GOVERNMENT OF
WESTERN AUSTRALIA

Department of **Biodiversity,**
Conservation and Attractions

Biodiversity and
Conservation Science

Report on genetic identification of unknown dolphin tissue sample T14438.

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The sample suspected to be an Indo-pacific bottlenose dolphin (*Tursiopsis aduncus*) was collected from Wonnerup and received from Kelly Waples July 2022.

DNA was extracted from the tissue sample using a TNES extraction method. The Displacement Loop primers Delph-D-Loop Forward /Delph-H00034 Reverse (Region 1) and Dip5 Forward/Dip1.5 Reverse (Region 2) were used to PCR non-coding mitochondrial D-Loop DNA using a Shaw hot start PCR program. PCR products were sequenced using the same Forward and Reverse primers at the Western Australian State Agricultural Biotechnology Centre (SABC) Murdoch University and sequence data for each region was aligned and edited using BioEdit Sequence Alignment Editor.

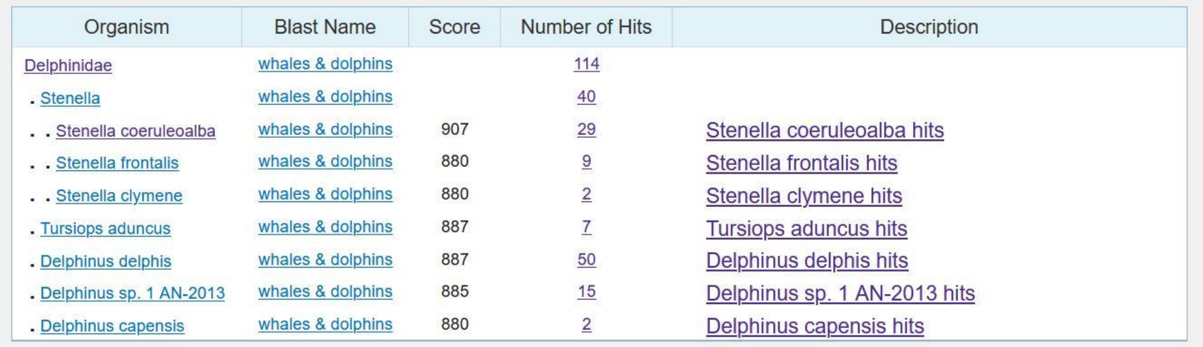
The Delph-D-Loop Forward primer produced a sequence read of 503bp while the Delph-H00034 Reverse primer produced a sequence read of intermittent low quality and was not included in further analysis. The Dip5 Forward/Dip1.5 Reverse sequences were combined to produce a contiguous read of 504bp. Each sequence region was queried using the blastn suite (Standard Nucleotide BLAST) for highly similar sequences.

Region 1

Region 1 was significantly aligned to 114 sequences of Delphinidae (marine dolphins). Alignments with the 13 highest Maximum Scores, highest Total Scores, highest Query Coverages, lowest E values, and highest Percent Identities were all with sequences from *Stenella coeruleoalba* (striped dolphin) (Fig 1). Region 1 was also significantly aligned to a number of sequences of *Delphinus delphis* (saddleback dolphin), *Stenella frontalis* (Atlantic spotted dolphin) and *T. aduncus* (Fig 2), although Maximum and Total Scores and Percent Identities were all lower for these alignments (Fig 1).

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Stenella coeruleoalba mitochondrial D-loop, haplotype pac_C	Stenella coerule...	907	907	100%	0.0	99.20%	627	AM498704.1
☑	Stenella coeruleoalba mitochondrial D-loop, haplotype atlan_A4	Stenella coerule...	907	907	100%	0.0	99.20%	627	AM498719.1
☑	Stenella coeruleoalba isolate NJNU0401 mitochondrial control region, complete sequence	Stenella coerule...	902	902	100%	0.0	99.01%	915	AY046540.1
☑	Stenella coeruleoalba mitochondrial D-loop, haplotype med2_A3	Stenella coerule...	902	902	100%	0.0	99.01%	627	AM498686.1
☑	Stenella coeruleoalba mitochondrion, partial genome	Stenella coerule...	902	902	100%	0.0	99.01%	16489	MT410956.1
☑	Stenella coeruleoalba isolate NJNU0400 mitochondrial control region, complete sequence	Stenella coerule...	896	896	100%	0.0	98.81%	914	AY046539.1
☑	Stenella coeruleoalba isolate NJNU0403 mitochondrial control region, complete sequence	Stenella coerule...	896	896	100%	0.0	98.81%	915	AY046542.1
☑	Stenella coeruleoalba isolate NJNU0404 mitochondrial control region, complete sequence	Stenella coerule...	896	896	100%	0.0	98.81%	915	AY046543.1
☑	Stenella coeruleoalba mitochondrion, complete genome	Stenella coerule...	896	896	100%	0.0	98.81%	16384	EU557097.1
☑	Stenella coeruleoalba mitochondrion, complete genome	Stenella coerule...	896	896	100%	0.0	98.81%	16384	NC_012053.1
☑	Stenella coeruleoalba mitochondrial D-loop, haplotype med2_A4	Stenella coerule...	891	891	100%	0.0	98.61%	627	AM498690.1
☑	Stenella coeruleoalba mitochondrial D-loop, haplotype atlan_B1	Stenella coerule...	891	891	100%	0.0	98.61%	627	AM498713.1
☑	Stenella coeruleoalba mitochondrial D-loop, haplotype atlan_D1	Stenella coerule...	891	891	100%	0.0	98.61%	627	AM498715.1
☑	Delphinus delphis mitochondrial partial D-loop, haplotype DdHap44, specimen voucher FC121	Delphinus delphis	887	887	99%	0.0	98.60%	774	HE680138.1
☑	Tursiops aduncus isolate SA101 mitochondrion, complete genome	Tursiops aduncus	887	887	99%	0.0	98.60%	16384	KF570353.1
☑	Tursiops aduncus isolate SA116 mitochondrion, complete genome	Tursiops aduncus	887	887	99%	0.0	98.60%	16384	KF570356.1

Figure 1. Sequences producing significant alignments for Region 1 of tissue sample T14438.



Organism	Blast Name	Score	Number of Hits	Description
Delphinidae	whales & dolphins		114	
. Stenella	whales & dolphins		40	
. . Stenella coeruleoalba	whales & dolphins	907	29	Stenella coeruleoalba hits
. . Stenella frontalis	whales & dolphins	880	9	Stenella frontalis hits
. . Stenella clymene	whales & dolphins	880	2	Stenella clymene hits
. Tursiops aduncus	whales & dolphins	887	7	Tursiops aduncus hits
. Delphinus delphis	whales & dolphins	887	50	Delphinus delphis hits
. Delphinus sp. 1 AN-2013	whales & dolphins	885	15	Delphinus sp. 1 AN-2013 hits
. Delphinus capensis	whales & dolphins	880	2	Delphinus capensis hits

Figure 2. Number of significant alignments (Hits) for Region 1 of tissue sample T14438.

Results were also visualised as a Fast Minimum Evolution distance tree (Fig. 3) although resolution among the clades is poor, due to the high degree of genetic similarity among the Delphinidae species in question.

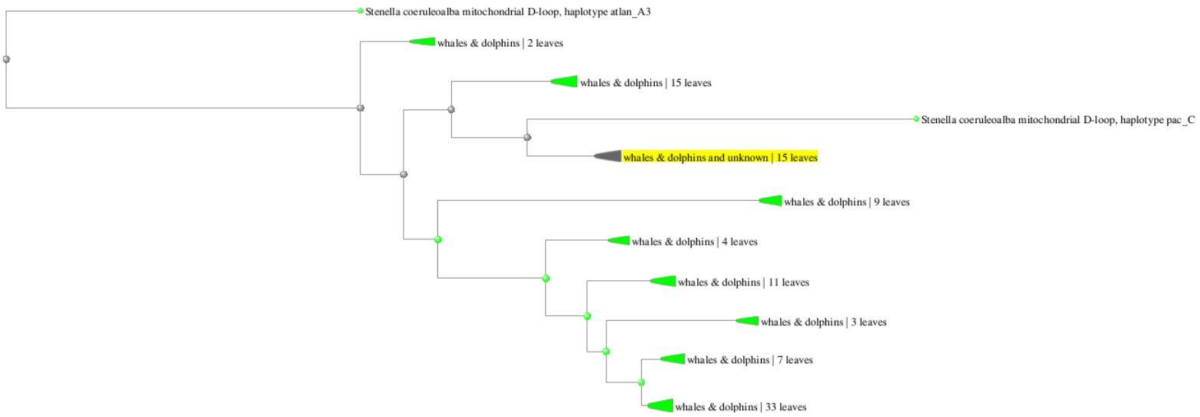
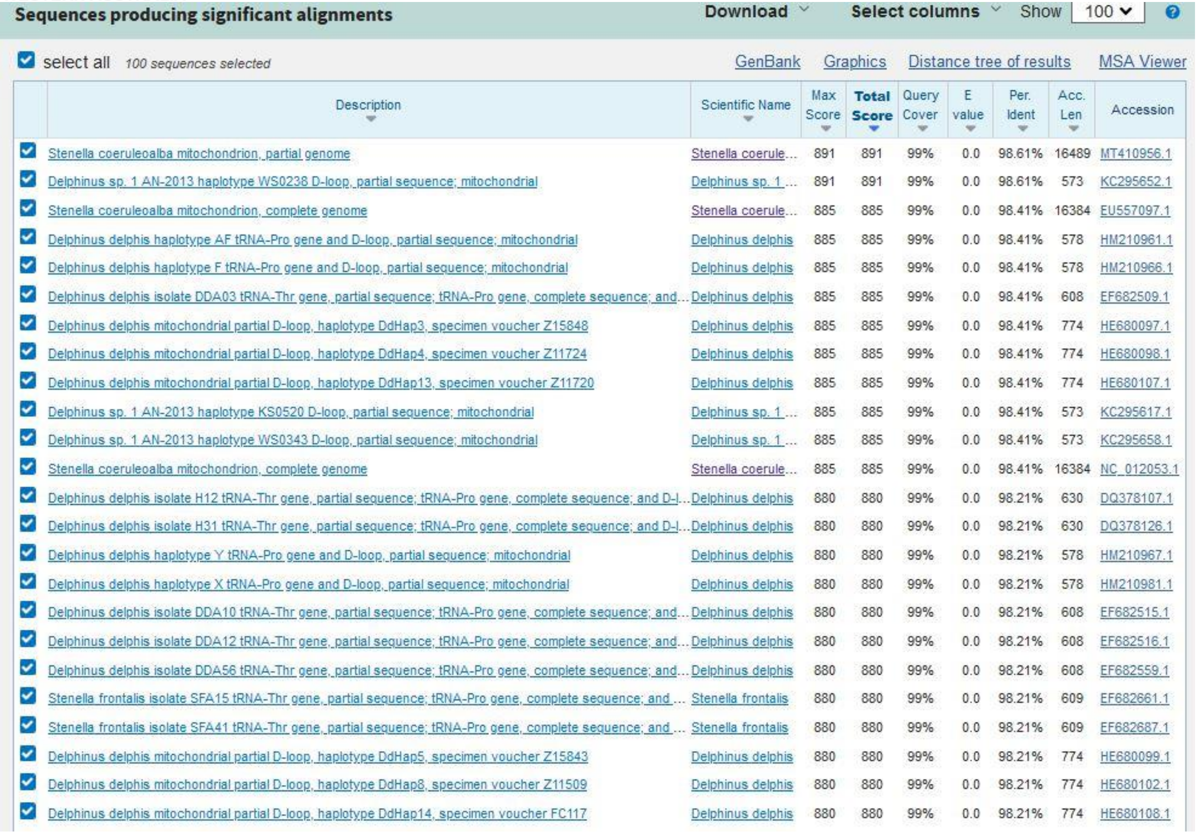


Figure 3. Yellow includes the queried (sample) region. Green are GenBank sequence accessions.

Region 2

Region 2 was significantly aligned to 130 sequences of Delphinidae. The alignment with the highest Maximum Score, highest Total Score, highest Query Coverage, lowest E value, and highest Percent Identity was again with sequences from *S. coeruleoalba* (Fig 4). Region 2 was also significantly aligned to sequences of *D. delphis*, *S. frontalis* and *T. aduncus* (Fig 5), often with high Maximum Scores, Total Scores, Query Coverages, low E values, and high Percent Identities (Fig 4.). Results for Region 2 were also visualised as a Fast Minimum Evolution distance tree (Fig. 6) although resolution of the clades again is poor.

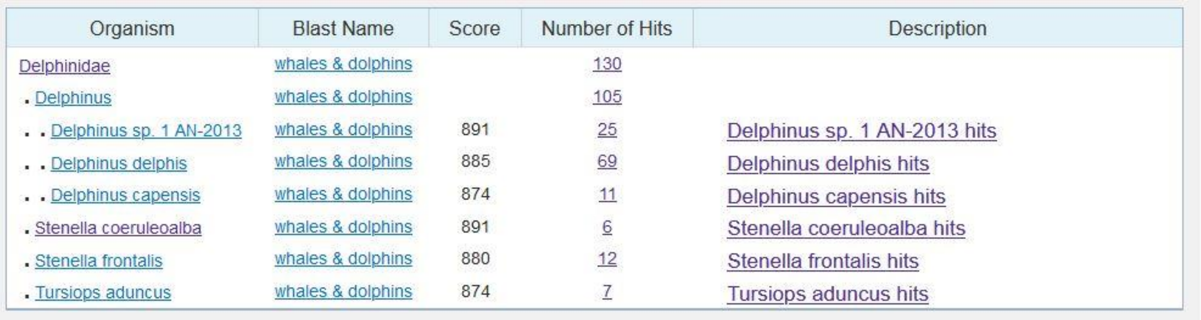


Sequences producing significant alignments

☑ select all *100 sequences selected*

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Stenella coeruleoalba mitochondrion, partial genome	Stenella coerule...	891	891	99%	0.0	98.61%	16489	MT410956.1
☑	Delphinus sp. 1 AN-2013 haplotype WS0238 D-loop, partial sequence; mitochondrial	Delphinus sp. 1 ...	891	891	99%	0.0	98.61%	573	KC295652.1
☑	Stenella coeruleoalba mitochondrion, complete genome	Stenella coerule...	885	885	99%	0.0	98.41%	16384	EU557097.1
☑	Delphinus delphis haplotype AF tRNA-Pro gene and D-loop, partial sequence; mitochondrial	Delphinus delphis	885	885	99%	0.0	98.41%	578	HM210961.1
☑	Delphinus delphis haplotype F tRNA-Pro gene and D-loop, partial sequence; mitochondrial	Delphinus delphis	885	885	99%	0.0	98.41%	578	HM210966.1
☑	Delphinus delphis isolate DDA03 tRNA-Thr gene, partial sequence; tRNA-Pro gene, complete sequence; and...	Delphinus delphis	885	885	99%	0.0	98.41%	608	EF682509.1
☑	Delphinus delphis mitochondrial partial D-loop, haplotype DdHap3, specimen voucher Z15848	Delphinus delphis	885	885	99%	0.0	98.41%	774	HE680097.1
☑	Delphinus delphis mitochondrial partial D-loop, haplotype DdHap4, specimen voucher Z11724	Delphinus delphis	885	885	99%	0.0	98.41%	774	HE680098.1
☑	Delphinus delphis mitochondrial partial D-loop, haplotype DdHap13, specimen voucher Z11720	Delphinus delphis	885	885	99%	0.0	98.41%	774	HE680107.1
☑	Delphinus sp. 1 AN-2013 haplotype KS0520 D-loop, partial sequence; mitochondrial	Delphinus sp. 1 ...	885	885	99%	0.0	98.41%	573	KC295617.1
☑	Delphinus sp. 1 AN-2013 haplotype WS0343 D-loop, partial sequence; mitochondrial	Delphinus sp. 1 ...	885	885	99%	0.0	98.41%	573	KC295658.1
☑	Stenella coeruleoalba mitochondrion, complete genome	Stenella coerule...	885	885	99%	0.0	98.41%	16384	NC_012053.1
☑	Delphinus delphis isolate H12 tRNA-Thr gene, partial sequence; tRNA-Pro gene, complete sequence; and D-l...	Delphinus delphis	880	880	99%	0.0	98.21%	630	DQ378107.1
☑	Delphinus delphis isolate H31 tRNA-Thr gene, partial sequence; tRNA-Pro gene, complete sequence; and D-l...	Delphinus delphis	880	880	99%	0.0	98.21%	630	DQ378126.1
☑	Delphinus delphis haplotype Y tRNA-Pro gene and D-loop, partial sequence; mitochondrial	Delphinus delphis	880	880	99%	0.0	98.21%	578	HM210967.1
☑	Delphinus delphis haplotype X tRNA-Pro gene and D-loop, partial sequence; mitochondrial	Delphinus delphis	880	880	99%	0.0	98.21%	578	HM210981.1
☑	Delphinus delphis isolate DDA10 tRNA-Thr gene, partial sequence; tRNA-Pro gene, complete sequence; and...	Delphinus delphis	880	880	99%	0.0	98.21%	608	EF682515.1
☑	Delphinus delphis isolate DDA12 tRNA-Thr gene, partial sequence; tRNA-Pro gene, complete sequence; and...	Delphinus delphis	880	880	99%	0.0	98.21%	608	EF682516.1
☑	Delphinus delphis isolate DDA56 tRNA-Thr gene, partial sequence; tRNA-Pro gene, complete sequence; and...	Delphinus delphis	880	880	99%	0.0	98.21%	608	EF682559.1
☑	Stenella frontalis isolate SFA15 tRNA-Thr gene, partial sequence; tRNA-Pro gene, complete sequence; and ...	Stenella frontalis	880	880	99%	0.0	98.21%	609	EF682661.1
☑	Stenella frontalis isolate SFA41 tRNA-Thr gene, partial sequence; tRNA-Pro gene, complete sequence; and ...	Stenella frontalis	880	880	99%	0.0	98.21%	609	EF682687.1
☑	Delphinus delphis mitochondrial partial D-loop, haplotype DdHap5, specimen voucher Z15843	Delphinus delphis	880	880	99%	0.0	98.21%	774	HE680099.1
☑	Delphinus delphis mitochondrial partial D-loop, haplotype DdHap8, specimen voucher Z11509	Delphinus delphis	880	880	99%	0.0	98.21%	774	HE680102.1
☑	Delphinus delphis mitochondrial partial D-loop, haplotype DdHap14, specimen voucher FC117	Delphinus delphis	880	880	99%	0.0	98.21%	774	HE680108.1

Figure 4. Sequences producing significant alignments for Region 2 of tissue sample T14438.



Organism	Blast Name	Score	Number of Hits	Description
Delphinidae	whales & dolphins		130	
. Delphinus	whales & dolphins		105	
. . Delphinus sp. 1 AN-2013	whales & dolphins	891	25	Delphinus sp. 1 AN-2013 hits
. . Delphinus delphis	whales & dolphins	885	69	Delphinus delphis hits
. . Delphinus capensis	whales & dolphins	874	11	Delphinus capensis hits
. Stenella coeruleoalba	whales & dolphins	891	6	Stenella coeruleoalba hits
. Stenella frontalis	whales & dolphins	880	12	Stenella frontalis hits
. Tursiops aduncus	whales & dolphins	874	7	Tursiops aduncus hits

Figure 5. Number of significant alignments (Hits) for Region 2 of tissue sample T14438.

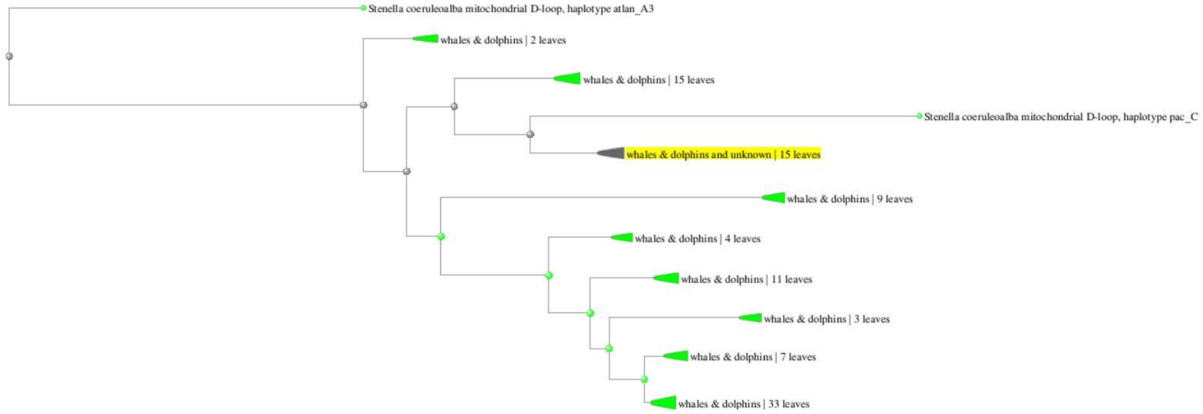


Figure 6. Yellow includes the queried (sample) region. Green are GenBank sequence accessions.

Conclusion

Taking the results from the two sequenced regions into consideration, T14438 has the greatest sequence alignment with, and is identified as *S. coeruleoalba*. The Delphinidae species returned in the top 100 significant alignments for each sequenced region have a high degree of genetic similarity and while it cannot be definitively ruled out that the sample may belong to *D. delphis*, it is unlikely to be *T. aduncus* as originally suspected.