Supplement 4. Microbotanical Sample Extraction and Slide Mounting Procedures.

Sample numbers, weights, and volumes were recorded prior to pretreatment. To deflocculate the calculus and ease dispersal, ~1mL of 0.1% Alconox solution was added to each sample; Alconox solution was substituted for 10% sodium hexametaphosphate ((NaPO3)6) solution (the surfactant used by Henry and Piperno 2008) as the former surfactant is commonly used in the UCSB ISL to deflocculate sediment samples prior to microbotanical analysis and thus was more readily available. Tubes were then capped and stored in the fume hood for 24 hours. Samples were sonicated in distilled water for five minutes and centrifuged for three minutes, after which each supernatant was pipetted off using a separate glass Pasteur pipette that had already been sterilized in the UCSB ISL according to standard protocols (pressure cooking in distilled water for two hours and then drying under the fume hood in a sterile environment; Crowther et al. 2014).

Tube contents were rinsed in distilled water in two repetitions of the following protocol: ~1mL of distilled water was added, tubes were vortexed briefly then centrifuged for three minutes, and the supernatant was removed with a sterile pipette. Chemical pretreatment consisted of the addition of ~1mL of 10% hydrochloric acid (HCl) to each tube, after which they were stored in the fume hood for 24 hours. The hydrochloric acid was removed by a series of two rinses in distilled water, identical in procedure to the first set of water rinses and ending with the removal of the final supernatant.

To suspend the microbotanicals for slide mounting, one to two drops of 1:1 glycerin (C3H8O3) to distilled water solution were added to each microcentrifuge tube and the entire contents was mixed using a metal toothpick sterilized via the lab procedures outlined above. Each extract was examined in its entirety. The contents of each tube were drawn up into a glass Pasteur pipette and the liquid was deposited on a series of sterilized slides, each of which was covered with a sterilized glass cover slip. Edges of the cover slip were sealed with clear nail polish and left to dry for a few minutes.

**References Cited**

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