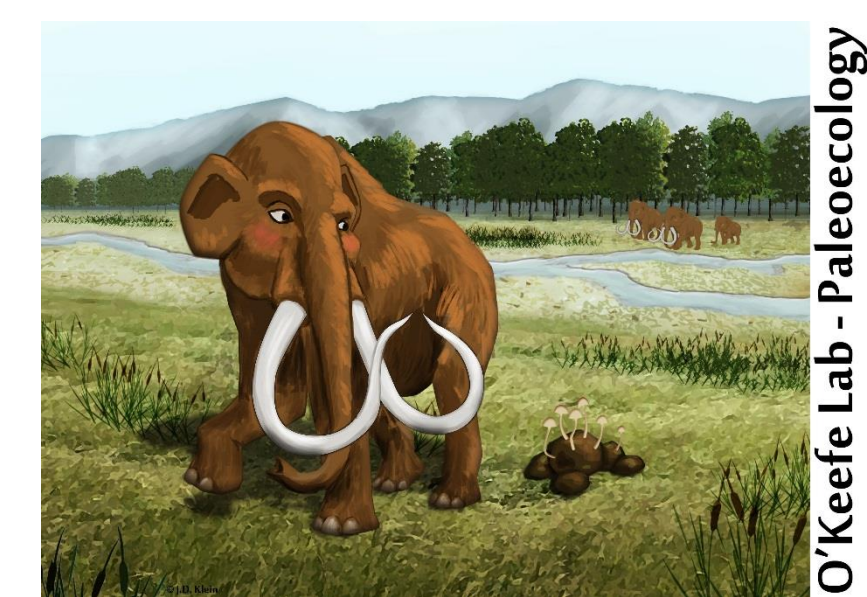
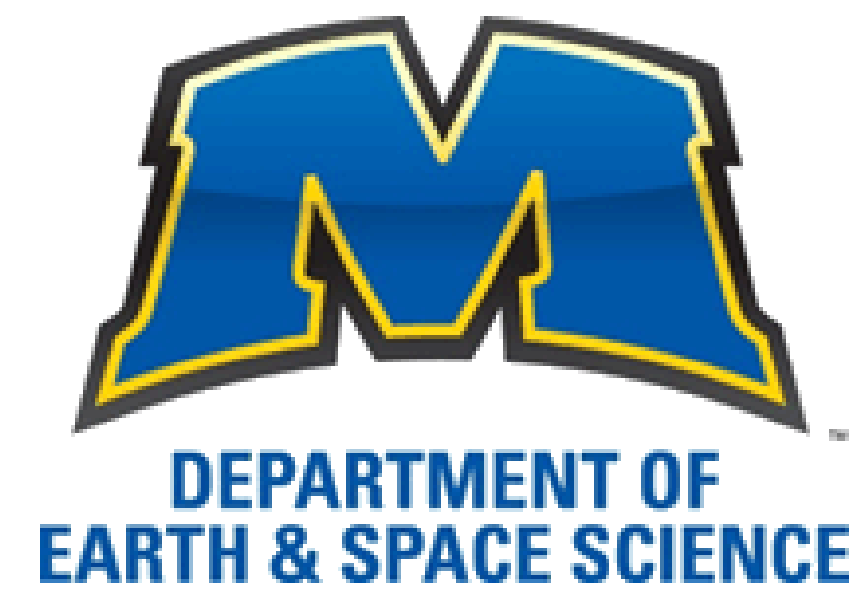


# Palynology of Big Bone Lick: Evidence for Megaherbivores and High Salinity

Sweeney, Carissa\*<sup>1</sup>, Jett, Tucker\*<sup>1</sup>, O'Keefe, Jen<sup>2</sup> (mentor)

<sup>1</sup>The Craft Academy for Excellence in Science and Mathematics

<sup>2</sup>Geology Program, Department of Earth and Space Science, College of Science

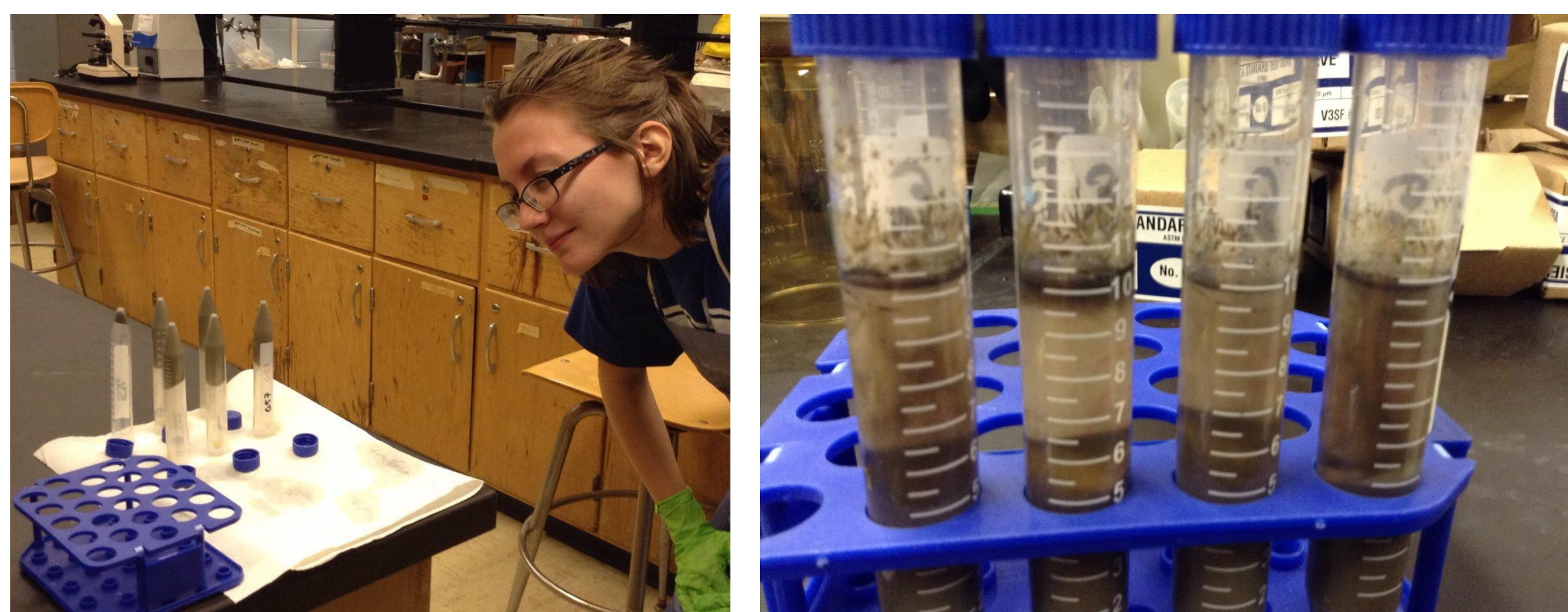


## Abstract

We know a lot about the plants that grew at Big Bone Lick State Historic Site in the Pleistocene because of macrobotanical remains, but no previous study has successfully extracted pollen from the sediments. The enzymatic technique developed in the OPaL lab was utilized and resulted in successful extraction of pollen, spores, and algal remains. These microfossils include abundant dung fungi, from which megaherbivore density can be estimated. Additionally, the first direct evidence for both provenance of the sediment and salinity of the environment at the time of deposition are present. This project reflects a major advance in our understanding of the paleoecology of the birthplace of vertebrate paleontology in the United States.

## Processing Success

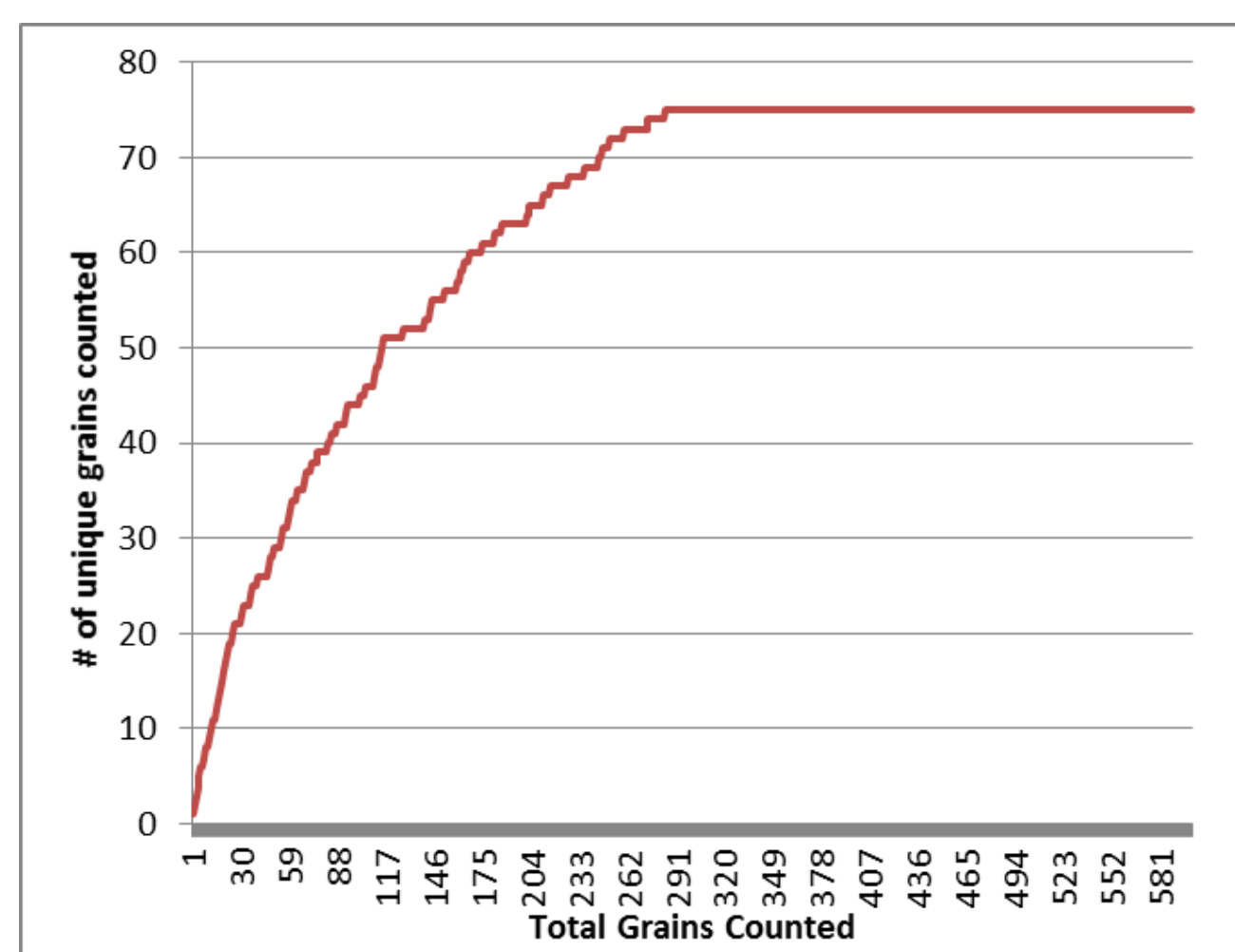
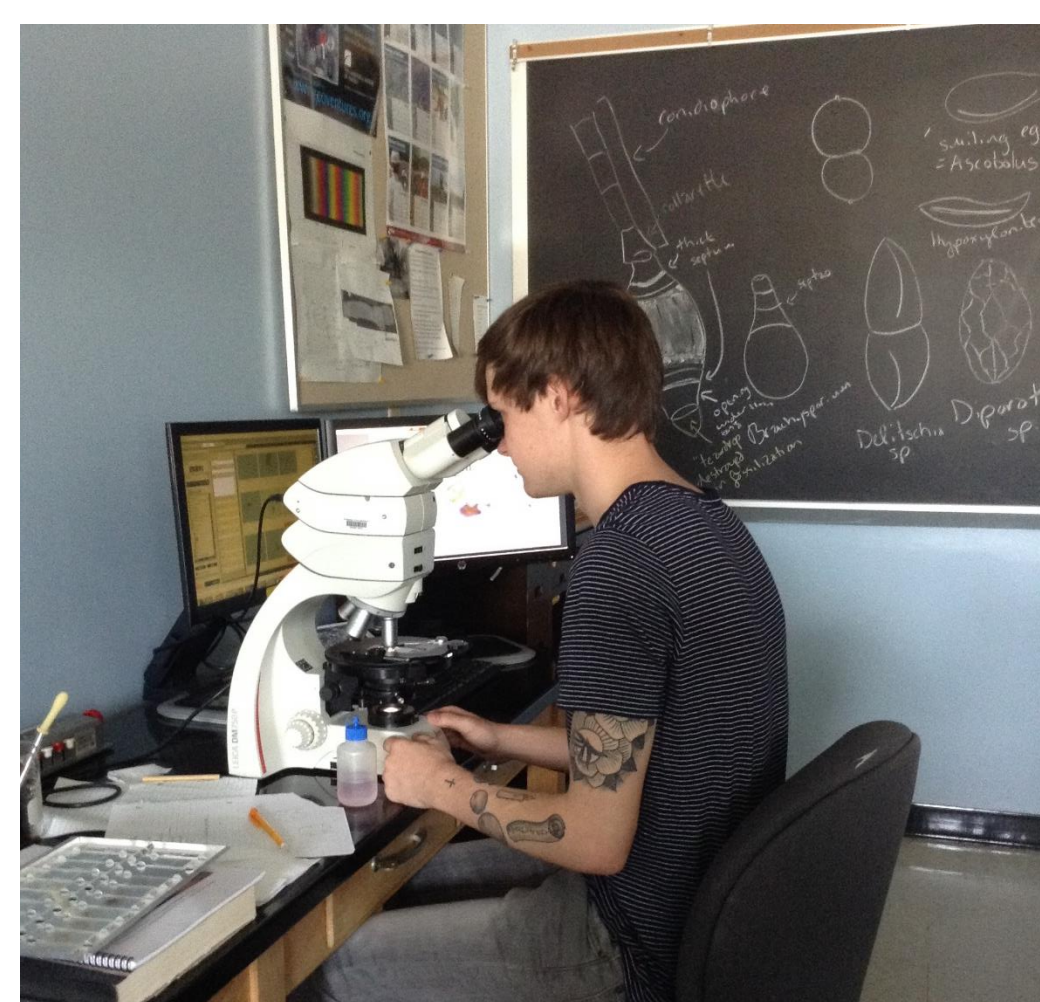
To date, no successful palynology has been completed for the Quaternary and early Holocene sediments in or near Big Bone Lick (Tankersley et al., 2015; Von Mann et al., 2005); in fact, most investigators thought processing for palynology in this area was a wild goose chase. We processed (Figure 2) our samples using the enzymatic methods developed by O'Keefe and Wymer (2017) and refined by Black et al. (in preparation), which resulted in abundant recovery of diverse (Figure 3) pollen, plant spores, dinoflagellates, algae, diatoms, tardigrade eggs, protist eggs, thecate amoebae, and fungal remains...including spores from a fungus known only from goose pool!



**Figure 2.** We didn't know if processing was successful until after very nerve-wracking heavy-density separation. The brown sludge on top of the fluid in the test tubes meant success!

## Analyzing Our Samples

We spent many intense hours learning to tell palynomorphs from junk, and training our eyes for microscopy (Figure 3). By the end of March, we'd found over 180 taxa in our eight samples. Counting was completed on a Leica DM750-P Microscope and images were captured using a Leica ICC50 W camera and annotated using Leica LAS version 4.8 software. Counts were tallied in Microsoft Excel®, and continued for each sample until a rarefaction curve reached its asymptote, indicating that we had documented 99% of the biodiversity preserved in the sample (Figure 3).



**Figure 3.** Left, examining a slide in the microscopy lab; right, an example rarefaction curve showing the high diversity and abundance found in sample 550.

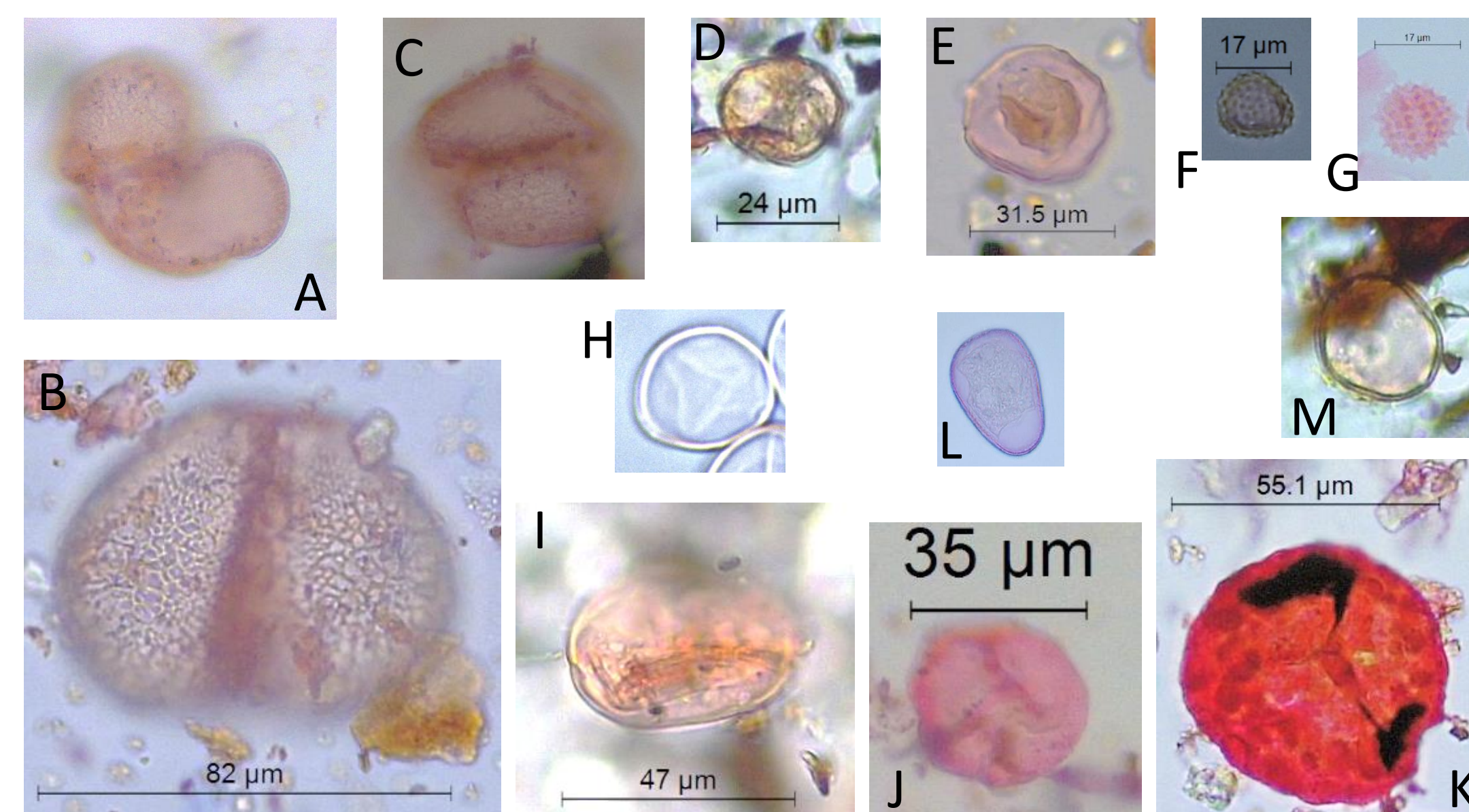
## Introduction

Big Bone Lick State Historical Park is internationally known as the "Cradle of American Paleontology" (Hedeon, 2010). It is especially known for bones of Pleistocene-age mammals, but also plant remains (primarily seeds, twigs, nuts, leaves, and charcoal), as well fish and invertebrate remains. To date, attempts at palynological analyses of the sediments at Big Bone Lick have been unsuccessful. Our goal was to process samples using modern techniques to see if they were productive.

Eight sediment samples from "profile 2" of the 2012 University of Cincinnati archaeological dig at Big Bone Lick were provide for this preliminary study. Charcoal from two of the samples provided rangefinder dates, indicating that sediments were deposited between 25,550 to 24,550 years BP and 18,560 years BP (Matthew Maley, pers. comm., 2016; Tankersley et al., 2015), placing our samples in the early Middle Pleistocene or "Oldest Dryas."

## Dominant Plant Palynomorphs

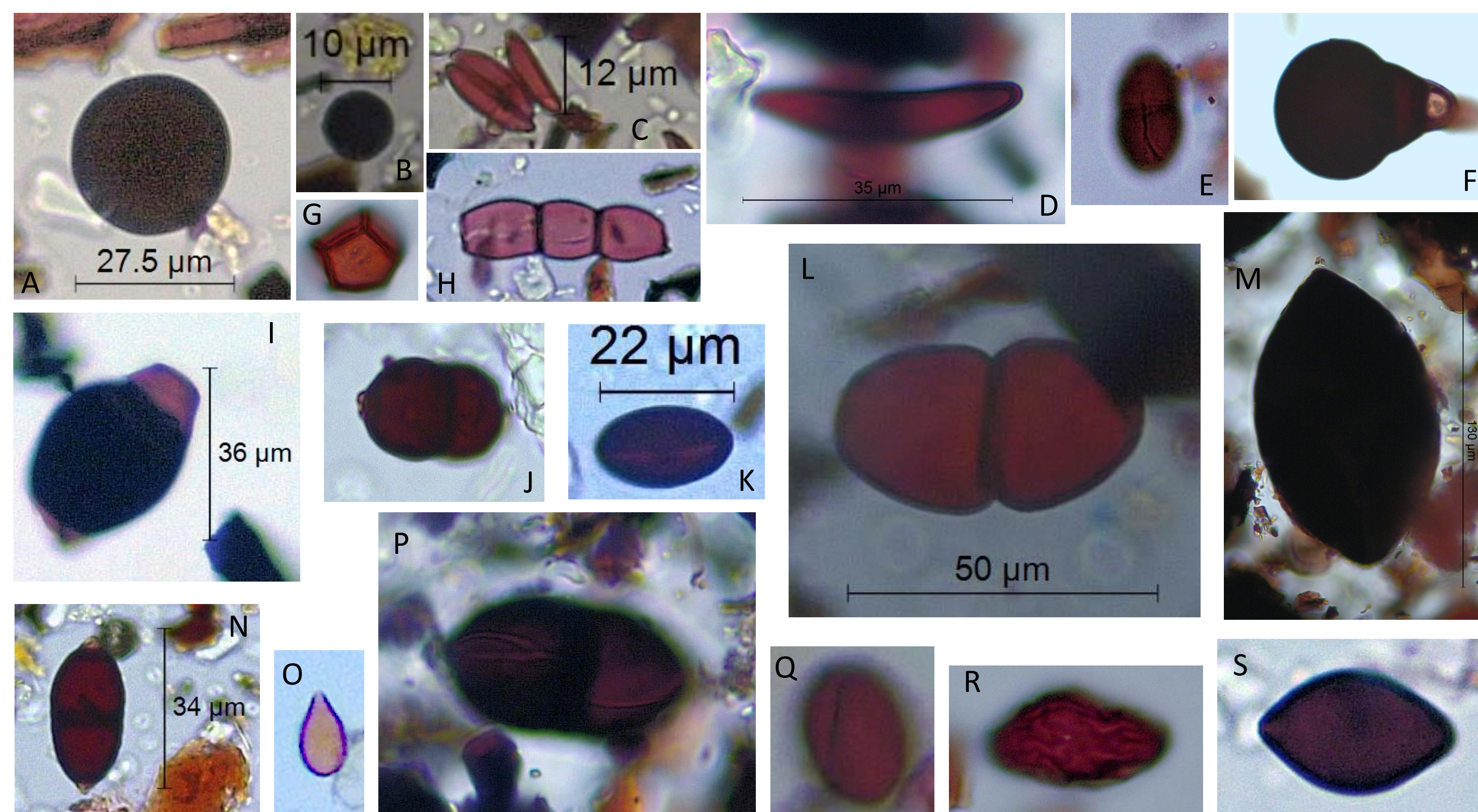
All of our samples were dominated by varying mixtures (Figure 5) of Pine (*Pinus*), Fir (*Abies*), Spruce (*Picea*), Oak (*Quercus*), Hickory (*Carya*), and other tree pollens, while understory plants included Ragweed (*Ambrosia*), Black-eyed Susans (*Rudbeckia*), Nettles (*Urtica*), Chain Ferns (*Woodwardia*), Bracken Ferns (*Pteris*), Water-fern (*Salvinia*), and Sedges (*Carex*). Surprisingly, grasses (*Poaceae*) were rare.



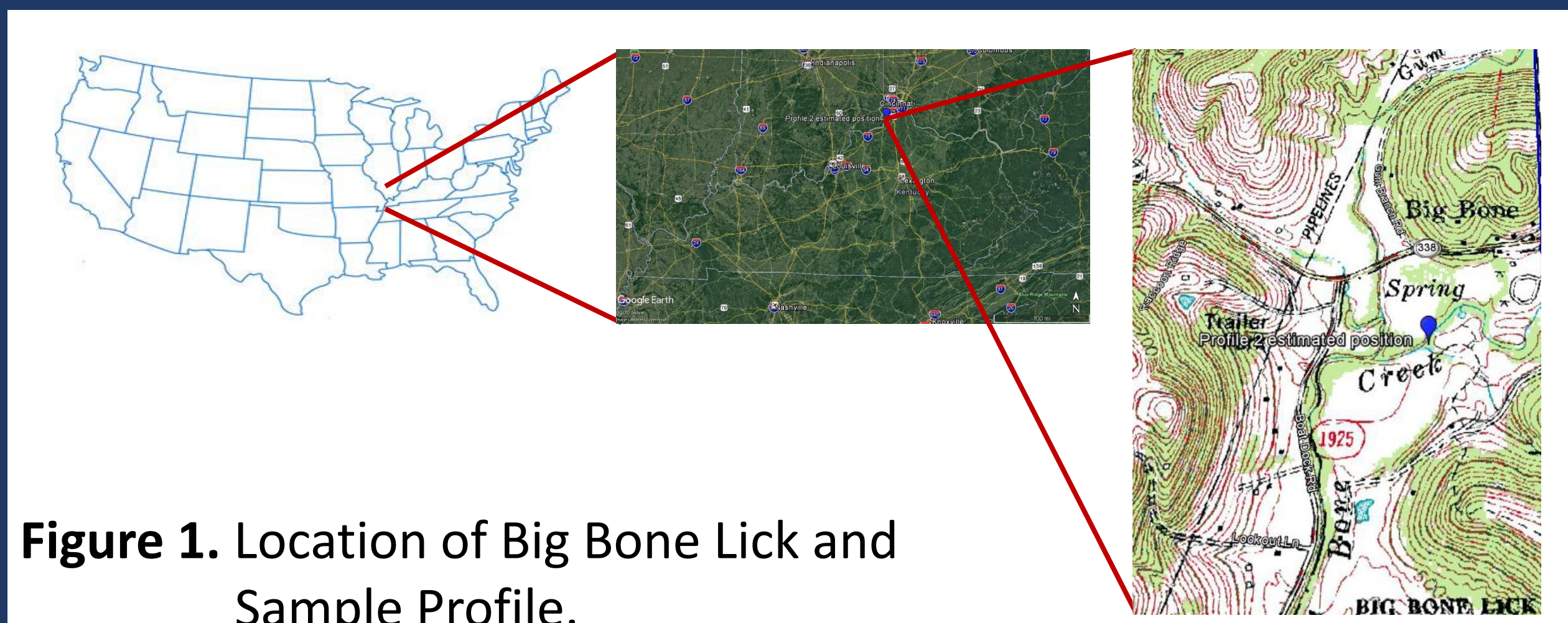
**Figure 5.** Dominant Plant Palynomorphs. A) *Pinus*, B) *Abies*, C) *Picea*, D) *Quercus*, E) *Carya*, F) *Ambrosia*, G) *Rudbeckia*, H) *Urtica*, I) *Woodwardia*, J) *Pteris*, K) *Salvinia*, L) *Carex*, M) *Poaceae*.

## Fungi

Fungal remains predominated the samples. They range from mycorrhizal forms to saprophytic forms to coprophilous (dung) fungi. The coprophilous fungi point toward the existence of a variety of other animals, ranging from mammals (Gill et al., 2013) to geese (*Saccobolus*; L.Novak, pers comm. 2017). Of great importance is the presence of *Lacrimasporites*, which is a salt-marsh indicator.



**Figure 9.** Representative fungi from Big Bone Lick. A) *Glomus*; B) *Monoporisporites*; C) *Hypoxylonites*; D) *Rosellinia*; E) *Pseudodelitschia*; F) *Brachysporium* type 1; G) *Thecaphora*; H) *Sporormiella*; I) *Brachysporium* type 2; J) *Puccinia*; K) *Semidelitschia*; L) *Dyadosporites*; M) *Podospora*; N) *Caryospora*; O) *Lacrimasporites*; P) *Delitschia feracea*; Q) *Saccobolus*; R) *Diporotheca rhizophilia*; S) *Cercophora* cf. *C. coronate*.



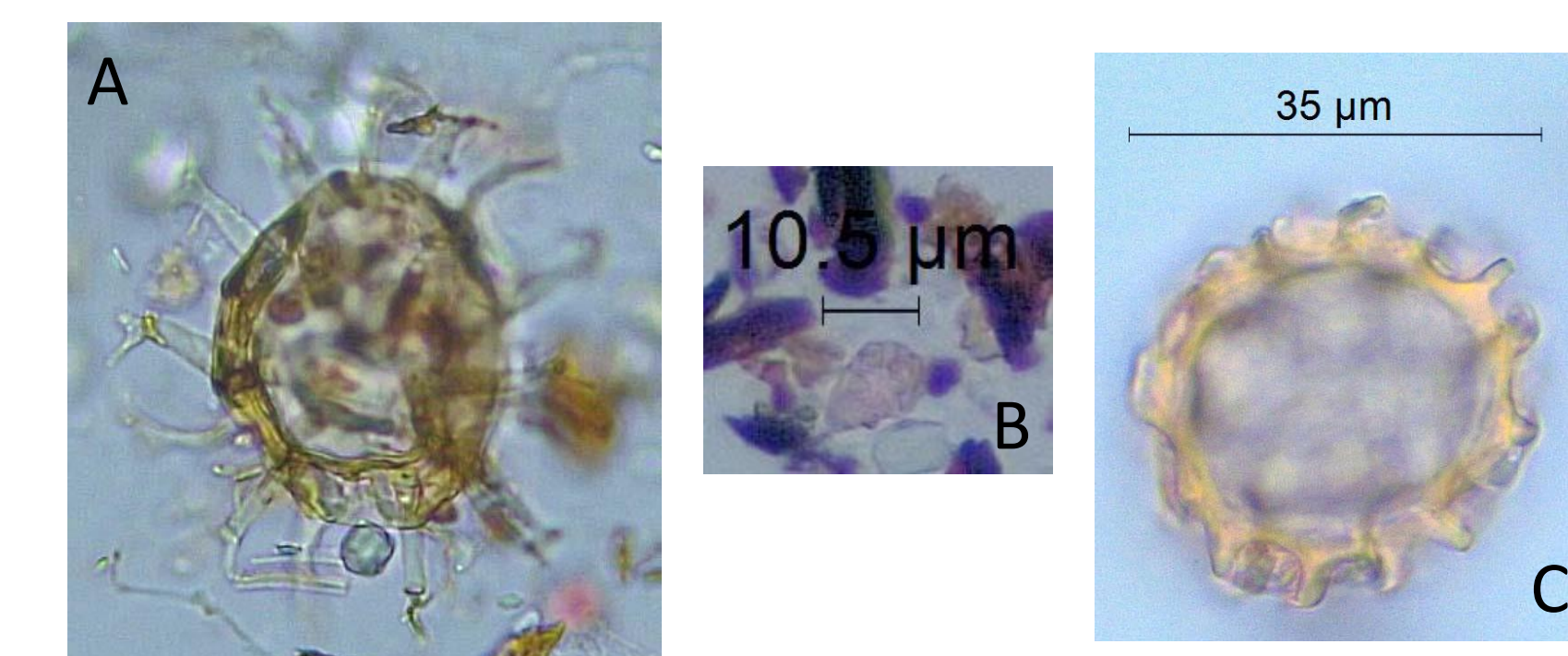
**Figure 1.** Location of Big Bone Lick and Sample Profile.



**Figure 4.** Tucker (left) and Carissa (right) with their chalkboard of (primarily) fungal spores found in Big Bone Lick sediments.

## Evidence for Saline Conditions

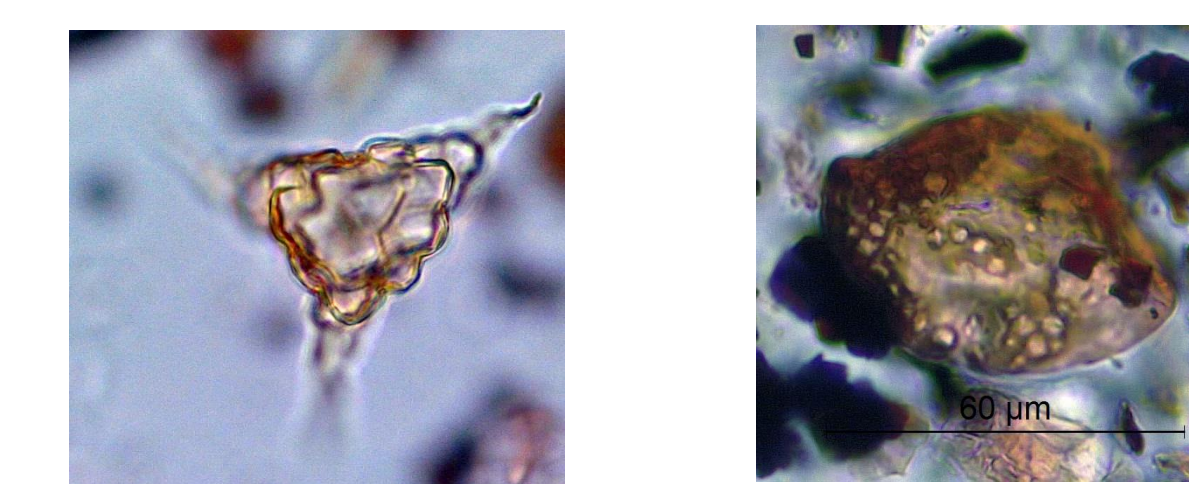
It has been presumed that saline conditions existed at Big Bone Lick for tens of thousands of years (Tankersley et al., 2015). We found important palynomorphs better known from saline lakes & shallow seas (Figure 6). These are the first direct indicators of very saline conditions present at Big Bone Lick during the Oldest Dryas.



**Figure 6.** Saline-indicator palynomorphs. A) the dinoflagellate *Spiniferites*, B) unnamed thecate amoebae, C) Saline-tolerant alga.

## Sediment Source

While some of the sediment is known to be reworked from the underlying Kope Formation (Ordovician), we found two palynomorphs that indicate at least some re-working from upper Devonian – Mississippian age sources.



**Figure 8.** *Veryhachium* sp. (left) and *Tasmanites* sp. (right)

## Acknowledgements

- Matthew P. Maley, MS, CEE, for samples and reports on previous analyses.
- "Rosie," for her long-suffering service to palynology completed by the OPaL group.
- Harry Arthur Dade, British mycologist who painstakingly painted the first "dungscape" in 1966, our inspiration for study of the fungi at Big Bone Lick.

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