

# Biocultural Implications from Scanning Electron Microscopy of Prehistoric Human Dental Calculus, Ohio

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## Abstract

Dental calculus, though it provides a unique calcium reservoir of bacteria and dietary debris seldom preserved elsewhere in the archaeological record, is an overlooked source of data in anthropological literature. Small particles of archaeobotanical debris can adhere and become incorporated into unmineralized plaque on teeth during mastication. The physical inclusion of bacteria and archaeobotanical debris in the matrix of calculus is direct evidence for the contemporaneity of both structures, because the mineralization of dental plaque occurs only in the presence of saliva. This research reassesses the value of collecting human dental calculus from archaeological remains and provides insight into the oral health and diet of prehistoric Native Americans in northern Ohio.

Samples of dental calculus (n=19) were collected from the Danbury site (33OT16) in Ottawa County, Ohio inhabited from the Late Archaic through Late Prehistoric periods. Analysis yielded a variety of noticeable inclusions, including both mineralized bacteria, calcium-phosphate (CaP) crystalline structures, and numerous phytoliths (amorphous, grass, and non-grass types). Most importantly, cellulose fibers consistent with cotton were found in the calculus of four Late Woodland individuals and the implications of long-distance interaction suggested by this find are discussed. SEM analysis of was limited, however, by the disaggregation of debris and the lack of standardization of phytolith and starch grain classification.

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## Introduction

Dental calculus is a mineralized dental plaque, the etiology of which is not fully understood, covered in life by an unmineralized plaque biofilm with active bacteria. Though calculus provides a unique calcium reservoir of bacteria and dietary debris seldom preserved elsewhere in the archaeological record, is an overlooked source of data in anthropological literature. Small particles of archaeobotanical debris can adhere to and become incorporated into the unmineralized plaque on teeth during mastication and oral manipulation. The physical inclusion of bacteria and archaeobotanical debris in calculus is direct evidence for the contemporaneity of both structures, because the mineralization of dental plaque occurs only in the presence of saliva. An innovative approach to the study of ancient diet and oral health is microscopy analysis of dental calculus. If the microscopic inclusions within dental calculus can be identified, then analysis of calculus from archaeological remains can provide direct insight into the oral health, diet, and paleoethnobotanical history of prehistoric individuals and populations.

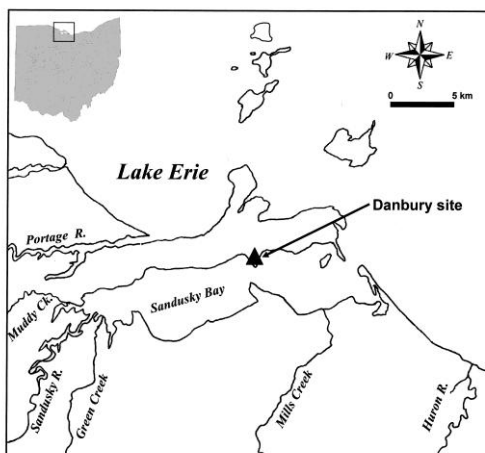


Figure 1. Location of the Danbury site (33OT16). Adapted from Redmond, 2006.

Here I report the results of an analysis of dental calculus applied to specimens from the Danbury site (33OT16), a pre-Columbian multicomponent habitation site, located on the south shore of Lake Erie in Ottawa County, Ohio (Fig. 1). There have been 42

burial features (BF), containing 152 individuals, excavated from the Danbury site since 2003. Accelerator mass spectrometry (AMS) dating of carbon and bone collagen samples indicate

habitation at the site during the Late Archaic (2900-2500 B.C.), Early Woodland (A.D. 800-1000), Late Woodland (A.D. 900-1100), and Late Prehistoric (A.D. 1450-1550) periods.

The site has a wide range of nonperishable burial goods, the majority being marine shell beads (Redmond, 2006a, 2006b). Prominent among these artifacts and associated with the three

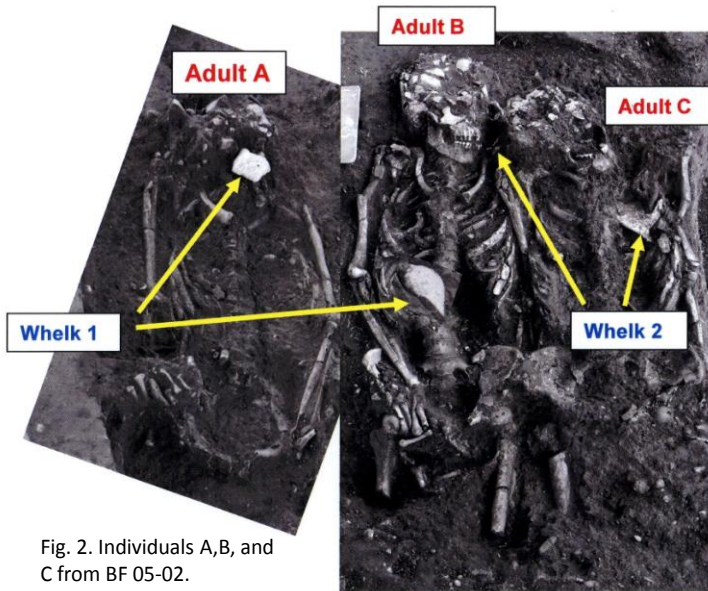


Fig. 2. Individuals A,B, and C from BF 05-02.

individuals from BF 05-02 (Fig. 2), are portions of two drilled pendants made from lightning whelk shell (*Busycon sinistrum*), a species whose ranges extend from the Gulf of Mexico to the southern Atlantic (Fig. 2) (Trubitt, 2003; Redmond, 2006b). AMS dating of bone collagen samples from individuals

B and C from BF 05-02 produced two sigma date ranges of Cal. A.D. 880-1010 (Beta-207989) and Cal. A.D. 880-1020 (Beta-207990), respectively. These dates place the individuals and the associated shell artifacts in the Late Woodland period, a time when such artifacts appear to be rare in the Ohio region (Seeman and Dancey, 2000:601; Stothers and Abel, 2002:76). Consequently, the whelk shell burial inclusions from the Danbury site provide some of the best documented evidence of exchange between the Late Woodland societies of northern Ohio and the Gulf Coast. Results from this study not only provide valuable information about the diet and health of Late Woodland and Late Prehistoric Ohioans, but shed new light on the interactions of Native Americans throughout North America in those periods.

## Materials and Methods

A total of 19 supragingival dental calculus specimens were collected from 8 individuals from the Danbury site. Demographic information about each individual was assessed using Standards techniques (Table 1) (Buikstra and Ubelaker, 1994). Calculus was identified as a light buff to chocolate-brown stained encrustation above the gingival line of each tooth. The amount

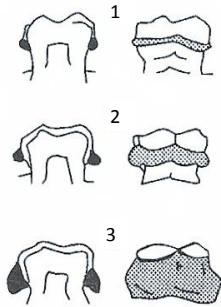


Figure 3. Brothwell's (1981) dental calculus scoring index.



Figure 4. Dental calculus before sampling from BF 05-06

of calculus was scored according to Brothwell's (1981) index and the location of collected calculus was recorded (Fig. 3). Each tooth was cleaned with a soft toothbrush to eliminate loose debris. Pieces of calculus were removed with a dental scalar directly

into sterilized vials. Each specimen was diluted with 5% hydrochloric acid (HCl) to remove surface carbonates and postmortem contamination.

Specimens were allowed to digest for 2 hours stored within a dessicator cabinet. After drying, specimens were affixed to aluminum stubs with sterile tweezers

and carbon tape. Specimens were sputter-coated with two layers of carbon and examined using a JEOL JSM 820 scanning electron microscope (SEM) under 15Kv. Specimens were scanned at both low and high magnification. Micrographs were obtained using Oxford Instrument's INCA platform for SEM imaging. Phytoliths were easily identified from their silica content via x-ray microanalysis. Inclusions were identified using reference collections by comparison of morphological features.

## Results and Discussion

Inclusions and demographic data are shown in Table 1. Due to the disaggregated nature of the calculus matrix, not all specimens yielded identifiable inclusions. Calculus was most abundant on the lingual surfaces of the mandibular teeth and on the buccal surfaces of the maxillary teeth. Given the frequency of caries in the population and the low overall calculus scores (1), there does not seem to be a correlation between calculus and caries development. Low calculus scores also suggest the consumption of abrasive foods, such as a diet rich in maize (primarily abrasive due to grit from processing), while diets high in protein provide an alkaline environment conducive to plaque mineralization. Isotopic results of individuals A and B from BF 05-02, which indicate the consumption of significant amounts of maize ( $^{13}\text{C}/^{12}\text{C}$  ratio of 13.2‰ - 12.7‰) and aquatic fauna ( $^{15}\text{N}/^{14}\text{N}$  ratio of 11.31‰ - 13.61‰), support this.

A variety of calcium-phosphate crystals were visible in the calculus matrix (Fig. 5). The exact composition and sequence in which these types of crystals form is unclear especially from archaeological populations. The presence of these OCP and DCPD crystal structures and their lack of involvement with any adjacent bacteria suggests these samples represent immature calculus which developed six or less months prior to the time of death of these individuals. The oral flora of archaeological populations is almost entirely unknown. Calcified chained cocci (possibly *Streptococcus*) and bacillus bacteria outlines were present in several samples (Fig. 6). The bacteria are homogenous and represent normal healthy oral flora. The mineralization of some of these microorganisms suggests the maturity of those specimens to be six months or older prior to the death of the individual. The maturity of the calculus allows for a time frame of inclusion of dietary debris.

BF No.	Sex	Age	Tooth	Score	Loc.	Inclusions				
						Bacteria	CaP Crystals	Phytoliths/ Starch	Unid. Fiber	Gossypium Fiber
05-02A	F (P)	42-52 (A)	LrP4	1	L	x				x
05-02B	M (P)	29-33 (A)	UrM1	1	B					
			UrM2	2	B	x		x		
			UrP3	1	B			x		x
			UrI2	1	B					
			UrC	1	B				x	
05-02C	M (P, Pb)	35-40 (Pb, A)	UII2	1	L		x			x
			UrM2	1	B					
05-06	F (P, Pb)	25-30 (Pb, A)	LIM2	1	B		x			
			UIM1	1	B					
			LrC	1	L	x				
			LrI2	1	L				x	
			LIC	1	L				x	
05-07	M (O)	Adult (O)	LrP3	1	L					
				1	L		x			
			LM1	2	L			x	x	x
06-01B	F (P)	12-14 (D, F)	UIP4	1	B					
06-02A	M (P)	13-14 (D, F)	UrI1	1	B					
06-03A	M (P, Pb)	50+ (Pb, A)	LI2	1	L	x	x			

Table 1. Data including BF (burial feature) number, sex ( P= pelvis; Pb= pubis; O=other) and age (P=pelvis; Pb=pubis; A=auricular; O=other; D= dental stage; F= epiphyseal fusion), calculus score (1=mild, covering less than one third of the crown; 2=moderate, covering from one third to two thirds of the crown), location of the calculus on each tooth (L=lingual surface; B=buccal surface), and the presence (indicated by 'x') of inclusions such as bacteria, calcium phosphate (CaP) crystals, phytoliths or starch grains, unidentified fibers, and *Gossypium sp.* fibers within the calculus specimens.

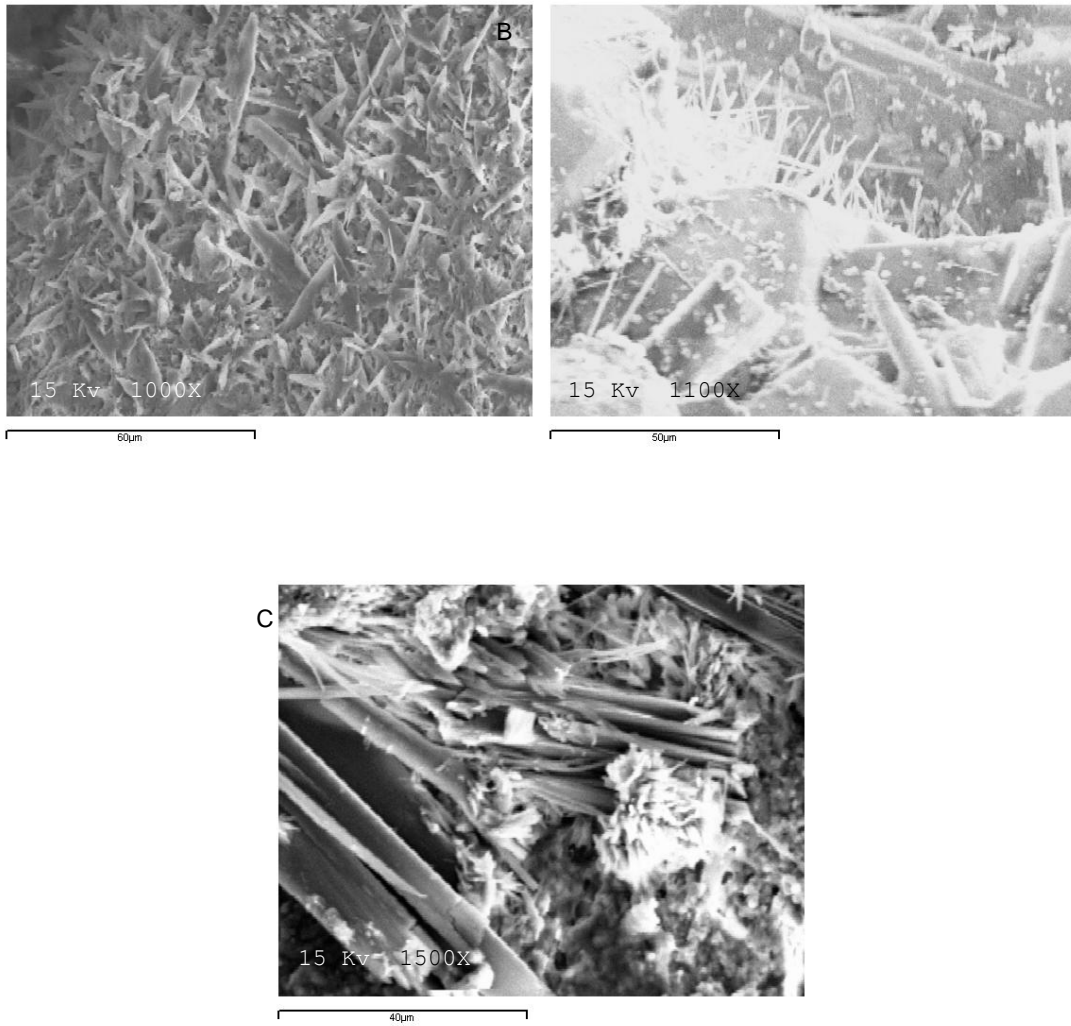


Figure 5. SEM micrographs of calcium-Phosphate crystal structures within the dental calculus matrix from: A) Danbury BF 05-02B (Sample 3); B) Danbury BF 05-02C (Sample 1); C) Danbury BF 06-02A (Sample 25).

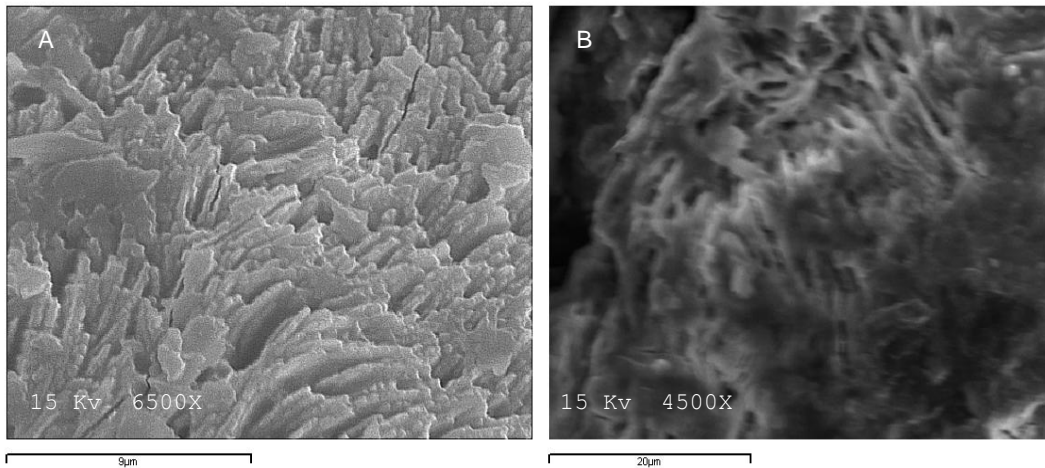


Figure 6. SEM micrographs of mineralized bacteria and bacteria outlines. A) Chained cocci bacteria (possibly *Streptococcus*) from Danbury BF 05-02B (Sample 5), B) *Bacillus* bacteria outlines from Danbury BF 05-02A (Sample 25).

The majority of recovered phytoliths were amorphous, grass, and non-grass types, but were usually too broken or covered with calculus for clear genus identification. Both monocot and dicot phytoliths were present. These included short-cell grass rondel forms, which occur abundantly in the *Eragrostoidae* family and hair cells phytoliths, commonly found in the epidermis of leaves or seeds (Piperno, 1988), which are most commonly found in dicotyledons and herbaceous and woody plants (Bozarth, 1992).

Cellulose fibers were also embedded within the calculus matrix of individuals A, B, and C from BF 05-02 and from BF 05-07 (Fig. 8). These fibers show the characteristic flattened, ribbon-like, and elongated distinct appearance of cotton seed fibers (*Gossypium spp.*), with raised edges, rolled in a helicoidal

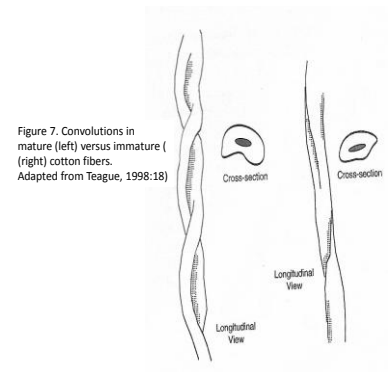


Figure 7. Convolution in mature (left) versus immature (right) cotton fibers. Adapted from Teague, 1998:18)

manner with mostly underdeveloped convolutions (Fig. 7). Of the four domesticated cotton species found worldwide, two have been domesticated in the New World. *Gossypium hirsutum* is



widely distributed in Central and South America and the Caribbean. *Gossypium barbadense* is centered in northwestern South America but overlaps with the range of *G. hirsutum* in the Caribbean. By A.D. 500 *Gossypium hirsutum* is first seen in the Gila and Salt River regions of Arizona (Kent, 1983; Lee, 1984; Teague, 1998). While there is some archaeological evidence of contact between the Caribbean islands and southern Florida during the Late Prehistoric period (A.D. 1000-1650), there is no evidence of pre-Columbian cotton with eastern North America (Bullen, 1974; Seidemann, 2006). Knowledge of the role and extent of cotton exchange between Southwestern Pueblo and Plains groups and others has been limited due to a lack of comprehensive archaeobotanical evidence.

Though societies in Ohio were producing yarns made from twinned native plant fibers at least as early as the Middle Woodland, it was quite unexpected to find multiple fibers with the physical attributes of mature and immature *Gossypium spp.* embedded in the dental calculus of four Late Woodland individuals from the Danbury site. The cultural implications of this discovery are far-reaching and suggestive of long range interaction between either Southwestern Pueblo groups, or northern Mesoamerican societies, and groups living in northern Ohio during the Late Woodland period, a time in Ohio when trade of exotic goods is generally regarded as having been attenuated (Seeman and Dancey, 2000).

Several explanations are possible for how the cotton fibers became embedded into the dental calculus of these individuals. Fluffing (separating the fibers) is an important part of spinning preparation and without modern carding tools, pulling fibers while anchoring with ones teeth is an easy solution. It is also possible that while splitting plied cotton fibers by hand the fibers were anchored in the mouth and became incorporated into calculus. Most likely their presence represents the reprocessing of cotton fibers for the production of textiles or fishing line.

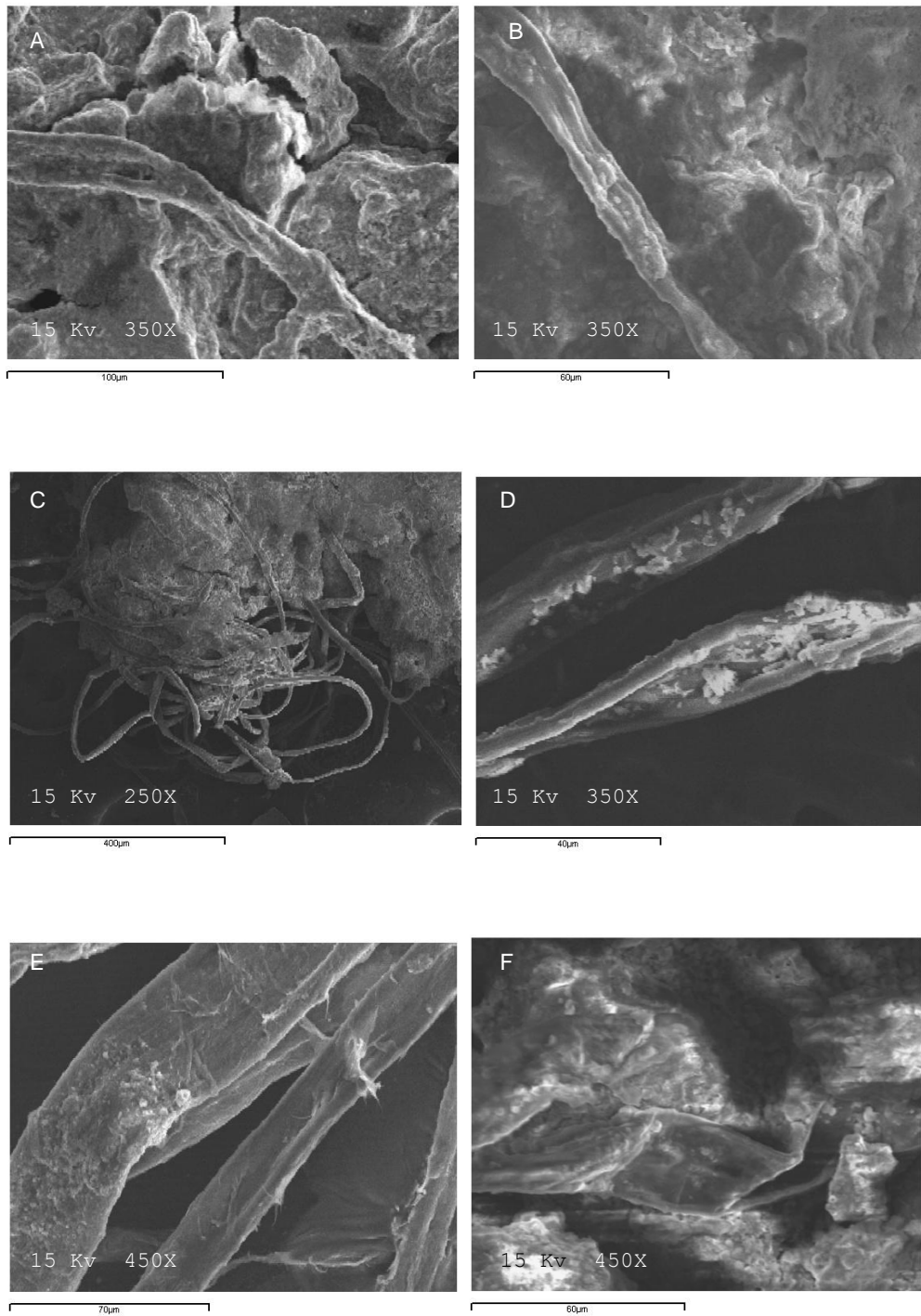


Figure 8. SEM micrographs of cotton fibers from: A and B) BF 05-02B; C) 05-02C; D) BF 05-02A; E) and F) BF 05-07.

The interaction between northern Ohio and the Southwest or Plains is best supported by the evidence of both cotton and marine shell exchange between these groups. The whelk shells do not point to the Southwest as a source *per se*, rather they point to another avenue of long distance exchange (from lower Great Lakes to the Gulf of Mexico) which was most likely channeled, along with cotton, through an intermediary like the Spiro Mounds site (A.D. 850 to 1450 ) in Oklahoma (King *et al.*, 1981). The Spiro site is considered one of the most important locations through which exotic goods were funneled, and there have been many artifacts from Spiro indicating long-distance trade including shell cups made from lightning whelks (*Busycon sinistrum*) from the Gulf of Mexico (Phillips and Brown 1978:26) just like the pendants from BF 05-02 from Danbury and lithics from the Spiro were made of chert from the Cahokia, Illinois region. Although the precise variety of cotton found at the Danbury site and the circumstances behind such an undertaking are unclear, the ramifications of such broad-based exchange in prehistoric North America are far-reaching.

## **Conclusions**

The analysis of human dental calculus provides an innovative and direct assessment of ancient diet and has advantages in comparison to stable isotope studies, because it provides information about foods eaten a short time before death (from days to months), is non-destructive, less expensive, and generally more precise with regard to identifying specific plants. The methodology used in this paper, however, provides the potential for analysis of plant materials that would otherwise be digested or lost from employing the multiple washes or higher hydrochloric acid concentrations suggested by other authors.

It is hoped that the data presented here will alert the anthropological community to the value of microscopic analysis of dental calculus in supplementing our understanding of

paleoethnobotanical histories. It is anticipated that additional studies of this kind will result in the identification of cotton at sites outside the Southwest, and yield more detailed results beyond those of this report. In light of these new data, further research is definitely needed to gain a more comprehensive view of the extent trade of exotic goods in prehistoric North America.

While the identification of plant fibers from archaeological contexts is essential for understanding ethnobotanical history, identification is limited by the wide range of plant material in use, the absence of plant parts used for standard identification, and the alteration of fibers from processing, use, and degradation within the burial environment.

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