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Response to Comment by Goldberg *et al.* on "DNA from Pre-Clovis Human Coprolites in Oregon, North America"

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Goldberg *et al.* use data from micromorphological and Fourier transform infrared analyses to argue that Paisley Cave pre-Clovis coprolite 1374-5/5D-31-2 is of herbivore, rather than human, origin. We argue that the diagnostic capability of the techniques used by Goldberg *et al.* are limited, and we present new genetic data that support our original claims.

Goldberg *et al.* (*1*) present micromorphological and Fourier transform infrared (FTIR) spectroscopy analyses to challenge our assertion that coprolite specimen 1374-5/5D-31-2 from Paisley Caves, Oregon, is of human origin (*2*). Their claim assumes that micromorphology, mineralogical content, and the FTIR signature of carbonate hydroxyl apatite (CAP) can clearly segregate herbivorous mammal coprolites from vegetal-containing human coprolites. To test this, we applied FTIR analyses to three human Nubian coprolites (removed from the base of the pelvises in a position consistent with postmortem evacuation of the colon) dated between 550 and 1450 C.E. These are dominated by plant matter but also contain animal bones and/or fish scale (*3*). In addition to containing an abundance of phytoliths, none yielded a CAP FTIR signature (Fig. 1), two characteristics Goldberg et *al.* use to exclude a human origin of coprolite 1374-5/5D-31-2 (*1*).

That the criteria put forward by Goldberg *et al.* (*1*) are inconclusive for identifying human coprolites is not surprising given that dietary, diagenetic, and even health variation contribute to coprolite content [e.g., (*4*)]. Two authors (Bryant and Cummings) have analyzed several hundred human and animal coprolites, including many from the Americas (*5*). Human coprolite content varies immensely and can include phytoliths, grass fibers, seeds, hair, feathers, shell, bone and insect fragments, and pollen. Of specific relevance, Bryant has noticed that in some regions, human coprolites can contain such large quantities of ground and chewed plant fibers and phytoliths that, if not for their other omnivorous diet contents, they could easily be mistaken for ruminant coprolites [e.g., (*6*)]. Others have similarly noted that the amount of calcium, phosphates, and salts in coprolites varies with diet and water source. Abnormally low calcium excretions (some <1 mg per gram of stool) have been observed in Great Basin Native American coprolites dominated by vegetal remains (*7*), and the amount of phosphates has been shown to vary with infectious diseases and pregnancy (*8*). With regard to content, although large quantities of phytoliths are consistent with herbivory, they are also consistent with diets of some humans. Native American populations from the Great Basin have been shown to eat seeds from 161 plant species, 39 of them grasses with high phytolith content in the surrounding glumes (*9*); consequently, phytoliths are common in such human coprolites [e.g., (*10*)]. It is a misconception, therefore, that humans do not eat phytolith-rich foods. Phytoliths are an abundant component of the glumes or chaff surrounding grass seeds and are also present in a variety of other foods.

Given the lack of compelling evidence that the above techniques are definitive, we present a genetic approach as an alternative test. Mammalian feces contain DNA derived from both recently ingested food and from the host (*11*). This enables us to test several hypotheses with regard to coprolite 1374-5/5D-31-2. First, should no authentic DNA survive, we should only be able to recover human DNA [the sample is contaminated with modern human DNA (*2*)]. Should ancient DNA survive, however, and should the sample be of herbivore origin, we would expect to recover both plant and herbivore DNA. In contrast, should the coprolite be derived from a

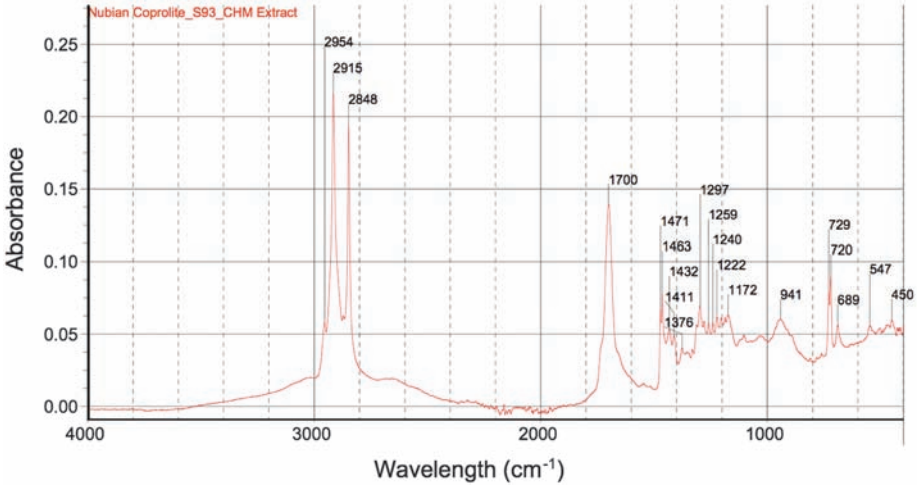

Fig. 1. Nubian coprolite from a child about 5 years of age, from an Early Christian cemetery (21-S-46), used between 500 and 750 C.E., located at Kulubnarti, on the Nile River in the *Batn el Hajar* of Sudanese Nubia. Wave numbers of infrared light are represented along the bottom, expressed as cm^{-1}. Absorbance (amplitude) is represented on the left scale. Amplitude varies with preservation, as well as original presence of the compounds. CAP yields peaks at 610 and 566 wave numbers, as well as a broad peak between 1530 and 1400 wave numbers, representing the C-O stretching vibration and a peak at 878 wave numbers, representing the C-O out-of-plane vibration. These peaks are missing from this human coprolite, which yielded pollen, phytolith, macrofloral, and/or faunal evidence for dates, sorghum, and fish scales (*3*). Although Goldberg *et al.* (*1*) note the presence of silicates in the wave-number range of 450 to 1100, we highlight that peaks in the low 1000s can also represent carbohydrates and cellulose, whereas various polysaccharides (also carbohydrates) are denoted by numerous peaks between 800 and 1170 wave numbers. Goldberg *et al.* (*1*) also mention that wave numbers 1300 to 1400 represent organics, implying that this is the only portion of the spectrum that represents organic matter. However, there are many portions of the mid-infrared spectrum that are associated with organic matter, which include the above-mentioned carbohydrates and polysaccharides, fats/lipids/plant waxes between 3000 and 2800 wave numbers, aromatic and saturated esters at multiple locations (including 1705 to 1750 cm^{-1} and 1130 to 1030 cm^{-1}), and proteins in multiple locations, including 1350 to 1490 cm^{-1}, 1500 to 1600, and others (*15*).

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Table 1. Number of sequences obtained from coprolite 1374-5/5D-31-2 and identification through BLAST against NCBI GenBank.

Primers	No. of sequences	Highest GenBank match	Similarity
16*S*mam1/16*S*mam2	53	*Homo sapiens*	94–100%
16*S*A&MFv2/	594	*Homo sapiens*	96–100%
16*S*A&MRv2*		*Bos taurus*	96–100%
RbcL F/RbcL R	No product	–	–
trnLh/trnLg†	286	*Pinus contorta*	100%
		Hesperostipa comata	100%

*Including applying human blockers (table S1). †Examples of plant findings, also found as pollen, macrofossils, and/or phytoliths in coprolite 1374-5/5D-31-2.

human that had been only eating vegetal matter, we would expect plant and human DNA, but no herbivore DNA.

To test these scenarios, we performed polymerase chain reaction (PCR) for both mammalian mitochondrial DNA (mtDNA) and plant chloroplast DNA (cpDNA) on DNA extracted from coprolite 1374-5/5D-31-2 using generic primers. The universal mammal primers yield amplicons of 140 to 142 base pairs (bp) and 69 to 70 bp, respectively, across species that include humans, rodents, carnivores, and ovibovids (table S1). Thus, both are expected to PCR-amplify DNA from all mammal species relevant to Paisley Caves. Given the concern that the coprolite is contaminated with modern human DNA, there is a risk of contaminant human DNA "masking" endogenous herbivore DNA. We therefore incorporated a 10-fold excess of human-specific blocking probes (*12*) into the shorter 16*S* analysis (table S1). A previous test of these probes in a solution of modern human and elephant DNA demonstrated that this method greatly reduces levels of amplified human DNA. The generic plant primers amplify ~140 bp and 83 to 103 bp, respectively, of cpDNA across angiosperm and gymnosperm taxa (table S1). PCR amplicons were sequenced in depth using both traditional cloning/Sanger sequencing and Genome Sequencer FLX sequencing. Assignment of the sequences to taxa was carried out using the Basic Local Alignment Search Tool (BLAST) against the nucleotide database of National Center for Biotechnology Information (NCBI) GenBank.

The sequence data are inconsistent with an herbivorous origin for the coprolite. All identifiable sequences (45 of 53) from the longer 16*S* mtDNA fragment were human (table S1). Given that the plant cpDNA PCR targeting a similar length product (~140 bp) was unsuccessful, these are likely contaminants (table S1). These results indicate that the endogenous DNA in coprolite 1374-5/5D-31-2 is heavily fragmented, typical of ancient DNA and consistent with our original study (*2*). More important, the 594 sequences amplified from the shorter 16*S* mtDNA fragment only contain identifiable sequences of human and *Bos taurus* (cow) (table S1 and fig. S1); the latter was not present in the Americas before European contact and is a common contaminant in laboratory reagents (*13*). No additional taxa were found when applying the human primer blocking approach (Table 1). The cow sequences differ distinctly from bison, the closest native relative, differing by at least two bison-specific mismatches (over 30 bp) (fig. S1). *B. taurus* is always the highest BLAST match against GenBank (Table 1), and the few differences observed (fig. S1) are as expected from sequencing errors or DNA damage.

The absence of mtDNA derived from a putative authentic herbivore is unlikely to be due to DNA degradation, because the shorter cpDNA primers yielded 286 sequences (ranging from 83 to 103 bp in size), relating to plants such as *Pinus* and the grass *Hesperostipa*—taxa that are found among the macrofossils, pollen, and/or phytoliths in coprolite 1374-5/5D-31-2 (Table 1). Given this, and the other evidence of mammalian mtDNA survival in these, and other, even older coprolites from similar dry caves of the southwest (*14*), ancient DNA survival seems certain. In conclusion, our findings are inconsistent with specimen 1374-5/5D-31-2 being of herbivore origin as claimed by Goldberg *et al*. (*1*) but consistent with its being human derived.

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Supporting Online Material

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Fig. S1
Table S1
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