Quaternary International xxx (2017) 1–7

Contents lists available at ScienceDirect

Quaternary International

journal homepage: www.elsevier.com/locate/quaint

Molecular identification of paleofeces from Bechan Cave, southeastern Utah, USA

Emil Karpinski [a, b, *], Jim I. Mead [c], Hendrik N. Poinar [a, b, d]

[a] Department of Biology, McMaster University, 1280 Main St. West, Hamilton, Ontario, L8S 4K1, Canada
[b] McMaster Ancient DNA Centre, Department of Anthropology, McMaster University, 1280 Main St. West, Hamilton, Ontario, L8S 4L9, Canada
[c] The Mammoth Site, Hot Springs, SD 57747, USA
[d] Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, 1280 Main St. West, Hamilton, Ontario, L8N 3Z5, Canada

ARTICLE INFO

Article history:
Received 18 August 2016
Received in revised form
15 March 2017
Accepted 30 March 2017
Available online xxx

Keywords:
Mammuthus
Paleofeces
aDNA
Enrichment
Bechan Cave
Colorado Plateau

ABSTRACT

Recent advances in ancient DNA methodologies have enabled the retrieval of highly degraded DNA from contexts with poor preservation conditions. While paleofeces have previously been shown to contain endogenous DNA of the defecator, the preserved DNA is composed of a mixture of diverse microbial, floral and fungal constituents, with limited DNA from the host. However, in situations where skeletal remains are unavailable, paleofeces can serve as an important alternative genetic source, allowing for the molecular identification of the target species and diet. Here, we describe the extraction of ancient DNA from a paleofecal sample found within Bechan Cave (southeastern Utah, USA). Previous work in the cave has suggested that these remains likely stem from *Mammuthus*. We used a comprehensive proboscidean bait set which was used to enrich a nearly complete mitochondrial genome (81.6%) at an average coverage depth of 8.1×. Phylogenetic analysis of the derived consensus sequence revealed that the Bechan Cave bolus does indeed derive from *Mammuthus*, and its sequence falls within Clade 1 (haplogroups F or C), most similar to specimens identified as *Mammuthus columbi*.

1. Introduction

Desiccated paleofecal remains from extinct and extant mammals are well known from the dry caves and rock shelters of the arid American Southwest (see overview in Mead and Swift, 2012). Some of the earliest discoveries and analyses come from the dung of the extinct herbivore *Nothrotheriops shastensis* (Shasta ground sloth; Eames, 1930; Lull, 1930; Laudermilk and Munz, 1934; Martin et al., 1961). The predominant means of identification of these dried paleofecal remains (*Nothrotheriops*, *Oreamnos* [mountain goat]) is using external morphology and/or the size fraction of the contents (Long and Martin, 1974; Spaulding and Martin, 1979; Mead et al., 1986; Mead and Spaulding, 1995). More recently, molecular analyses have enhanced our ability to identify unknown dung producers, as well as elucidate aspects of their diets (e.g., shrub ox, *Euceratherium*, Harrington's mountain goat, *Oreamnos harringtoni*; *Nothrotheriops shastensis*) (Poinar et al., 1998; Hofreiter et al., 2003; Kropf et al., 2007; Campos et al., 2010a,b).

Large accumulations of paleofeces have been found in a rock shelter known as Bechan Cave, along the Colorado Plateau, Utah (Davis et al., 1984; Mead et al., 1984). (Fig. 1). The overall morphology of the dung boluses, their size (230 × 170 × 85 mm) (Fig. 2) and plant constituents, suggest that the producer was likely to be *Mammuthus* (mammoth) (Mead et al., 1986), however, due to the large size of the boluses, there are at minimum two other possible producers in that vicinity and at that time: mastodon (*Mammut*) and two species of ground sloths (*Mylodon* and *Megalonyx*). Here we provide a molecular analysis of a single bolus from Bechan Cave to further augment the putative identification of the paleofeces.

2. Regional setting

Bechan (the Navajo word for 'big feces') Cave is a large sandstone rock shelter on the southern Colorado Plateau of southeastern Utah. Excavation of the sand floor deposit exposed a rich organic layer upwards of 40 cm thick with a total volume in the cavern estimated to be 300 m^3 (Mead et al., 1986; Agenbroad et al., 1989). A minimum

* Corresponding author. McMaster Ancient DNA Centre, Department of Anthropology – CNH 412, McMaster University, 1280 Main St. West, Hamilton, Ontario, L8S 4L9, Canada.
E-mail address: karpine@mcmaster.ca (E. Karpinski).

http://dx.doi.org/10.1016/j.quaint.2017.03.068
1040-6182/

Fig. 1. The entrance to Bechan Cave, southeastern Utah. The entrance is approximately 10 m high.

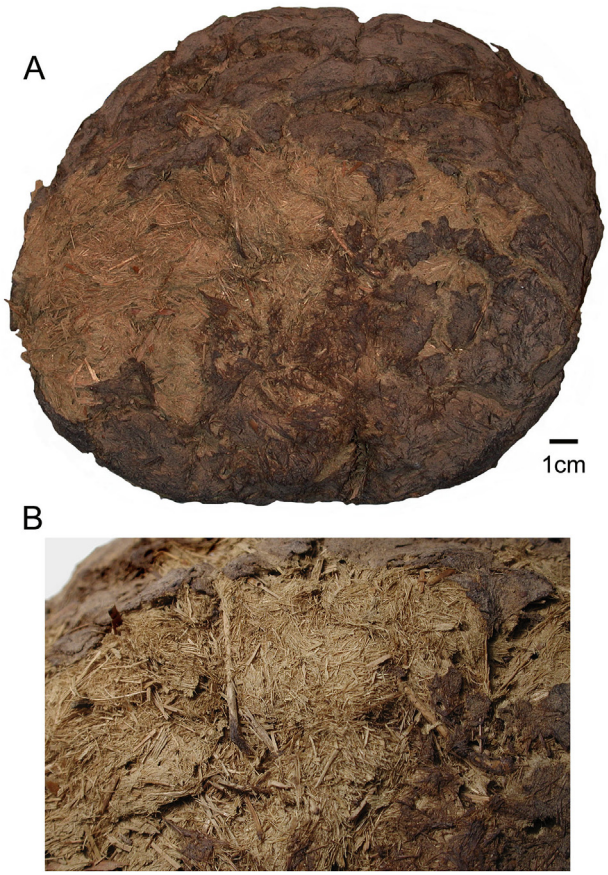

Fig. 2. **(A)** Single dung bolus presumed to be *Mammuthus* from Bechan Cave. **(B)** Close-up of bolus showing the size of poorly-chewed contents.

of eight different morphologies of dung were recovered, with the dominant form (trampled and entire) identified as belonging to mammoth (see Mead et al., 1986 for details about the morphology and identification). No skeletal remains of mammoths have been recovered from the cave.

3. Chronology

Paleofeces attributed to *Mammuthus* based on morphology have been recovered from a number of dry alcoves on the Colorado Plateau including Grobot Grotto, Mammoth Alcove, Oak Haven, Shrubox Alcove, Wither's Wallow, Bechan Cave, and possibly in Cowboy Cave (Agenbroad and Mead, 1992; Mead and Agenbroad, 1992). *Mammuthus* dung from Bechan Cave produced radiocarbon dates on six isolate boluses ranging in age from 11,670 ± 300 to 13,505 ± 580 uncorrected radiocarbon years before present. The contents of an additional *Mammuthus* bolus were separated (by O.K. Davis and P.S. Martin) into different taxonomic groups (e.g., grass culms, *Atriplex*, sedge achenes) and AMS dated, producing 14 ages between 11,630 ± 150 to 13,040 ± 280 yr BP (Mead and Agenbroad, 1992). The paleofecal specimen used here for the molecular analysis is from the same dung bed unit that yielded the radiocarbon dates listed above.

4. Materials and methods

A sample of the presumed mammoth bolus (based on characters outlined in Mead et al., 1986) was selected by JIM and provided to HP as a 'blind test' to determine the identification of the dung producer. All work done prior to indexing amplification was completed in the dedicated ancient DNA clean rooms of the McMaster Ancient DNA Centre (Hamilton, ON). All post amplification work was done in the McMaster Ancient DNA Centre's Bio-Bubble, a post-amplification clean room in a different building.

4.1. Subsampling and extraction

We took two subsamples (10 mg and 35 mg) from the bolus (labelled sample #SP442) and as a control, a subsample of *Mylodon darwinni* bone (40 mg) as well as an extraction control (a tube with no sample) to act as a carrier and extraction blank respectively (Appendix A: Subsampling). All samples were washed with 500 μl of 0.5 M EDTA for 1.5 h at 400 rpm and room temperature in an Eppendorf ThermoMixer followed by an 18 h demineralization with 1 ml of 0.5 M EDTA under identical conditions. Samples were then spun down, supernatants removed and then digested using 500 μl of a proteinase K buffer (Table A.1) at 50 °C with rotation in a hybridization oven for 3 h.

Supernatants were extracted with 900 μl of PCI (phenol/chloroform/isoamyl alcohol) (pH 8) and 600 μl of chloroform. Demineralized supernatants underwent an additional round of PCI extraction due to substantial discoloration of the organic fraction. Both aqueous phases were then concentrated over a 30 kDa Amicon Ultra 0.5 Centrifugal Filter tubes (EMD Millipore) and washed three times with 1xTE (pH 8–8.5). 15 μl of each extract was then additionally purified through MinElute PCR Purification Kit spin columns (Qiagen) using 6:1 vol of Buffer PB, two washes with 750 μl of

Please cite this article in press as: Karpinski, E., et al., Molecular identification of paleofeces from Bechan Cave, southeastern Utah, USA, Quaternary International (2017), http://dx.doi.org/10.1016/j.quaint.2017.03.068