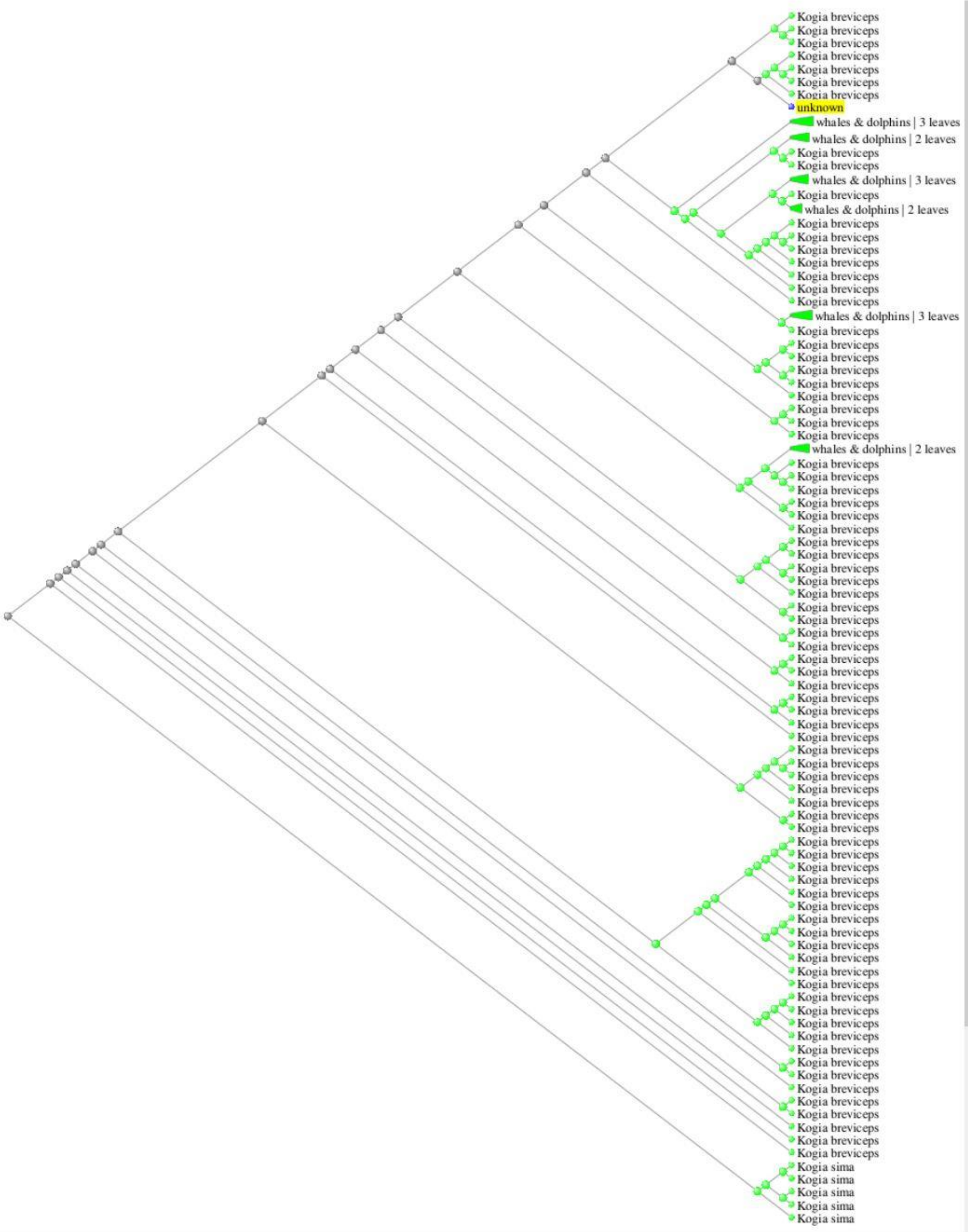

Figure 3. Yellow indicates the queried (sample) region. Green are GenBank sequence accessions.

## Conclusion

T15104 has the greatest sequence alignment with and is identified as a pygmy sperm whale *K*. *breviceps*. As pygmy sperm whales are of the Kogiidae Family and pilot whales are of the Delphinidae Family this conclusion is supported by the failure of Region 2, which is a Delphinidae specific region, to amplify for this sample.