

Evaluating next-generation sequencing utility for genotyping and sex determination using historical and contemporary sloughed skin samples from humpback whales (*Megaptera novaeangliae*)

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Abstract

Non-invasively collected samples such as sloughed skin has proven viable for genetic analysis using microsatellites and mitochondrial DNA, even when degraded. Next-generation sequencing techniques using single nucleotide polymorphisms (SNPs), offer a favourable alternative for degraded DNA, advancing our capacity to address important evolutionary, ecological and conservation questions for long-term studies of globally distributed species. This study utilised a next-generation sequencing approach for the first time to investigate SNP genotyping on 91 humpback whale (*Megaptera novaengliae*) sloughed skin tissues from both historical (2001 and 2003) and contemporary (2022) collections. DNA quality and quantity assessments confirmed successful long-term preservation and viability of historical skin tissues (>20 years old) with no significant degradation over time. SNP genotyping using sloughed skin produced reliable SNP markers (i.e., high quantity and quality) applicable for future genetic assessments. Three sex-linked markers associated with maleness were identified, suggesting heterogametic male (XX/YY) sex determination, although these markers alone are not reliable for accurate sexing. This study confirms the viability of SNP genotyping from historical and contemporary sloughed skin samples, supporting a collaborative, non-invasive approach to genetic and conservation research on humpback whales and other wide-ranging species.

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	<i>diff</i>	<i>lwr</i>	<i>upr</i>	<i>p</i>	<i>bonferroni</i>
<i>2003-2001</i>	0.05413043	-0.50424406	0.6125049	0.9709355	1.000
<i>2022-2001</i>	0.83259259	0.10405886	1.5611263	0.0210467	0.023
<i>2022-2003</i>	0.77846216	0.05902662	1.4978977	0.0307652	0.035

Locus	Allele sequence	Heterozygous proportion		Homozygous REF allele		Heterozygous loci allele		Homozygous alternative allele	
		Male	Female	Male	Female	Male	Female	Male	Female
83554469-7-T/C	TGCAGGT T TGCTGCTTCTGGCGACAACCTCCCATCTGTCAATTTCATGTCATCTCTTG TGATTTT	1	0.056	0	17	5	1	0	0
	TGCAGGT C TGCTGCTTCTGGCGACAACCTCCCATCTGTCAATTTCATGTCATCTCTTG TGATTTT								
8357554-41-G/A	TGCAGGCCCCAGAGCTCTCTGTGGGGCGCGGGGGCTACCC G AGCGAGCCGTGGTCTTTCCCTG	1	0	0	10	1	0	0	0
	TGCAGGCCCCAGAGCTCTCTGTGGGGCGCGGGGGCTACCC A GAGCGAGCCGTGGTCTTTCCCTG								
83568686-20-G/A	TGCAGGTGATGTTAGATAC G TGTTGATGGTAAACCAATAAGACAGGGGATTCATACCTTTGACGT	1	0	0	4	1	0	0	0
	TGCAGGTGATGTTAGATAC A TGTTGATGGTAAACCAATAAGACAGGGGATTCATACCTTTGACTT								







