

Ancient DNA sampling report Walrus bones from Alþingisreitur

Albína Hulda Pálsdóttir, Sanne Boessenkool & Bastiaan Star



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Ancient DNA sampling report: Walrus bones from Alþingisreitur

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Cover photo: Partial walrus pelvis from the Alþingisreitur excavation. Photo by Albína Hulda Pálsdóttir.

Contents

Ancient DNA sampling report: Walrus bones from Alþingisreitur	4
Bones sent for ancient DNA sampling	4
Explanation of numbers	4
Photos of bones before sampling.....	6
Laboratory methods.....	9
Results of ancient DNA analysis.....	10
Photos of bones after ancient DNA sampling.....	11
Funding.....	13
Acknowledgements	13
References.....	14

Table of Figures

Figure 1: 2008-32-685 Walrus pelvis with chop marks from context# 30482, bone# 711. The small loose fragment broke off during ZooMS sampling.	6
Figure 2: 2008-32-685 Walrus pelvis with chop marks from context# 30482, bone# 711. The four holes are from ZooMS sampling.	6
Figure 3: 2008-32-709 Context# 33690, walrus skull fragment, #1051. (Photo by Brynja Guðmundsdóttir).....	7
Figure 4: 2008-32-709 Context# 33690, walrus skull fragment, #1051. Hole from ZooMS sampling.	7
Figure 5: 2009-32-2419, context# 214775 walrus vertebra, #1052. Hole from ZooMS sampling.	8
Figure 6: 2009-32-2419, context# 214775 walrus vertebra, #1052. Holes from ZooMS sampling.	8
Figure 7: Two views of a fragment of the walrus pelvis. 2008-32-685 Walrus pelvis with chop marks from context# 30482, bone# 711, lab number WLR036 after ancient DNA sampling. Only the small loose fragment which broke off the pelvis during ZooMS sampling was sampled for ancient DNA to minimize damage to this well-preserved bone.	11
Figure 8: 2008-32-709 Context# 33690, walrus skull fragment, #1051, lab number WLR035. The bone after sampling for ancient DNA. Here a chunk was cut out from the middle of the bone.	11
Figure 10: 2008-32-709 Context# 33690, walrus skull fragment, #1051, lab number WLR035. The bone after sampling for ancient DNA. Here a chunk was cut out from the middle of the bone.	12
Figure 11: 2009-32-2419, context# 214775 walrus vertebra, #1052, lab number WLR037. Two views of the bone after ancient DNA sampling.	12

List of Tables

Table 1: List of the walrus bones selected for ancient DNA and isotope sampling from the Alþingisreitur excavation in Reykjavík, Iceland	5
Table 2 Results from ancient DNA analysis of the three walrus bones. * n.a.; WLR035 did not yield a DNA library suitable for sequencing. Endogenous DNA content is measured as the percentage of reads (with a minimum MapQ value of 25) aligning to the Pacific walrus nuclear reference genome.....	10

Ancient DNA sampling report: Walrus bones from Alþingisreitur

The walrus bones were sampled as part of three research projects:

- Northern Journeys: Reimagining the Medieval Revolution
- The Impact of Art: Investigating Artifacts using Ancient DNA to Trace Historic Trade and Exploitation of Atlantic Walrus
- Tracking Viking-assisted Dispersal of Biodiversity using Ancient DNA

The projects are led by Dr. James Barrett, University of Cambridge (PI: Northern Journeys), Dr. Bastiaan Star, University of Oslo (PI: The Impact of Art) and Dr. Sanne Boessenkool, University of Oslo (PI: Viking-assisted Dispersal).

Bones sent for ancient DNA sampling

The bones that were sampled for ancient DNA analysis are from three of the four phases¹ of the Alþingisreitur excavation in downtown Reykjavík Iceland (Garðarsdóttir, 2010, 2013; Pálsdóttir, 2010, 2013). Their dating is as follows: Phase IV AD 871-1226, Phase III AD 1226-1500, Phase II AD 1500-1800. The bones were previously sampled for ZooMS (Buckley et al., 2014, 2015). The preservation of the bones varies but two of them have blue patches, likely the mineral vivianite (McGowan & Prangnell, 2006; “vivianite (mineral),” 2013), formed due to the partially water logged conditions at the site along with the high iron content of the soil. Detailed information about each bone is listed in Table 1.

Explanation of numbers

Each bag and Tyvek label should have three separate numbers in this order:

- The first number 2009-32-4060 is the National Museum of Iceland number. There can be many bones under each National Museum number. (The first part is the year excavated, the second part is the number the museum gives the excavation, the third part is a unique number given to each find/sample/bone in the Intrasys system which all the excavation information has been recorded and stored in).
- The second number C#4019892 the context number where the bone was found.
- The #1042 is the number given to all measured bones/bones sent for special analysis/bones with pathology in the NABONE Access database for the animal bones from the Alþingisreitur excavation. There is usually only one bone behind a number like this (it might be in multiple fragments which are all obviously from the same specimen although that is rare).

¹ There were very few bones retrieved from the youngest phase of the site which dates to between 1800 and around 1980 (Phase I).

Table 1: List of the walrus bones selected for ancient DNA and isotope sampling from the Alþingisreitur excavation in Reykjavík, Iceland

Site	Context #	bjms number	Date	Find unit	Area	Phase	Species	Element	End	Count	Frag	Fusion	Texture	Butchery	Bone #	Measurments and comments.
Alþingisreitur, Reykjavík, Iceland	30482	2008-32-685	7.10.2008	30920	A	3 (1226–1500 AD)	Walrus	Pelvis	f	1	11	f	2	ch	711	Size 30x13x6 cm, weight 313 gr. Measurements: 4. ca. 89,64 5.ca 55,79 5b. 55,16 6. 18,08 7. 136,88 8. 56,72 9. ca. 127,42 10. ca. 45,83 11. ca ca 43,11. Some blue vivianite patches on surface. A small fragment had previously broke off during ZooMS sampling.
Alþingisreitur, Reykjavík, Iceland	33690	2008-32-709	13.10.2008	34346	A	4 (871–1226 AD)	Walrus	Skull	f	1	11		1		1051	Size 18x10x9 cm, weight 427 gr. Some blue vivianite patches on surface.
Alþingisreitur, Reykjavík, Iceland	214775	2009-32-2419	19.5.2009	214920		4 (871–1226 AD)	Walrus	Vertebra	f	1	10	u	2		1052	Size 6x6x7 cm weight 57 gr. Greatest length at least 63,39. Breath of Processus articularis at least 61 mm.

Photos of bones before sampling

More views of each bone available if needed. All photos by Albína Hulda Pálsdóttir unless otherwise noted.



Figure 1: 2008-32-685 Walrus pelvis with chop marks from context# 30482, bone# 711. The small loose fragment broke off during ZooMS sampling.



Figure 2: 2008-32-685 Walrus pelvis with chop marks from context# 30482, bone# 711. The four holes are from ZooMS sampling.



Figure 3: 2008-32-709 Context# 33690, walrus skull fragment, #1051. (Photo by Brynja Guðmundsdóttir).



Figure 4: 2008-32-709 Context# 33690, walrus skull fragment, #1051. Hole from ZooMS sampling.



Figure 5: 2009-32-2419, context# 214775 walrus vertebra, #1052. Hole from ZooMS sampling.



Figure 6: 2009-32-2419, context# 214775 walrus vertebra, #1052. Holes from ZooMS sampling.

Laboratory methods

All DNA extraction and pre-PCR library protocols were performed in the dedicated ancient DNA laboratory at the Department of Biosciences, University of Oslo following strict aDNA precautions (Allentoft et al., 2012; Cooper & Poinar, 2000). Laboratory protocols, data processing and filtering are described in Star et al. (2018). In short, after UV expose on all sides, samples were milled using a custom designed stainless-steel mortar (Gondek, Boessenkool, & Star, 2018) or a Retsch MM400 mixer mill. Extraction used a combined bleach and pre-digestion protocol (Boessenkool et al., 2016). Specifically, bleach washes were performed in duplicate (150-200 mg of powder each) (Boessenkool et al., 2016), washed with H₂O and pre-digested, which was followed by an overnight, second digestion (Gamba et al., 2015). The two eluates were concentrated (Amicon-30kDA Centrifugal Filter Units) after the overnight digestion and combined, extracting DNA using Minelute (Qiagen) according to manufacturer's instructions. Using 60 µl pre-heated (60°C) EB buffer, DNA was eluted by incubating for 15 min at 37°C (Star et al., 2014). After this, blunt-end Illumina libraries were built (Meyer & Kircher, 2010; Schroeder et al., 2015) and library quality was assessed using a Bioanalyzer 2100 (Agilent). Libraries that passed quality control were sequenced (125 bp paired-end) on an Illumina HiSeq 2500 and demultiplexed allowing zero mismatches in the index tag.

Endogenous DNA content and coverage on the mitochondrial genome were assessed for each sample. Sequencing reads were processed using PALEOMIX (Schubert et al., 2014), collapsing forward and reverse reads using AdapterRemoval v1.5 (Lindgreen, 2012), and aligned to the Atlantic walrus mitogenome (Arnason et al., 2002) with BWA *aln* v.0.7.5a-r405 (Li & Durbin, 2009) and the Pacific walrus (*O. r. divergens*) nuclear genome (Foote et al., 2015). Alignments with a quality score (MapQ) of <25 were removed. Endogenous DNA content is measured as the number of unique reads aligning to the Pacific walrus reference genome.

Results of ancient DNA analysis

Unfortunately, the DNA libraries from the three walrus specimens did not yield sufficient endogenous DNA preservation for further analyses within this study (Table 2). Three sheep bones sampled for ancient DNA from the Alþingsreitur excavation have also had either very low or no endogenous DNA content (unpublished results). These results suggest DNA preservation at this site is poor.

Table 2 Results from ancient DNA analysis of the three walrus bones. * n.a.; WLR035 did not yield a DNA library suitable for sequencing. Endogenous DNA content is measured as the percentage of reads (with a minimum MapQ value of 25) aligning to the Pacific walrus nuclear reference genome.

Museum number	Internal lab number	Sequenced reads	Endogenous DNA (%)
2008-32-685	WLR036	11565670	0.0013
2008-32-709	WLR035	*	*
2009-32-2419	WLR037	17763778	0.0006

Photos of bones after ancient DNA sampling

More views of each bone available if needed. All photos by Agata Gondek unless otherwise noted.

The photos are presented in the same order as the photos before sampling.



Figure 7: Two views of a fragment of the walrus pelvis. 2008-32-685 Walrus pelvis with chop marks from context# 30482, bone# 711, lab number WLR036 after ancient DNA sampling. Only the small loose fragment which broke off the pelvis during ZooMS sampling was sampled for ancient DNA to minimize damage to this well-preserved bone.



Figure 8: 2008-32-709 Context# 33690, walrus skull fragment, #1051, lab number WLR035. The bone after sampling for ancient DNA. Here a chunk was cut out from the middle of the bone.



Figure 9: 2008-32-709 Context# 33690, walrus skull fragment, #1051, lab number WLR035. The bone after sampling for ancient DNA. Here a chunk was cut out from the middle of the bone.



Figure 10: 2009-32-2419, context# 214775 walrus vertebra, #1052, lab number WLR037. Two views of the bone after ancient DNA sampling.

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