Black, S.L.
1986 *The Clemente and Herminia Hinojosa Site, 41 JW 8; A Toyah Horizon Campsite in Southern Texas.* Center For Archaeological Research. The University of Texas at San Antonio, Special Report 18.

BIOSILICA ANALYSIS (Ralph L. Robinson)

Poor preservation of organic microfossil and macrofossil evidence of prehistoric vegetation has hampered efforts to reconstruct paleoenvironments in many areas of Texas, including the geographical area in which the study area is located. The Hinojosa site, 41 JW 8, is in an ecotone between the western margin of the Western Gulf Coastal Plain and the eastern margin of the Rio Grande Plain. Gould (1969) placed Jim Wells County in two vegetational regions, with a small portion of the eastern edge of the county in the Gulf Prairies and Marshes Region and the greatest part of the county in the South Texas Plains Region. Ecotones are ideal research areas because of the dynamic equilibrium of biotic communities.

Macrofossil evidence of silica accumulator biota--biosilica, or phytoliths as they are commonly known,--was found to be well preserved and abundant in the sediments of the Hinojosa site. Scanning Electron Microscopy (SEM) and Light Microscopy were used to analyze the biosilica assemblage extracted from four sediment samples. The biosilica assemblage was deposited by: the Poaceae (Grass Family); three grass subfamilies, the Panicoideae (tall grasses), the Pooideae (humid and/or cool environment grasses), and the Chloridoideae (short grasses); the cf. Cyperaceae (Sedge Family); at least three species within the Ulmaceae (Elm Family), **Ulmus crassifolia** Nutt. (Cedar Elm), **Celtis pallida** Torr. (Grajeno), and **Celtis** cf. **laevigata** Willd. (Texas Sugarberry); the Boraginaceae (Borage Family), **Ehretia anacua** (Teran and Berl.) I.M. Johnst. (Anacua); and the Spongillideae (Freshwater Sponge Family). Based upon the environmental requirements of the biota which produced this biosilica assemblage, it is suggested that the environment of the Hinojosa site at A.D. 1350-1400 was similar to that of today but with more available moisture. It is also suggested that the January mean minimum temperature was probably not below 36 to 40°F.

BIOSILICA ANALYSIS: MATERIALS AND METHODS

The materials and methods used in this analysis are discussed in five divisions: Processing of Sediment Samples, Processing of Flora and Fauna, Mounting of Samples for Light Microscopy, Mounting of Samples and Operating Procedures for Scanning Electron Microscopy, and Analysis Methodology.

Processing of Sediment Samples

Step 1. Sample Selection. Four sediment samples from the Hinojosa site were selected for processing. Samples 1, 2, and 3 were from Col. 1, and Sample 7

Note: Robinson did not complete the final stage of his analysis: the quantification of the biosilica assemblage from each sample. Thus, the interpretations in this section are based on relative impressions rather than statistically valid counting procedures. The sample provenience of the illustrated specimens (Figs. 16 and 17) has not been identified.

was from F.6. Table 21 summarizes Step 1 of the processing method and describes the samples. Before volume and weight measurements were taken, all roots and macrofossils except very small charcoal and snail shell fragments were removed and described.

Step 2. Removal of water soluble organic and inorganic compounds. Each sample was dispersed with a solution of 5.56 g of sodium hexametaphosphate per liter of distilled water and decanted after 1.5 to 3.75 hours of sedimentation. This step was repeated three times. Standard Sedimentation Tables were used in all stages of processing, including rinses.

Step 3. Removal of carbonates and the less than 5 μm fractions. Carbonates were removed in two stages: (a) 3% hydrochloric acid at room temperature for 2.5 hours; (b) concentrated hydrochloric acid was then added and heated at 100°C for 1.5 hours. Samples were stirred every 15 minutes during this step. The samples were then rinsed and decanted after sedimentation to remove the less than 5 μm fraction.

Step 4. Removal of organic compounds. A 3:1 mixture of concentrated nitric acid/saturated solution of sodium chlorate in water, heated at 100°C for 6.5 hours was used to remove organic compounds. Each sample was then rinsed five times with sedimentation time allowed to retain the greater than 5 μm size fractions.

Step 5. Exotic known. One **Lycopodium** spore tablet containing 12,500 ± 250 spores was added to each sample, dissolved with 10% hydrochloric acid, and rinsed to remove chlorides.

Step 6. Sedimentation. The Step 2 dispersant solution was used to isolate and remove the 5 μm to less than 10 μm size fraction. Standard Sedimentation Tables were used. Dispersal and sedimentation were repeated 10 times, and the remaining fraction (10 μm and larger) was rinsed twice. The 5 μm to less than 10 μm fraction was then microscopically examined for biosilica.

Step 7. Heavy density separation. A 2.3 specific gravity solution of zinc bromide, distilled water, and hydrochloric acid was used for the heavy density separation of biosilica from the heavier quartz and other minerals. The 10 μm and greater fraction was placed in bent, U-shaped sections of 3/8-inch (inside diameter) Nalgene clear plastic tubing. The amount of water present in each sample was calculated, and the necessary amount of 2.5 specific gravity solution was added to each sample and vortexed to bring the specific gravity to 2.3. The heavy liquid with a specific gravity of 2.3 was added, vortexed, and the tubing placed in a 50-ml centrifuge tube containing water. The samples were then centrifuged at 1500 gravities for 10 minutes. After centrifuging, the plastic tubing was removed from the centrifuge tube and clamped between the lower, heavy fraction and the upper, lighter fraction. This step was repeated three times. The light fraction of the samples, which contains the biosilica, and the exotic known was then placed in 250-ml centrifuge containers, diluted with water and hydrochloric acid to a specific gravity of less than 1.5, and decanted after centrifuging. After repeated rinses to remove the bromide and hydrochloric acid, the light fraction was transferred to three-dram glass vials. For a more detailed description of biosilica processing techniques, refer to Robinson (1982).

TABLE 21. SUMMARY OF STEP 1 OF BIOSILICA PROCESSING

Sample Number	Provenience	Lot Number	Biosilica Processing Number	Volume Processed	Weight of Sample (g)	Description
1	WTA Col. 1 L.1	361	192	10 cc	12.70	light gray, sandy loam; **Celtis** spp. seed, roots, and charcoal
2	WTA Col. 1, L.2	362	193	10 cc	12.70	light gray, sandy loam; snail shell fragments (**Rabdotus** spp.), worm casts and roots
3	WTA Col. 1, L.3	403	194	10 cc	13.90	light gray, sandy loam; snail shell fragments (**Rabdotus** spp.), complete snail shell, discoidal, whorls on same plane; rodent bone and roots
7	F.6 (matrix area B)	406	195	10 cc	13.80	light gray, sandy loam; snail shell fragments (**Rabdotus** spp.), mammal bone fragment, roots and charcoal

Processing of Flora and Fauna

During the past seven years an extensive comparative collection of biosilica has been prepared using the following methods:

A. Processing of Flora

Step 1. Plants are collected, identified, and pressed. A detailed record is kept in a permanent record log of exact location, collection date, soil type, and associated plants. Several plants of the same species are collected so that an example of the plant remains are on permanent file. Selected plant parts are removed, washed, dried at 100°C, and weighed.

Step 2. Phytoliths are separated from the plant tissues by oxidation using the same solution used in Step 4 of the sediment processing procedure, centrifuged to retain all size fractions, and rinsed.

Step 3. Phytoliths are stored in three-dram glass vials.

B. Processing of Fauna

Step 1. Sponges are collected, washed, and dried as detailed above. A record is kept in a permanent record log of collection date, location, and environmental conditions. Accurate identification can only be made after processing and microscopic analysis of spicules.

Step 2. Nitric acid is used to destroy the tissues, leaving only the diagnostic spicules and a few adhering diatoms and phytoliths. Samples are rinsed and centrifuged until all traces of acid are removed.

Step 3. The spicules are transferred to three-dram glass vials for storage.

Mounting of Samples for Light Microscopy

Two types of slides were prepared for light microscopy: (1) liquid, and (2) solid mounting media. (1) Several drops of the sample, biosilica in distilled water, were pipetted on a cleaned microscope slide, covered with a cleaned coverslip, and sealed with fingernail polish. (2) Several drops of the sample were pipetted on a cleaned coverslip and allowed to dry for several hours. Four drops of Permount were placed on a cleaned microscope slide and allowed to dry for several minutes to allow excess toluene to evaporate. The microscope slide was then inverted and placed on the coverslip, and allowed to dry. This method of mounting insures that the biosilica will be on one plane, on or near the coverslip, and therefore will require minimum racking of the microscope during transects. Liquid mounting media (1) was used for 41 JW 8 biosilica samples and comparative biosilica samples; solid mounting media (2) was used only for comparative biosilica samples. A Nikon Optiphot Microscope with Hoffman Modulation Contrast was used for all light microscopy.

Mounting of Samples and Operating Procedures for Scanning Electron Microscopy (SEM)

A cleaned glass coverslip was attached to a cleaned, polished SEM stub with double stick tape. Four drops of the sample were pipetted on the coverslip and allowed to dry for 12 hours at 90°C. This is an effective method of attachment as biosilica adheres to the glass coverslip as desiccation occurs. Carbon paint was applied to the upper edge of the SEM stub and the underneath side of the attached coverslip to enhance electrical conduction. This prevents the buildup of electrons and the resulting charging of samples. The samples were sputter coated with 20 nm of gold-palladium, using a Technics, Inc., Hummer. All photomicrographs were made on a Jeol-25S II Scanning Electron Microscope using Kodak Tri-X Pan film. The operating conditions of the SEM which remained constant are as follows: working distance of 10 mm, 190 m objective aperture, and zero degrees of tilt. Variable operating conditions such as keV of accelerating voltage, condenser setting, and magnification are listed in the captions of Figures 16 and 17.

Analysis Methodology

The analysis was conducted in five steps: 1. Light Microscopy Analysis, 2. SEM Analysis, 3. Classification, 4. Calculation of Biosilica Sum, and 5. Analysis of Spectra and Diagrams.

Step 1. Light microscopy analysis. Light microscopy of the 41 JW 8 biosilica and **Lycopodium** spores mounted in water was the first step of microscopic analysis. All samples were scanned in transects at 100X, 200X, and 400X. 1000X was used to examine individual, small phytoliths from grasses. As biosilica was observed, a probe was pressed against the coverslip, rotating the specimen so the morphology of all surfaces could be seen. Unusual types were drawn. Biosilica and **Lycopodium** spores were counted.

Step 2. SEM analysis. One 41 JW 8 sample (Sample 3) was selected for SEM analysis as light microscopy had revealed that a wide variety of diagnostic phytolith types were present. A four-drop sample of Sample 3 was scanned with the SEM, and 27 photomicrographs were taken of selected types of the hundreds of phytoliths examined. Biosilica counts were not made during SEM scans because: (1) the expense of SEM analysis; (2) many types of biosilica are difficult to identify without the observation of three-dimensional morphological characteristics which can be seen in a liquid mounting medium; and (3) SEM reveals only the external morphology of biosilica which is transparent in transmitted light, and internal morphology is valuable taxonomically. The wide range of magnification and the depth of field of SEM photomicrographs does make them extremely valuable for taxonomic analysis when used in combination with light microscopy.

Step 3. Classification. The types of biosilica observed during light microscopy and SEM were compared to comparative samples (II). Light microscopy was used to examine comparative samples in liquid and solid mounting media. Drawings made using light microscopy were utilized (Robinson 1982). Several hundred SEM photomicrographs were also utilized. Four of

these photomicrographs are shown in Figures 16,a,c,d and 17,g. The types of biosilica observed were classified according to (1) three-dimensional morphology; (2) the tissues in which they were deposited **in vitro**; and (3) the taxa of organisms which produced the tissues (Table 22). The resolution of the taxonomic level of identification (family to species of organism) is dependent upon taphonomic variables and the diagnostic value of a type or suite of types (assemblages) which could be identified with certainty.

Step 4. The counts made during light microscopy are used to calculate a valid biosilica sum of the biosilica assemblage. The biosilica sum is converted into three types of spectra and diagrams: (a) relative frequency; (b) concentration or actual frequency; and (c) influx.

(a) Relative frequency is simply a percentage frequency of types of biosilica or the number of individual members of one type of biosilica divided by the number of individual members of all types of biosilica considered.

(b) The concentration of biosilica per cubic centimeter of matrix is calculated using the formula shown:

$$\text{Actual biosilica frequency/Biosilica concentration per cubic centimeter of sediment} = \frac{\#\text{ of exotic added X biosilica counted}}{\#\text{ of exotic added X volume of sediment}}$$

(c) The biosilica influx is the actual frequency of biosilica concentration deposited per square centimeter per year. Influx is calculated as follows:

$$\text{Biosilica influx per square centimeter per year} = \frac{\text{Actual biosilica frequency}}{\text{rate of sediment deposition (years/cm)*}}$$

*Biosilica influx calculations are limited by the assumption that depositional rates within a stratigraphic unit are uniform. High resolution stratigraphic analysis is necessary for high resolution biosilica influx values.

Step 5. Analysis of spectra and diagrams. The three types of spectra and diagrams are then analyzed to determine the type of community of silica-accumulating biota which produced the biosilica assemblage, and therefore the local paleoenvironment or paleomicroenvironment. Relative vegetational biomass or change in vegetational biomass through time can be discerned with spectra and diagrams from reliably dated columns from stratified sediments. The carrying capacity of the environment can then be inferred from the relative biomass.

Figure 16. **SEM Photomicrographs of Phytoliths from 41 JW 8 and Comparative Collection Plants.**

a, comparative collection: **Tripsacum dactyloides** (L.) L., (Eastern Gammagrass), Poaceae, Panicoideae, Andropogoneae; bilobate panicoid short cell phytolith, ventral orientation length: 25 µm; 12.5 keV accelerating voltage, condenser setting 12;

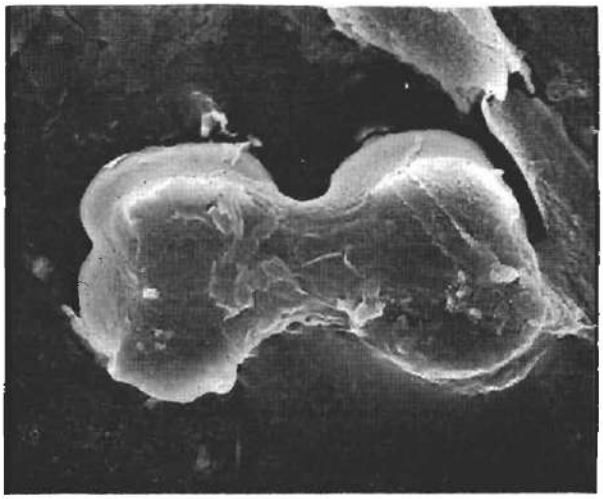
b, Poaceae, Panicoideae; bilobate panicoid short cell phytolith, dorsal or ventral orientation, length: 17 µm; 15 keV accelerating voltage, condenser setting 12;

c, comparative collection: **Phalaris caroliniana** Walt., (Carolina Canarygrass), Poaceae, Pooideae (Festucoideae), Aveneae; elongate festucoid short cell phytolith, dorsal/lateral orientation, length: 27 µm; 12.5 keV accelerating voltage, condenser setting 12;

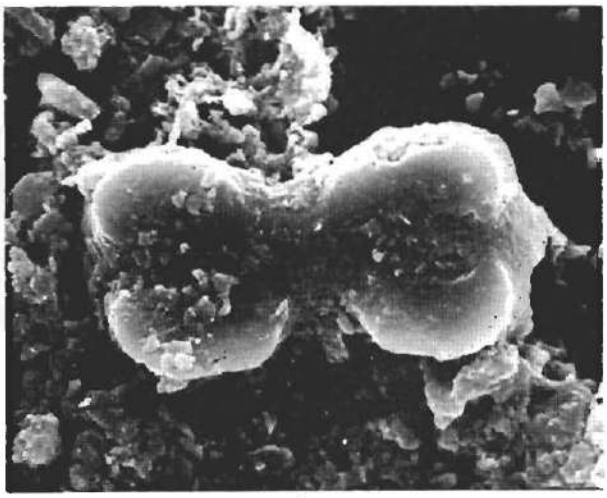
d, comparative collection: **Hordeum pusillum** Nutt., (Little Barley), Poaceae, Pooideae (Festucoideae), Triticeae; elongate festucoid short cell phytolith, dorsal/lateral orientation, length: 34 µm; 12.5 keV accelerating voltage, condenser setting 1;

e, Poaceae, Pooideae (Festucoideae); elongate festucoid short cell phytolith, dorsal/lateral orientation, length: 24 µm; 12.5 keV accelerating voltage, condenser setting;

f, Poaceae, Pooideae (Festucoideae); elongate festucoid short cell phytolith, ventral/lateral orientation, length: 83 µm; 12.5 keV accelerating voltage, condenser setting 1.



a



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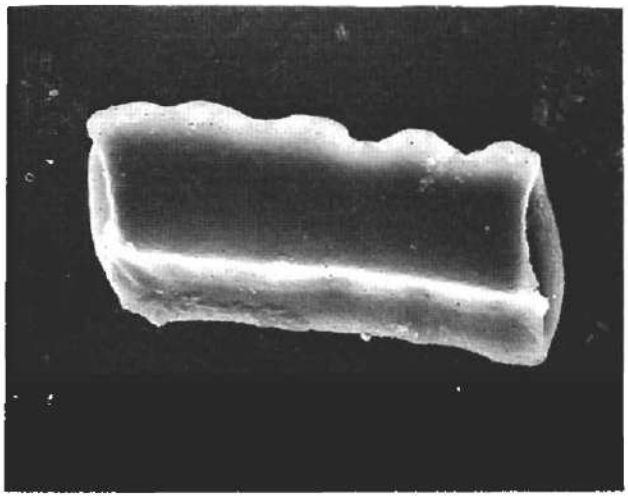

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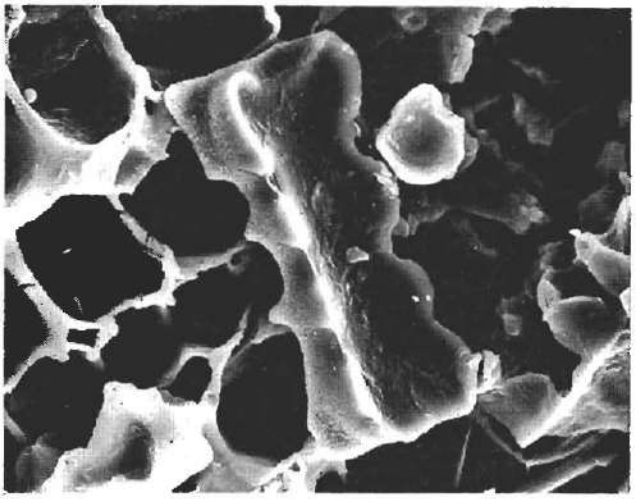

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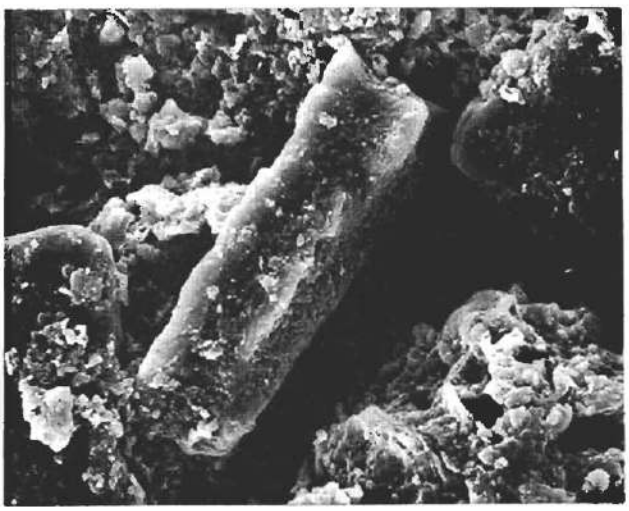

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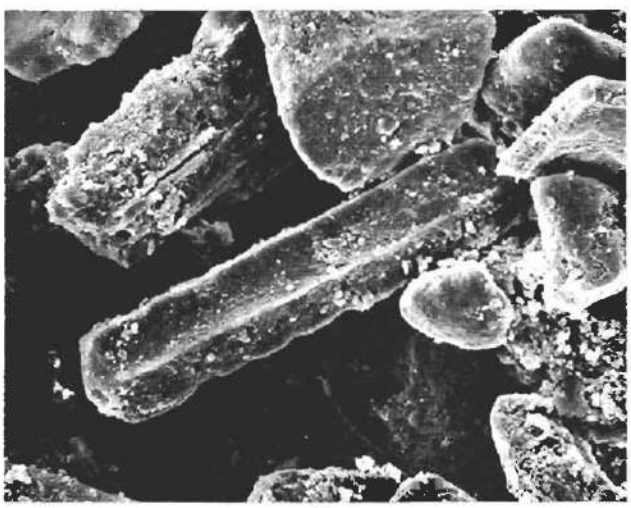


f

Figure 17. **SEM Photomicrographs of Phytoliths from 41 JW 8 and Comparative Collection Plants.**

a, comparative collection: **Phalaris caroliniana** Walt., (Carolina Canarygrass), Poaceae, Pooideae (Festucoideae), Aveneae; trichome, lateral orientation, length: 52 µm; 12.5 keV accelerating voltage, condenser setting 12;

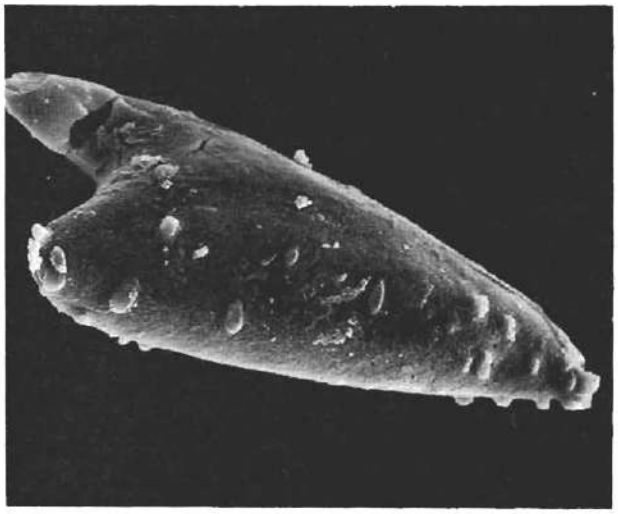
b, Poaceae; panicoid or chloridoid bulliform cell phytolith, dorsal or ventral orientation, width: 47 µm; 12.5 keV accelerating voltage, condenser setting 1;

c, center: Ulmaceae, **Celtis** spp.; trichome base, dorsal or ventral orientation, width: 40 µm; t: Poaceae, Pooideae (Festucoideae); festucoid trichome, lateral orientation, length of base: 35 µm; 12.5 keV accelerating voltage, condenser setting 1;

d, center: Ulmaceae, **Celtis** cf. **laevigata** Willd., (Texas Sugarberry); trichome base with surrounding epidermal cells (tissue fragment), dorsal orientation, width: 31 µm; 12.5 keV accelerating voltage, condenser setting 1;

e, cf. Ulmaceae, **Celtis** spp.; trichome, dorsal orientation, width: 53 µm; 15 keV accelerating voltage, condenser setting 12;

f, Ulmaceae; trichome, dorsal orientation, width of base: 33 µm; 12.5 keV accelerating voltage, condenser setting 1.



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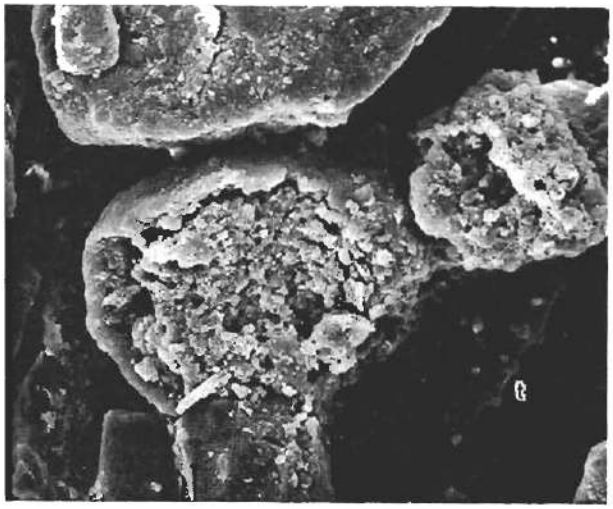

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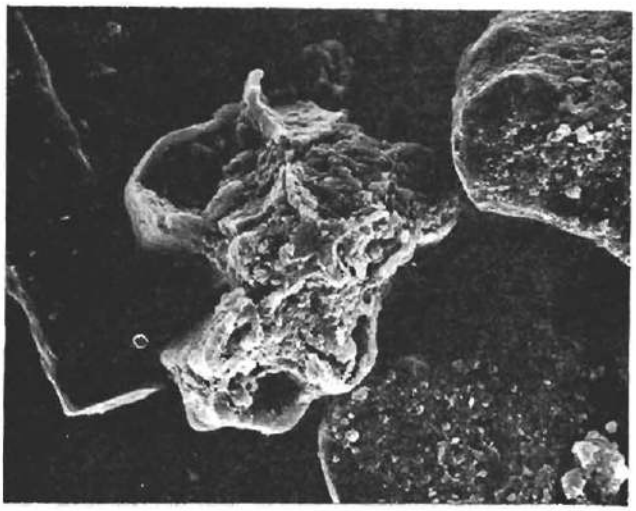

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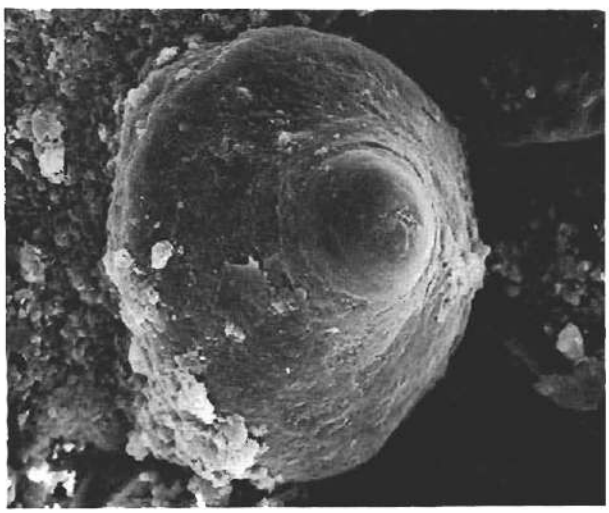

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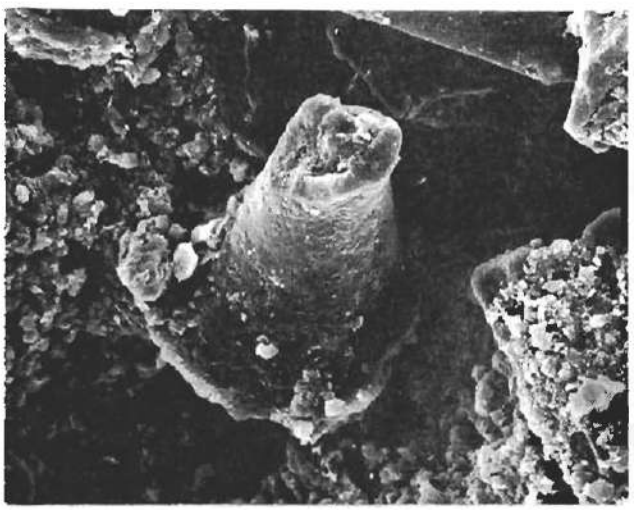

f

TABLE 22. TYPES OF BIOSILICA IN 41 JW 8 SEDIMENT

- Phytosilica
  - Vascular Plants--Phytoliths
    - Monocotyledoneae
      - Poaceae (Gramineae), Grass Family
        - Panicoideae, Subfamily of Tall Grasses, (C-4) 4-carbon photosynthetic pathway
          - Epidermal tissue
            - Panicoid short cells
              - Bilobates (Fig. 16,b)
              - Crosses
            - Tissue fragments
        - Chloridoideae, Subfamily of Short Grasses, (C-4) 4-carbon photosynthetic pathway
          - Epidermal tissue
            - Chloridoid short cells
            - Chloridoid bulliform cells
            - Tissue fragments
        - Pooideae (Festucoideae), Subfamily of humid and/or cool season grasses, (C-3) 3-carbon photosynthetic pathway
          - Epidermal tissue
            - Festucoid short cells
              - Conical
              - Elongate (Fig. 16,e,f)
              - Oblong
            - Trichomes (Fig. 17,c[t])
            - Tissue fragments
        - Poaceae, identified to taxonomic level of Grass Family
          - Epidermal tissue
            - Bulliform cells
            - Trichomes
            - Long cells
            - Tissue fragments
          - Mesophyll
        - cf. Cyperaceae (Sedge Family)
          - Epidermal tissue
            - Short cells
    - Dicotyledoneae
      - Ulmaceae (Elm Family)
        - Epidermis of Leaves
          - **Ulmus crassifolia** Nutt. (Cedar Elm)
            - Tissue fragments: trichome, trichome base with surrounding epidermal cells
            - Trichome
            - Trichome base
          - **Celtis pallida** Torr. (Granjeno)
            - Tissue fragments: trichome, trichome base with surrounding epidermal cells
          - **Celtis cf. laevigata** Willd. (Texas Sugarberry)
            - Tissue fragment: trichome base with surrounding epidermal cells
          - **Celtis** spp. (Hackberry)
            - Trichome (Fig. 17,e)
            - Trichome base (Fig. 17,c)
            - Tissue fragments (Fig. 17,d)
        - Ulmaceae, identified to taxonomic level of Elm Family
          - Trichomes (Fig. 17,f)
          - Trichome bases
          - Epidermal cells
          - Tissue fragments
      - Boraginaceae (Borage Family)
        - Epidermis of Leaves
          - **Ehretia anacua** (Teran & Berl.) I. M. Johnst. (Anacua)
            - Trichomes
            - Trichome bases
            - Epidermal cells
            - Tissue fragments
- Zoosilica
  - Spongillideae (Freshwater Sponge Family)
    - Megascleres (body spicules)

BIOSILICA ANALYSIS: RESULTS AND CONCLUSIONS

The results of this analysis are summarized in Table 22 and illustrated in Figure 16,b,e,f and Figure 17,h,i,j,k, and l. At this time, Step V of the analysis has not been completed; the results are a description of the assemblage of biosilica extracted from the 41 JW 8 sediments and the paleoecological implications.

As shown in Table 22, the Poaceae (Grass Family) is represented by phytoliths of several genera of the Panicoideae (tall grasses), several genera of the Pooideae (humid and/or cool environment grasses), and the Chloridoideae (short grasses). There is probably at least one genera of the Cyperaceae (Sedge Family) present. Phytoliths of two families of trees were identified, the Ulmaceae (Elm Family) and the Boraginaceae (Borage Family). The Ulmaceae are represented by **Ulmus crassifolia** (Cedar Elm), **Celtis pallida** (Granjeno), and **Celtis** cf. laevigata (Texas Sugarberry). One member of the Boraginaceae was present, **Ehretia anacua** (Anacua).

Spicules of the Spongillideae (Freshwater Sponge Family) were also observed. These aquatic animals were probably from Chiltipin Creek. As freshwater sponges attach themselves to available substrate, the spicules may have been introduced into the site sediment by local inhabitants in the act of bivalve mollusk procurement. I have found several freshwater sponges attached to the shells of living freshwater bivalves. The possibility also exists that the sponge spicules are present because of alluvial or aeolian deposition.

The biosilica assemblage from the Hinojosa site suggests an environment similar to that of today but with more available moisture. Most of the taxa identified are present in the South Texas Plains Region and/or the Gulf Coastal Plain Region in the modern environment. When individual taxa within the assemblage are considered, the presence of **Ehretia anacua** (Anacua) and the grasses in the subfamily Pooideae (Festucoideae) are especially important as environmental indicators. Anacua and the Pooideae grasses are extant in the modern environment.

Ehretia anacua (Anacua) is an indicator of paleotemperature, with a natural geographical range of: north to Hays and Travis Counties; east to Harris County; west into the South Texas Plains Region and the Mexican states of Nuevo Leon and part of Coahuila; and southward into the Mexican states of Tamaulipas, Guanajuato, and Veracruz (Correll and Johnston 1970; Vines 1977). A drought-resistant species, Anacua's main limiting environmental factor is temperature. At the northern boundary of its natural range, the Anacua is represented by a few, possibly relect, populations which are seasonally partially deciduous. The January mean minimum temperatures (JMMT) for Hays and Travis Counties are 40°F and 41°F, respectively. Planted as ornaments as far north as Dallas County with a JMMT of 36°F, the Anacua die in excessively cold winters (Correll and Johnston 1970). In the area of the Western Gulf Coastal Plain Region (Victoria County and part of Goliad County), where Anacua is most abundant (in Texas), the JMMT is 46°F. It is therefore suggested that the JMMT during the occupation of the Hinojosa site at A.D. 1350-1400 was probably not less than 36 to 40°F, or within 7 to 11°F of the present JMMT of 47°F for Jim Wells County. The possibility exists that winter temperatures went well below the projected means and that the

population of Anacua was reestablished by surviving relect populations and/or that reestablishment took place because of the food procurement activities of man, as the drupes of Anacua are sweet and edible.

The grasses within the subfamily Pooideae (Festucoideae) are important indicators of mesic, cool season, or cool humid environments. Of the 523 species of grasses in the extant flora of Texas, only 86 native species and varieties are of the subfamily Pooideae. Of these 86 taxa, only nine have been found in the South Texas Plains Region, and 17 in the southwestern part of the Gulf Prairies and Marshes Region (Gould 1969). Phytoliths from these grasses are usually uncommon in modern soil samples from most regions of Texas. When found in samples of sediments from archaeological sites, they are usually part of the biosilica assemblages from mesic environmental conditions (Robinson 1982).

The biosilica assemblage from 41 JW 8 is very similar to assemblages from mesic periods at 41 GD 21, Coleto Creek, Goliad County, Texas (Robinson 1979); and 41 LK 31/32 and 41 LK 201, Choke Canyon, Live Oak County, Texas (Robinson 1982). Both of these areas of study are also on the western margin of the Western Gulf Coastal Plain in south Texas.

Additional interdisciplinary research in this area of Texas will prove or disprove the proposed mesic interval of approximately A.D. 1350-1400. The investigation of carbon isotope ratios of bone collagens of **Bison bison** from archaeological sites could provide an independent test. An increase of the Pooideae (C3) grasses in the diet of **Bison bison** should be detectable in the $^{13}C/^{12}C$ ratios of their bone collagens.

ACKNOWLEDGMENTS

The facilities of the Palynology Laboratory, Archeobotany Laboratory, Department of Anthropology, Texas A&M University, College Station, Texas, were used for all stages of processing and analysis with the exception of Electron Microscopy.

The facilities of the Electron Microscopy Center, Texas A&M University, College Station, Texas, were used for Scanning Electron Microscopy, photomicrograph development, and printing.

Last, I would like to thank Suzanne G. Teel, College Station, Texas, for manuscript editing and typing.