Department of **Biodiversity, Conservation and Attractions**
GOVERNMENT OF WESTERN AUSTRALIA
Biodiversity and Conservation Science

Report on genetic identification of seal tissue sample T19238 (Fauna lab) T01043 (Marine)

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The tissue sample (T19238) was collected from an animal stranded at Rottnest Island, received from Kelly Waples in May 2025, and was identified by genetic sequencing as *Neophoca cinerea*, the Australian sea-lion.

DNA was extracted from the tissue sample using a TNES extraction method. The sample appeared very decomposed and was odorous. The DNA concentration from the extraction was low (2.11 ngµl^{-1}). The Displacement D-Loop mitochondrial primers L15925 Forward/H16499 Reverse were used to PCR non-coding mitochondrial D-Loop DNA using a Shaw hot start PCR program. The reaction failed to amplify a PCR product however as visualised on an agarose gel (Fig1).

Figure 1. Agarose gel showing two TNES DNA extractions of the sample T19238, a positive control of long-nosed fur seal DNA and a negative or no template control.

DNA was then extracted using a QIAGEN DNeasy blood and Tissue kit. The DNA concentration was again low (2.2 ngµl^{-1}); however, the PCR produced a strong single band for these extractions (Fig2).

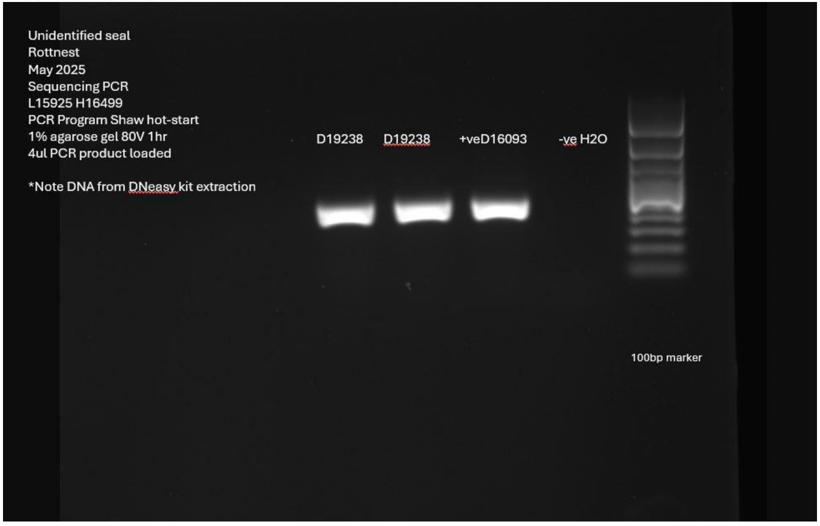
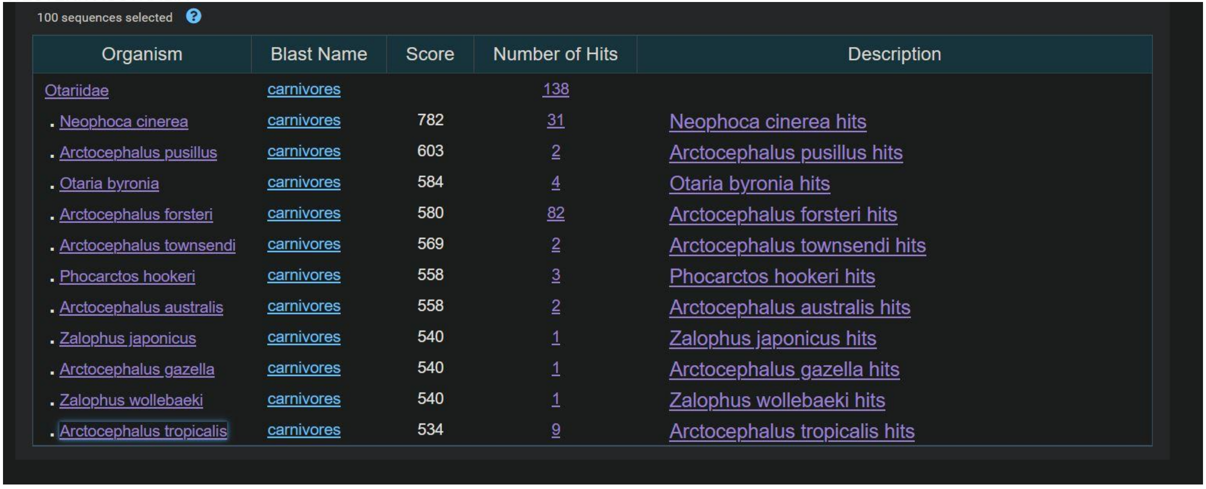


Figure 2. Agarose gel showing two QIAGEN DNeasy Blood and Tissue kit DNA extractions of the sample T19238, a positive control of long-nosed fur seal DNA and a negative or no template control.

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 541bp. This sequence region was queried using the blastn suite (megablast) for highly similar sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

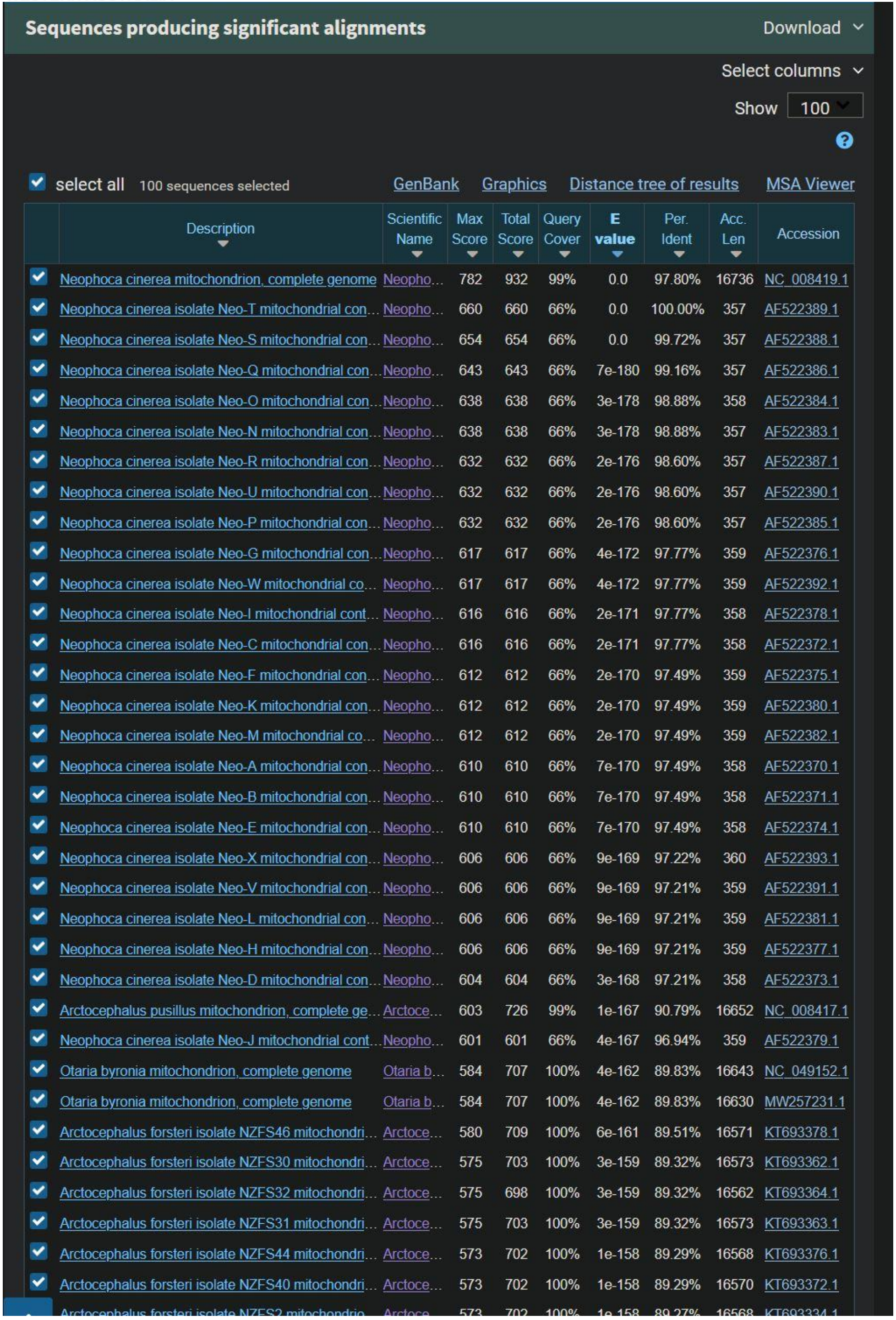
The queried sequence was significantly aligned to 138 sequences of the Otariidae family, (Fig 3). Of the sequences producing significant alignment, the top 24 hits with the highest Maximum Scores, highest Total Scores, highest Query Coverages, lowest E values, and highest Percent Identities, were with *Neophoca cinerea*, the Australian sea-lion (Fig 4).



100 sequences selected

Organism	Blast Name	Score	Number of Hits	Description
Otariidae	carnivores		138	
. Neophoca cinerea	carnivores	782	31	Neophoca cinerea hits
. Arctocephalus pusillus	carnivores	603	2	Arctocephalus pusillus hits
. Otaria byronia	carnivores	584	4	Otaria byronia hits
. Arctocephalus forsteri	carnivores	580	82	Arctocephalus forsteri hits
. Arctocephalus townsendi	carnivores	569	2	Arctocephalus townsendi hits
. Phocarctos hookeri	carnivores	558	3	Phocarctos hookeri hits
. Arctocephalus australis	carnivores	558	2	Arctocephalus australis hits
. Zalophus japonicus	carnivores	540	1	Zalophus japonicus hits
. Arctocephalus gazella	carnivores	540	1	Arctocephalus gazella hits
. Zalophus wollebaeki	carnivores	540	1	Zalophus wollebaeki hits
. Arctocephalus tropicalis	carnivores	534	9	Arctocephalus tropicalis hits

Figure 3. Number of significant alignments (Hits) for tissue sample T19238.



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Neophoca cinerea mitochondrion, complete genome	Neopho...	782	932	99%	0.0	97.80%	16736	NC_008419.1
☑	Neophoca cinerea isolate Neo-T mitochondrial con...	Neopho...	660	660	66%	0.0	100.00%	357	AF522389.1
☑	Neophoca cinerea isolate Neo-S mitochondrial con...	Neopho...	654	654	66%	0.0	99.72%	357	AF522388.1
☑	Neophoca cinerea isolate Neo-Q mitochondrial con...	Neopho...	643	643	66%	7e-180	99.16%	357	AF522386.1
☑	Neophoca cinerea isolate Neo-O mitochondrial con...	Neopho...	638	638	66%	3e-178	98.88%	358	AF522384.1
☑	Neophoca cinerea isolate Neo-N mitochondrial con...	Neopho...	638	638	66%	3e-178	98.88%	357	AF522383.1
☑	Neophoca cinerea isolate Neo-R mitochondrial con...	Neopho...	632	632	66%	2e-176	98.60%	357	AF522387.1
☑	Neophoca cinerea isolate Neo-U mitochondrial con...	Neopho...	632	632	66%	2e-176	98.60%	357	AF522390.1
☑	Neophoca cinerea isolate Neo-P mitochondrial con...	Neopho...	632	632	66%	2e-176	98.60%	357	AF522385.1
☑	Neophoca cinerea isolate Neo-G mitochondrial con...	Neopho...	617	617	66%	4e-172	97.77%	359	AF522376.1
☑	Neophoca cinerea isolate Neo-W mitochondrial co...	Neopho...	617	617	66%	4e-172	97.77%	359	AF522392.1
☑	Neophoca cinerea isolate Neo-I mitochondrial cont...	Neopho...	616	616	66%	2e-171	97.77%	358	AF522378.1
☑	Neophoca cinerea isolate Neo-C mitochondrial con...	Neopho...	616	616	66%	2e-171	97.77%	358	AF522372.1
☑	Neophoca cinerea isolate Neo-F mitochondrial con...	Neopho...	612	612	66%	2e-170	97.49%	359	AF522375.1
☑	Neophoca cinerea isolate Neo-K mitochondrial con...	Neopho...	612	612	66%	2e-170	97.49%	359	AF522380.1
☑	Neophoca cinerea isolate Neo-M mitochondrial co...	Neopho...	612	612	66%	2e-170	97.49%	359	AF522382.1
☑	Neophoca cinerea isolate Neo-A mitochondrial con...	Neopho...	610	610	66%	7e-170	97.49%	358	AF522370.1
☑	Neophoca cinerea isolate Neo-B mitochondrial con...	Neopho...	610	610	66%	7e-170	97.49%	358	AF522371.1
☑	Neophoca cinerea isolate Neo-E mitochondrial con...	Neopho...	610	610	66%	7e-170	97.49%	358	AF522374.1
☑	Neophoca cinerea isolate Neo-X mitochondrial con...	Neopho...	606	606	66%	9e-169	97.22%	360	AF522393.1
☑	Neophoca cinerea isolate Neo-V mitochondrial con...	Neopho...	606	606	66%	9e-169	97.21%	359	AF522391.1
☑	Neophoca cinerea isolate Neo-L mitochondrial con...	Neopho...	606	606	66%	9e-169	97.21%	359	AF522381.1
☑	Neophoca cinerea isolate Neo-H mitochondrial con...	Neopho...	606	606	66%	9e-169	97.21%	359	AF522377.1
☑	Neophoca cinerea isolate Neo-D mitochondrial con...	Neopho...	604	604	66%	3e-168	97.21%	358	AF522373.1
☑	Arctocephalus pusillus mitochondrion, complete ge...	Arctoce...	603	726	99%	1e-167	90.79%	16652	NC_008417.1
☑	Neophoca cinerea isolate Neo-J mitochondrial cont...	Neopho...	601	601	66%	4e-167	96.94%	359	AF522379.1
☑	Otaria byronia mitochondrion, complete genome	Otaria b...	584	707	100%	4e-162	89.83%	16643	NC_049152.1
☑	Otaria byronia mitochondrion, complete genome	Otaria b...	584	707	100%	4e-162	89.83%	16630	MW257231.1
☑	Arctocephalus forsteri isolate NZFS46 mitochondri...	Arctoce...	580	709	100%	6e-161	89.51%	16571	KT693378.1
☑	Arctocephalus forsteri isolate NZFS30 mitochondri...	Arctoce...	575	703	100%	3e-159	89.32%	16573	KT693362.1
☑	Arctocephalus forsteri isolate NZFS32 mitochondri...	Arctoce...	575	698	100%	3e-159	89.32%	16562	KT693364.1
☑	Arctocephalus forsteri isolate NZFS31 mitochondri...	Arctoce...	575	703	100%	3e-159	89.32%	16573	KT693363.1
☑	Arctocephalus forsteri isolate NZFS44 mitochondri...	Arctoce...	573	702	100%	1e-158	89.29%	16568	KT693376.1
☑	Arctocephalus forsteri isolate NZFS40 mitochondri...	Arctoce...	573	702	100%	1e-158	89.29%	16570	KT693372.1
	Arctocephalus forsteri isolate NZFS2 mitochondrio...	Arctoce...	573	702	100%	1e-158	89.27%	16568	KT693334.1

Figure 4. Sequences producing significant alignments for tissue sample T19238.

Results were also visualised as a Fast Minimum Evolution distance tree (Fig 5).

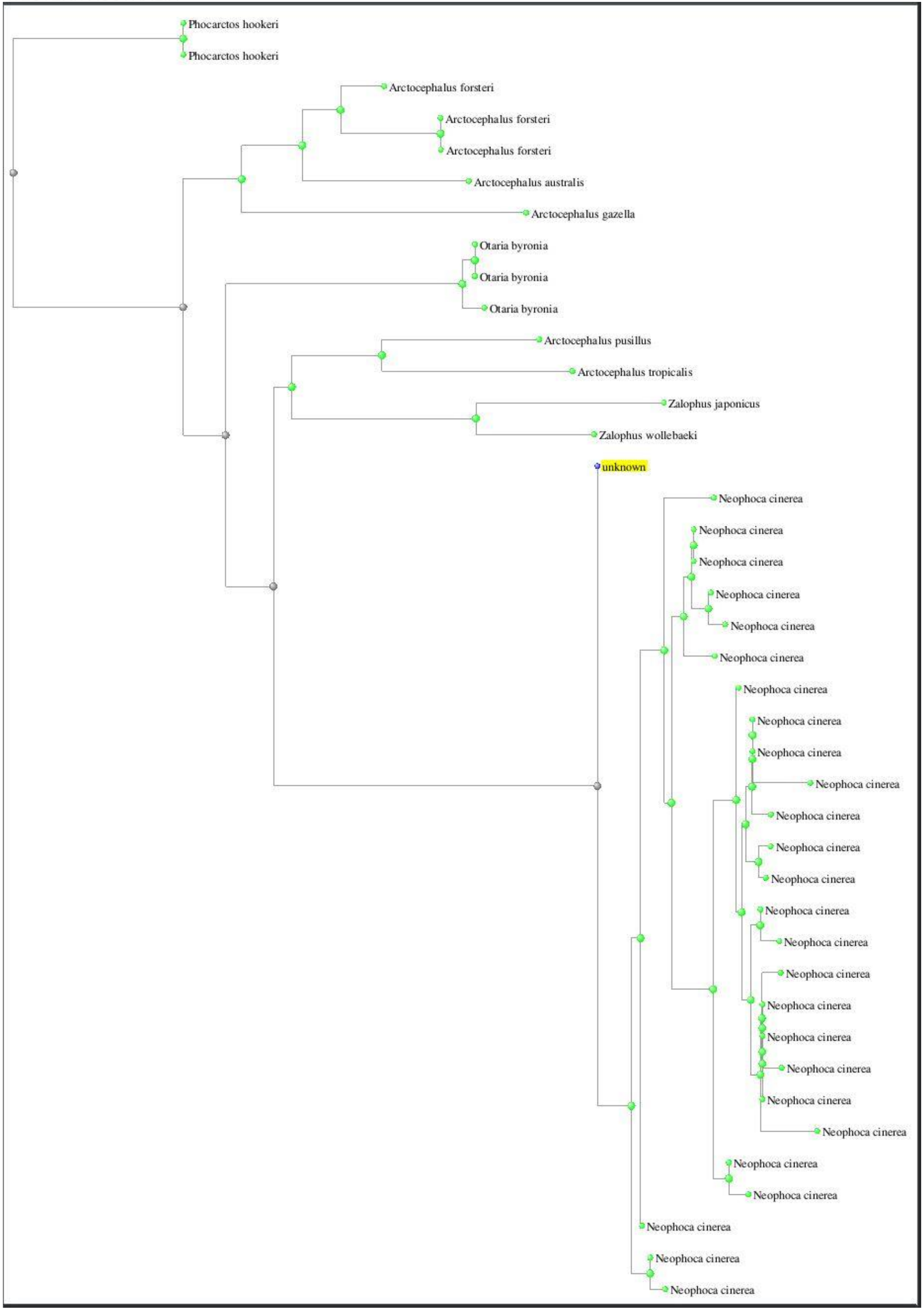


Figure 5. Yellow indicates the queried (sample) region. Green are GenBank sequence accessions with significant sequence alignment. Note that a large number of Arctocephalus forsteri accessions have been excluded for clarity.

Conclusion

T19238 has the greatest sequence alignment with and is identified as an Australian sea-lion, *N. cinerea*.