THECAMOEBIANS AND FORAMINIFERA IN MEXICAN ANCHIALINE CAVES

Paleoenvironmental Analysis Using Thecamoebians and Foraminifera in Mexican Anchialine Caves: A Focus On Aktun Ha (Carwash) Mexico

by

PETER J. VAN HENGSTUM, Hons. B.Sc., *summa cum laude*

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AUTHOR: Peter J. van Hengstum Honours B.Sc., *summa cum laude*
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SUPERVISOR: Associate Professor Dr. Eduard G. Reinhardt

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ABSTRACT

Thecamoebians (testate amoebae) and foraminifera have never been systematically investigated in a phreatic cave system. Foraminifera (marine to brackish water protists) and thecamoebians (brackish to freshwater protists) are cosmopolitan and have been studied in virtually all environments on Earth. Although foraminifera-bearing sediment has been observed from vadose cave environments in the last 20 years, there is no mention of their ecology – or mere existence – in flooded cave passages in any micropaleontology or microbiology textbooks. Only one published manuscript briefly mentions foraminifera in preliminary test samples from phreatic caves in Bermuda. The unprecedented ability of these organisms to colonize any environment on Earth led to the hypothesis that they are also exploiting global phreatic caves.

The karst landscape of the Yucatan Peninsula, Mexico, is an ideal setting for the formation of anastomosing, anchialine, phreatic cave systems. The stratified aquifer contains passages in both the superior freshwater lens (thecamoebian habitat) and marine water (foraminifera habitat) intruding below, creating an ideal location to test the hypothesis. Several important contributions have resulted from investigating surface samples and cores in this region. (1) Using thirty-three surface samples from three different cenotes, thecamoebians and foraminifera were found living in the sinkhole (cenote) environment, which are the physical entrance into the caves. An ecological boundary of ~3.5 ppt was determined for thecamoebians, with *Centropyxis constricta* "aerophilia" determined as the most euryhaline thecamoebian taxa. (2) Foraminifera and thecamoebians have been colonizing Carwash Cave since the Sangamon Interglacial (MIS 5e), based on seventy-five surface samples collected throughout the system. The recovered taxa have responded to changes in cave environment similarly to other coastal systems, thereby indicating their potential as paleoenvironmental proxies in phreatic caves. (3) In conjunction with stable isotopes ($\delta^{13}C_{org}$ and $\delta^{15}N_{org}$), thecamoebians and foraminifera were successful recorders of mid-late Holocene paleoenvironmental evolution in Carwash Cave. An important salinity transition in the freshwater lens (from >2 ppt to 1.5 ppt) of the aquifer at ~2760 Cal yrs BP is coincident with changes in the local Maya populations, indicating a possible environmental linkage between ancient freshwater resources and Maya cultural genesis. This thesis demonstrates unequivocally that thecamoebians and foraminifera exist in phreatic cave systems and hold wider potential as paleoenvironmental proxies in other global phreatic caves.

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"Love the organisms for themselves first, [and] with a little good fortune, discoveries will follow. If they don't, the love will have been enough."

Edward O. Wilson, 1994, *Naturalist*

I cannot take credit for finding this quote, for it is found at the front of an important textbook on foraminifera. However, it describes my interest—and serendipitous research—in thecamoebians and foraminifera perfectly. For my introduction into this discipline, I am indebted to Dr. Eduard G. Reinhardt. I have thoroughly enjoyed my learning experiences under his direction and will fondly remember cave diving with *The Foram Hunter*. The central research premise of this thesis is his conception, and I am privileged to be the first student-scientist to academically pursue it.

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CHAPTER 1

INTRODUCTION

Foraminifera and thecamoebians have been studied around the world for centuries, from the deep ocean abyss, to salt marshes, lakes, bogs, and woodland soils. However, they have never been systematically investigated in phreatic cave systems (Murray, 2007; Scott et al., 2001). Foraminifera are eukaryotic, unicellular, organisms that secrete (mostly calcium carbonate) or agglutinate foreign material to form a simple shell. Thecamoebians (testate amoebae) are also eukaryotic, unicellular organisms that either secrete (mucopolysaccharide or SiO_2) or agglutinate foreign particles to form a simple shell. Although the subtle differences between these taxonomic groups are many, the important feature for this research is that thecamoebians occupy freshwater to marginally brackish habitats, whereas foraminifera occupy brackish to hypersaline environments. Together, these protists occupy the entire salinity continuum, making them particularly suited as environmental proxies in coastal settings.

Cave passages can be classified according to their relative location in the water table. Vadose passages are air-filled and located above the water table, whereas phreatic passages are flooded and located below the water table. Previous research on vadose cave sediment using sedimentological, biological, and geochemical proxies has demonstrated their ability to record a primary signal of environmental change (i.e., Polk et al., 2007; Panno et al., 2004; Valen et al., 2007; Davis, 1990). Foraminifera have been found in vadose cave sediments, but they were interpreted as transported from the sea, as opposed to foraminifera actually living in the cave environment during the geologic past (*Bolivina, Ammonia*, Proctor and Smart, 1991).

The phreatic cave environment represents one of the last unexplored environments on earth. Since any time spent in these systems requires specialized SCUBA training, scientific investigations in these environments occurs less than in their vadose counterparts. Biological investigation into these systems routinely recovers new species, either phylogenetically unique, or extent taxa that have undergone regressive evolution (Iliffe et al., 1983; Iliffe, 1992). In all of the recent work on these systems, only one study mentions the finding of foraminifera and thecamoebians in a terrestrial phreatic cave, and these were only from limited test samples (Javaux and Scott, 2003). Foraminifera were also discovered in a submarine cave on a Japanese reef, but the taxa were not presented or the focus of the research (Kitamura et al., 2007).

Furthermore, the wider role of foraminifera and thecamoebians in phreatic cave ecosystems is currently unknown.

The Yucatan Peninsula of Mexico is one of several global locations with phreatic cave systems. This project generally focuses on Carwash (Actun Ha) Cave System, which is located just outside of the town of Tulum in Quintana Roo, Mexico. This cave is the ideal natural laboratory to begin systematic research on foraminifera and thecamoebians in phreatic caves for several reasons. First, the limited spatial extent of Carwash Cave allows for a feasible study of taxa throughout the entire cave system. Other regional cave systems on the Yucatan can have hundreds of kilometres of cave passage that span over tens of square kilometres, and therefore are not suitable for a short two-year study. Second, there is one main central cenote in the Carwash system, as compared to many cenotes in other regional cave systems. This allows for better correlation of taxa to a specific cenote to determine the influence of a cenote on final assemblages in the cave. Third, there is a moderate hydrologic flow (1 cm/s) in the freshwater lens at Carwash Cave. This hydrologic flow systematically transports nutrients and organic sediments into the cave. Lastly, there are cave passages that span the salinity continuum, thereby encompassing habitat for both thecamoebians and foraminifera.

1.1 CENTRAL RESEARCH QUESTIONS

This entire research project was generated on one simple premise. Since the cave systems are intrinsically aquatic, then foraminifera and thecamoebians should be colonizing this environment similarly to other coastal settings. A standard micropaleontological research design was employed to develop baseline data on modern conditions to better interpret possible down-core micropaleontologic profiles. Three central research questions guided this scientific investigation:

(1) What are the distributions and environmental controls on thecamoebians and foraminifera in the open water sinkhole (cenote) environment?

(2) Are the thecamoebians and foraminifera in an anchialine cave the expected fauna based on modern environmental conditions and the known ecology of the taxa?

(3) Can down core assemblages of foraminifera and thecamoebians be used to reconstruct environmental evolution in the phreatic cave?

1.2. GENERAL THESIS OUTLINE

The structure of the thesis follows the research questions and continually builds on knowledge in the subject area. Chapter two provides an introductory evaluation of distributions in three open water cenotes (sinkholes). Since the cenotes are the physical entrances into the phreatic caves, they are an ideal location to begin this research. Most of the initial hypotheses regarding this research placed significant value on bi-products of primary productivity from the cenote dominantly influencing the fauna in the adjoining cave (Fichez, 2004). Since the cenote is the central nutrient-pump supporting cave ecosystems, it was assumed that modern foraminifera and/or thecamoebians in the cave would be related to taxa found in the cenote. Surface samples (*n* = 33) were collected from three separate cenotes (Carwash, Maya Blue, and El Eden) in a landward transect with increasing salinity. This chapter contains more introductory information on the cenote environment and regional geology, which is not addressed in the subsequent chapters. The recovered faunal trends were correlated with salinity variations, as this abiotic factor was determined as the most relevant factor influencing the distributions.

Chapter three examines distributions of testate amoeba and foraminifera in surface samples (*n* = 75) transecting Carwash (Aktun Ha) Cave System. Since fauna were found in the cenote environment (Chapter 1), then the next step was to investigate fauna throughout one cave system. Sedimentary characteristics and carbonate isotopes ($\delta^{13}C_{carb}$ and $\delta^{18}O_{carb}$) on microfossil shells were also investigated to further understand the different cave assemblages.

The final scientific contribution, Chapter four, investigates the potential of testate amoeba and foraminifera as paleoenvironmental proxies in phreatic cave systems. Faunal assemblages—along with stable isotope analysis ($\delta^{13}C_{org}$ and $\delta^{15}N_{org}$) and particle size analysis—provide a mid-late Holocene environmental record of regional aquifer dynamics and local environmental change. Ten radiocarbon ages provide the chronological control for the reconstruction.

Each of the chapters in this thesis are independently prepared as a manuscript for publication. As such, some introductory material is repeated in chapters, but each chapter is designed to stand-alone research unit.

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CHAPTER 2

THECAMOEBIANS (TESTATE AMOEBAE) AND FORAMINIFERA FROM THREE ANCHIALINE CENOTES: LOW SALINITY FAUNAL TRANSITIONS (1.5-4.5 ‰)

**P.J. van Hengstum, E.G. Reinhardt, P.A. Beddows,
R.J. Huang, and J.J Gabriel**

This manuscript is prepared for submission to the Journal of Foraminiferal Research and generally follows the style required by the publisher of this journal.

2.1. Abstract

This study presents the first systematic documentation of thecamoebians and foraminifera from anchialine cenotes (sinkholes) from Quintana Roo, Mexico. Thirty-three surface sediment samples (upper 5 cm) were collected from cenotes Carwash (salinity 1.5‰), Maya Blue (2.9‰), and El Eden (>3.3‰). Based on Q- and R-mode cluster analysis of the faunal distributions, four low-diversity (Shannon Diversity Index 1.0 – 1.5) and salinity-controlled assemblages were identified. Assemblage 1 (1.5‰) was dominated by *Centropyxis aculeata* “aculeata” (53%) and *Arcella vulgaris* (21%) with limited amount of *Jadammina macrescens* (2%). Assemblage 2 (2.9 ± 0.2‰) was dominated by *Centropyxis aculeata* “discoides” (41%) and *Centropyxis aculeata* “aculeata” (27%). Dwarfed (~50 μm) *Centropyxis constricta* “aerophila” (20%) with an autogenous test and *J. macrescens* (29%) dominated Assemblage 3 (3.3 ± 0.2‰). Finally, *Ammonia tepida* (51%), *J. macrescens* (29%), and some *Elphidium gunteri* (11%) formed Assemblage 4 (3.3 ± 0.4‰). Thecamoebian and foraminifera populations in the cenotes are distributed according to salinity variations as found in other coastal systems, reiterating their usage as an indicator in low-salinity environments. Importantly, the transition from thecamoebian-dominated assemblages to foraminiferan-dominated assemblages is observed at a salinity of ~3.5‰ in the cenotes. The dwarfed, autogenous *Centropyxis constricta* was found to be the most euryhaline of the thecamoebians, persisting at the

ecological boundary of the taxonomic group (~3.3‰). The centropyxid taxa trended towards ecophenotypes without spines with increasing salinity and represents further evidence of morphological fluidity in thecamoebians and the 'strain' concept.

Keywords: Cenotes, anchialine, testate amoebae, marsh foraminifera, arcellaceans

2.2. INTRODUCTION

The Yucatan Peninsula of Mexico is an expansive (75 000km^2), low-lying limestone platform that separates the western Caribbean from the Gulf of Mexico (Fig. 2.1A). Speleogenesis has resulted in extensive and hydraulically active cave networks interconnected to coastal water. The karst landscape includes thousands of collapse sinkholes, known locally as cenotes (from Maya the word *dz'onot*), that provide physical access to the aquifer and subterranean cave systems. The cenotes are described as anchialine because they are tidally-influenced meromictic coastal aquatic environments where basal saline water is stratified from superior freshwater (Fig. 2.1B). The term 'anchialine' was coined by Holthuis (1973) to describe tidally-influenced surface pools with no surface connection to marine water that contain brackish to salt water.

Cenotes and the underlying anastomosing cave networks, collectively form unique ecosystems where primary productivity in the cenotes supports endemic cave populations (troglobites). Documentation of cenote biota began during scientific expeditions of the Carnegie Institution of Washington in the 1930's (Pearson and others, 1936). However, only recently have advances in SCUBA diving technology allowed for the documentation of cenotes and cave fauna resulting in new inventories of zooplankton, macrocrustaceans, and fish (Sanchez and others, 2002; Schmitter-Soto and others, 2002; Iliffe, 1992; Lopez-Adrian and Herrera-Silveira, 1994). However, neither foraminifera nor thecamoebians have been documented in the cenotes of Mexico even though foraminifera have been well documented in the Gulf of Mexico (e.g. Osterman 2003; Denne and Sen Gupta, 1993; Gary and others, 1989; Culver and Buzas, 1983; Poag, 1981 Bandy, 1956, Bandy, 1954). Recently, foraminifera and thecamoebians have been documented as being able to live in the unique phreatic cave environment of the Yucatan karst landscape (van Hengstum and others, 2008).

Foraminifera (marine to brackish water) and thecamoebians (freshwater to brackish) are environmentally sensitive, and form simple secreted ($CaCO_3$, mucopolysacharide, SiO_2) or agglutinated tests. Foraminifera are ubiquitous in most marine environments from the abyssal depths to upper salt marshes, and exhibit considerable ecological zonation with respect to various environmental parameters (Murray, 2007). In contrast, thecamoebians are found in most freshwater settings (i.e., from lakes, bogs, and soil) and are useful environmental indicators of moisture content, pH changes, and lake trophic status (Mitchell and others, 2007; Patterson and Kumar, 2002; Scott and others, 2001; Charman, 2001; Charman and others, 2000). Both taxonomic groups remain well preserved in the Holocene sedimentary record, thereby contributing to their wide usage as paleoenvironmental proxies.

An area of active research is to characterize the low-salinity zonation between these two taxonomic groups. Both fauna occur in regions of estuaries, lagoons, and salt marshes (Scott and others, 2001). However, the rapid fresh-to-saline transition in lagoons and estuaries makes the study of subtle taxonomic trends in extremely low salinity more feasible in the salt marsh (i.e., Gehrels and others, 2001; Riveiros and others, 2007). The sharp zonation of these two groups in salt marshes has provided significant insight into the ecological boundary of thecamoebians with respect to salinity, but additional environments are needed to study these faunal transitions. First, the known dominant factors that control thecamoebians are salinity, water saturation/percent moisture (Charman and others, 2000; Scott and others, 1980), and pH (Escobar and others, 2008). As such, isolating salinity as the dominant factor controlling thecamoebian taxa in an environment with large temporal fluctuations in all these parameters is challenging. Second, soil thecamoebian assemblages become established in the extreme high marsh zone settings where pedagenic processes begin. These additional taxa are not directly related to salinity tolerances off lacustrine thecamoebians and there is potential for these taxa to taphonomically bias lower salt marsh assemblages. Third, the sharp faunal gradations observed in salt marshes are not as inter-gradational as required to observe taxonomic subtleties in thecamoebians (Gehrels and others, 2001). Since the salt marsh cannot be directly compared to the cenote due to the many differences between the two environments, the cenote may be another low-salinity environment in conjunction with the salt marsh to help us delineate environmental effects on thecamoebians and foraminifera.

The objectives of this study are to: (1) characterize and develop baseline data on distributions of thecamoebians and foraminifera in Yucatan anchialine cenotes; and (2) investigate the gradation between thecamoebian and foraminiferan taxa in the unique low salinity cenote environment.

2.3. CENOTE PHYSIOGRAPHY AND STUDY SITES

The Yucatan Peninsula is composed of diagenetically immature reefal limestone with Eocene rock in the interior grading out to a Quaternary coastline overlain by unconsolidated Holocene sediments (Ward and others, 1985). The limestone retains ~25% porosity (Harris, 1984) allowing effective infiltration and drainage of precipitation and subsequently contributes to a lack of rivers in the Yucatan. Speleogenesis has been an ongoing process on the Yucatan Peninsula since the late Neogene in both phreatic and vadose settings (Smart and others, 2006). The geologic processes controlling cenote formation are: (1) sub-aerial dissolution of limestone in the vadose zone through carbonic acid percolation during precipitation; (2) subterranean dissolution and collapse of cave ceilings from karst dissolution at the CO_3^- under-saturated halocline (Smart and others, 1988); and (3) dominantly through the collapse of cave ceilings due to the removal of a hydraulic buoyant force during sea level regressions (Smart and others 2006; Schmitter-Soto and others, 2002). Cenote morphology is most commonly a circular/elliptical basin overlying the cave beneath with limestone blocks forming a central breakdown pile at the cenote base. Sediment accumulations at the bottom of the cenote have been recently investigated by Gabriel and others (2008) and determined to record the evolution of the cenote in response to Holocene sea level rise.

The hydrogeologic setting of the Yucatan receives a mean annual rainfall of 1.5 m/yr with 85% lost through evapotranspiration based on mean annual air temperatures. The remaining 15% of annual rainfall is drained through the freshwater lens (Alcocer and others, 1998; Moore others, 1992; Hanshaw and Back, 1980). The modern aquifer on the Yucatan is density-stratified; a freshwater lens is buoyant above the marine water intruding from the coast (Moore and others, 1992). Both fresh and saline waters circulate through the caves at 1-10 km/day. While fresh water migrates to the coast, the marine water incursion exhibits phases of landward migration within larger subsurface convection cells. (Beddows and others, 2007; Moore and others, 1992). The halocline separating the fresh and marine water dissolves the limestone in a phreatic setting resulting in anastomosing phreatic caves (Smart and others, 1988; 2006). Neogene sea level oscillations have shifted the relative position of the halocline causing cave development at different elevations in the subsurface. Due to local variations in hydrogeology, there is significant variability in cenote water chemistry across the Yucatan (e.g., pH: 6.3 - 10.4, dissolved oxygen: 0.82 – 10.6 mg/L, temperature: 22 – 33.5 °C, chlorophyll-*a*: 0.11 – 97.4 mg/m^3; Schmitter-Soto and others, 2002).

2.3.1. CARWASH CENOTE

Carwash Cenote (CW) is the main access into the Carwash (Actun Ha) Cave System, located ~8.5 km west of the Caribbean coast, within 40 m from the highway. The sinkhole has a general elliptical morphology with an average length of 46 m, width of 15 m, and depth of 5 m. The submerged central breakdown pile on the bottom is generally flat, with only one large boulder located in the northern end of the sinkhole, although there are many submerged tree trunks and branches around the periphery. The sediment accumulation on the breakdown pile and its relation to rising Holocene sea-level was documented in Gabriel et al., (submitted) and the cave fauna in van Hengstum et al., (submitted). The benthic environment contains a dense cover of algal mats and abundant fish (especially *Astyanax mexicanus*). Algal blooms characterize the surface water during the rainy season, where active primary productivity creates vibrant green surface water. Dissolved oxygen (3.5 mg/L) is above the ecological requirements of most aerobic foraminifera (>1 mg/L), but the salinity of 1.5‰ will inhibit most foraminifera and thecamoebian taxa. The top of the local halocline is seasonally stationary at 21 m depth (Beddows, 2004), which is significantly below the sampling depth-range (Fig. 2.2.). Detailed water quality data for Carwash cenote is given in Alcocer and others (1998).

2.3.2. MAYA BLUE CENOTE

Maya Blue Cenote (MB) is one of several sinkholes in the Naranjal/Maya Blue Cave System, located ~5.6 km west of the Caribbean coast. The water filled sinkhole is crescent shaped, with an average length of 50 m, width of 10 m, and depth of 3.5 m. Large blocks of limestone are exposed on at the bottom (~6-10 m in width) that are interspaced with sediment visibly abundant in diatoms and organics. A larger area of exposed sediment occurs at the center of the cenote, where several macrophytes have become established. Maya Blue is more oligotrophic than Carwash, with a notable absence of reoccurring pelagic algal blooms and year-round water clarity. Salinity is 2.9‰ and dissolved oxygen is above aerobic foraminiferal requirements in the sampling depth-range (~1.5 mg/L; Fig. 2.2.).

2.3.3. EL EDEN CENOTE

El Eden Cenote (EE) is situated 1.8 km inland from the Caribbean coast and a popular tourist snorkelling attraction in Quintana Roo. This is one of several cenotes found in the larger Ponderosa Cave System. Numerous large blocks of limestone strata, collapsed during cenote-formation, characterize the benthic environment. Along the periphery, a few of these limestone blocks intersect the water interface, and have developed into miniature islands colonized by ferns and grasses. This creates a high degree of variability in depths across the cenote bottom from 1 to 8 m. The diversity of fish fauna is the greatest at El Eden compared to the other two cenotes. The halocline is the shallowest at this locale being at 11.5 m depth, with a subtle increase in salinity with depth from the surface water (~3.3 - 3.4‰). Dissolved oxygen (>3.6 mg/L) and hydrologic flow rates (3 cm/s) of the freshwater lens are the greatest at El Elden as compared to the other two sites. The configuration of the breakdown blocks, which span down into the saline water, combined with increased flow rates, visibly induces turbulence and enhanced mixing of the saline water into the freshwater lens at El Eden.

2.4. METHODS AND ANALYSIS

Thirty-four surface sediment samples (upper 5 cm) were collected from Carwash Cenote (CW, x=7), Maya Blue Cenote (MB, x=12), and El Eden Cenote (EE x=14) on SCUBA in August 2006, capturing the range of cenote sub-environments (Fig. 2.3). Salinity profiles through the aquifer at each locale were collected with a submersible multi-parameter probe (YSI 600XLM) to characterize modern hydrologic conditions. Sediment samples (5 cc) were washed over a 45 μm screen to retain thecamoebians and foraminifera. Thecamoebian taxonomy follows Medioli and Scott (1983) and Reinhardt and others (1998). A total of 8 thecamoebian and 11 foraminifera taxa were identified in the three cenotes (Plate 2.1). Thecamoebians and foraminifera were considered collectively in this study, where relative fractional abundance (F_i) of each taxonomic unit was calculated by:

$$F_i = \frac{C_i}{N_i}$$

where C_i is the species count and N_i is the total population (individuals) in the sample.

As an exploratory tool, shell categories (thecamoebians, agglutinated foraminifera, and calcite foraminifera) were plotted on a ternary diagram, a useful technique for describing different foraminiferal environments (e.g., Murray 2007). With thecamoebians, agglutinated-test foraminifera, and calcite-test foraminifera representing the lacustrine to marine salinity continuum, a ternary diagram can provide insight into the distributions in environments with the most marginal salinity. The recovered taxa were categorized as above using the fractional abundance, and plotted on a standard ternary diagram.

The standard error (S_{Fi}) for each taxon was used to determine statistically significant taxa for further multivariate analysis (Patterson and

Fishbein, 1989). Using the relative fractional abundance, standard error was calculated through:

$$S_{F_i} = 1.96\sqrt{\frac{F_i(1-F_i)}{N_i}}$$

Additionally, the Shannon-Weaver Diversity Index (SDI; Shannon and Weaver, 1949) was calculated for each sample to quantify the environmental stability at each sample location (stable conditions: >2.5, transitional conditions: 1.5 - 2.5, and 'harsh' conditions: <1.5; Patterson and Kumar, 2002). The index is calculated through the following formula, where S is the species richness in each sample:

$$SDI = -\sum_{I}^{S}\left(\frac{F_i}{N_i}\right) * \ln\left(\frac{F_i}{N_i}\right)$$

Q-mode and R-mode cluster analysis is used to characterize assemblage associations using the statistical freeware package *PAST.* Q-mode clustering identifies statistically similar populations, whereas, R-mode clustering identify statistically affiliated fauna. Of the total 21 taxonomic units recovered, three thecamoebian taxa (*Bulinularia indica*, *Difflugia oblonga*, *Lagenodifflugia vas*) and two foraminiferan taxa (*Bolivina variabilis* and *Textularia earlandi*) were omitted from the analysis because they were statistically insignificant (Fishbein and Patterson, 1993). Ward's method of minimum variance with a Euclidian distance was used and the results are displayed in a hierarchical dendrogram, which indicates four salinity-controlled associations (Fig. 2.4). The biocoenosis was not quantified, but the thanatocoenosis is thought to better characterize average conditions at a sample site (Osterman, 2003; Scott and Medioli, 1980).

2.5. RESULTS

The dendrogram produced by the cluster analysis indicates four distinct salinity-controlled clusters that are interpreted as separate assemblages (Fig. 2.4). All seven samples recovered from CW (7) form Assemblage 1 (A1) from a mean depth of 4.6 m. A1 is characterized by an abundance of *C. aculeata* (74%) and *Arcella vulgaris* (21%). Notably, the thecamoebian strain *Centropyxis aculeata* "aculeata" (with spines, 53.8%) dominates over the strain without spines (*Centropyxis aculeata* "discoides" 53.8% to 21.2%). Only one sample in CW contained a small abundance of *J. macrescens* (2%). Very low diversity (mean SDI = 1.05, Table 1) indicates that the environment deviates from more optimal growth conditions for both lacustrine thecamoebians and foraminifera (where SDI >2). On the ternary diagram, all the samples plot at (or near) the apex due to large relative abundance of thecamoebians (mean 99.6%; Fig. 2.5).

Assemblage 2 (A2) is dominantly identified from MB, although one sample is from EE (E6). The A2 samples were found at a mean depth of 3.5 m and a salinity of 2.9 ± 0.2‰. Centropyxid taxa are still dominant (mean 82%) similar to A1; however, there are subtle taxonomic shifts at the species and strain (ecophenotype) level. *Centropyxis aculeata* "aculeata" decreases (mean 27%), *C. aculeata* "discoides" increases (41%), and there is a significant decrease in the contribution of *Arcella vulgaris* (mean 2 ±3%; high SD due to larger inter-sample variability) to the populations. *Centropyxis constricta* "aerophila" (mean 9%) and *Centropyxis constricta* "spinosa" (mean 3%) increase as compared to A1, but the tests of this species were exceptionally dwarfed, and only autogenous tests were found. The absence of the xenogenous test form is an interesting observation, as this type of test is very abundance in other lacustrine settings (i.e. see Patterson and Kumar, 2002). Euryhaline agglutinated foraminifera *J. macrescens* (mean 9%) and *Millammina fusca* (mean 4%) are present in A2, placing these samples along the left axis on the ternary plot. Similarly as A1, optimal growth conditions are not present as indicated by low diversity (mean SDI 1.367).

Assemblage 3 (A3) contains six samples from EE and one sample from MB. The average depth for the samples from EE is 2.5 m and the sample from MB is from a depth of 1.8 m (M7). The mean salinity for all samples increased to 3.4 ± 0.2‰, and faunal diversity still remains low (mean SDI = 1.509), albeit slightly more elevated than the other assemblages, indicating that faunal populations are still experiencing some ecological stress. From A2 to A3, the centropyxid taxa continue to decrease in dominance (mean 48%), agglutinated foraminifera increase (mean 36%), and there is the notable presence of hyaline foraminifera (mean 14%). The relative contribution of these taxa to the assemblage skews the position of the samples to the bottom left and center of the ternary diagram (Fig. 5.5). *Centropyxis aculeata* is no longer a dominant species in A3, and the abundance of the dwarfed *Centropyxis constricta* is doubled (mean 28%). The abundance of *Jadammina macrescens* (30%) and *M. fusca* (3%) also increase. *Tritaxis fusca* (mean 4 ±4%) and *Ammonia tepida* (mean 13 ±10%) contribute to populations, but there is large intra-sample variability. Test aberrancy was recorded in four samples (E1, E8, E10, E12), where 2% of *Ammonia tepida*, 35% of *Elphidium gunteri* and 5% of *J. macrescens* had irregular test morphologies.

All the samples forming Assemblage 4 (A4) are from EE at mean depth of 4.5 m and salinity of 3.7 ± 0.4‰. Thecamoebian taxa decrease significantly (mean 9%) with large intra-sample variability (± 13%). *Ammonia tepida* (mean 51%) is the dominant taxa with *T. fusca* (21%), and *E. gunteri* (mean 11 ±12%) contributing to the assemblage and increasing the diversity. The dominance of hyaline foraminifera in A4 causes the samples to plot more skewed to the lower right of the ternary diagram. Diversity is low (SDI = 1.284) from continual environmental stress in low salinity conditions. In samples where test aberrancy was recorded (E5, E7, E9), 17% of *E. gunteri* contained irregular chambers, and 25% of *J. macrescens* in E9.

2.6. DISCUSSION

2.6.1. COMPARISON BETWEEN DIFFERENT CENOTES

The greatest dominance of thecamoebians occurs in CW (mean 99.6%), which is to be expected, as this cenote has salinity (1.5‰) values closest to the ideal thecamoebian habitat of fresh water. In the ternary diagram, all the samples from CW plot at the apex, reflecting the dominance of thecamoebians over foraminifera in that cenote (Fig. 5.5). One sample plots slightly down the left axis, reflecting the minor contribution of *J. macrescens* at that sampling site. At MB, there is a larger contribution of agglutinated foraminifera (13%) causing the samples from that location to plot along the left axis of the ternary diagram. Thecamoebians are still the dominant fauna (85%) in MB, although the slightly higher salinity (2.9‰) is more conducive to textularid foraminiferal populations than in CW. Since both of these cenotes have similar benthic conditions (Fig. 2.2.), hydrologic flow (1.5 cm/s), pH, oxygen, and temperature, the difference in salinity is likely the dominant abiotic factor contributing to the observed faunal variations.

In contrast to MB and CW, the samples from EE are more evenly distributed over the ternary diagram than the other two cenotes. In addition to thecamoebians (mean 28%) and agglutinated foraminifera (mean 29%), EE also is a habitat for hyaline foraminifera (mean 43%). The halocline is the shallowest at EE, and hydrologic flow is doubled (3 cm/s as compared to the other sites. The higher salinity at EE (>3.3‰), associated with the close proximity to the coast, allows euryhaline hyaline taxa to become established (i.e., *A. tepida*) and precludes a larger thecamoebian population. Intrasample variability can be explained through: (1) subtle increases in salinity with depth in the freshwater lens; (2) complex bathymetric topography creating many microenvironments that may be influenced through seasonal temperature changes, and (3), sediment re-suspension and transport through tourist disturbance and higher flow rates. These environmental variables are biologically relevant for amoebae, which can temporarily encyst during ecologically unfavourable conditions. Since these characteristics are not as prevalent at MB or CW, more consideration is given to these intrinsic characteristics when interpreting samples from EE.

2.6.2. ASSEMBLAGE 1 (1.5‰)

The CW environment is the only location where A1 is located, suitable for only the most euryhaline of the thecamoebians. Therefore, A1 is dominated by *C. aculeata* and precluding the presence of other more stenohaline thecamoebians (e.g., *Difflugia oblonga* "triangularis"). The increased primary productivity in CW seems to promote larger populations of *Arcella vulgaris*. This qualitative assessment is based on other studies where *A. vulgaris* becomes more dominant taxa when there is increased nutrient loading or primary productivity (Reinhardt and others, 2005). *Jadammina macrescens* with test aberrancy is rare, indicating some environmental stress on the species. These results are similar to other coastal and lacustrine studies where: (1) *Centropyxis* sp. is a known opportunist and the most euryhaline of the testate amoebae (i.e., Riveiros and others, 2007; Scott and others, 2001); and (2) *Arcella vulgaris* is an indicator of environmental stress from nutrient-loading (i.e., Roe and Patterson, 2006; Reinhardt and others, 2005; Patterson and others, 2002); and (3) rare *J. macrescens* are persisting at their ecological freshwater boundary of 1.5‰ (Murray, 2007).

2.6.3. ASSEMBLAGE 2 (2.7 ‰ – 3.1‰)

Between A1 and A2, there are taxonomic and environmental transitions. All the samples plot in the top-most region of the ternary diagram, indicating that thecamoebians are still dominant contributors to the overall assemblage. *Jadammina macrescens* is still the only foraminiferan, with only a minor increase in abundance from A1. In the thecamoebians, there is a change in the dominant strain of the species *C. aculeata*. In the A2, there is a change to a dominance of *C. aculeata* "discoides" (41%) over *C. aculeata* "aculeata" (27%).

The change to a dominance of *C. aculeata* "discoides" over *C. aculeata* "aculeata" from A1 to A2 is a noteworthy observation. Reinhardt and others (1998) divided *C. aculeata* and *C. constricta* into ecophenotypes or 'strains' based on the presence or absence of spines: (1) *C. aculeata* "aculeata" is ornamented with >1 spines on the aboral (fundus) region, we have observed up to 18; (2) *C. aculeata* "discoides" is circular, without spines; (3) *C. constricta* "spinosa" is ornamented with 1-3 aboral spines, and (4) *C. constricta* "spinosa" is ornamented with >3 aboral spines. In previous lake studies, attributing these centropyxid strains to a definitive abiotic variable has proven problematic, although evidence does suggest that multiple environmental factors play a role in the phenotype expressed by a species (e.g., Reinhardt and others, 1999; Medioli and Scott, 1983).

To retain consistency among the entire *Centropyxis* genus in this study, our taxonomic strain concept of *C. aculeata* is consistent with Reinhardt and others (1998), but *C. constricta* is modified to: (1) *C. constricta* "spinosa"; identified based on the presence of spines on the aboral region of the test (>1, we have observed up to 8), (2) C. constricta "aerophila" has no spines on the aboral region. In A2, which has slightly elevated salinity conditions over A1, the *C. aculeata* test morphology without spines predominates (*C. aculeata* "discoides"). This is evidence that shells with the presence or absence of spines are different ecophenotypes of the same species. Evidence of this relationship is also presented elsewhere on the globe in non-tropical settings. In British Columbia salt marshes presented in Riveiros and others, (2007), where the strains "discoides" (mean 12.5%) and "aerophila" (14%) dominate over "aculeata" (7%) and "spinosa" (8%) in their High Marsh Assemblage. In UK saltmarshes, Gehrels and others (2001) *C. constricta* "aerophila" (designated as *C. platystoma*, but reported by the authors as equivalent to the "aerophila" morphotype) is the most euryhaline of the thecamoebians (see Figure 5 in Gehrels and others, 2001). These results indicate that in environments with marginal-salinity, the expression of spines on the thecamoebian test is related to salinity. However, since spines are also expressed on thecamoebians in lacustrine environments, other ecological or environmental factors may be controlling the expression of spines on the phenotype (such as pH, percent moisture, or factors related to reproduction).

2.6.4. ASSEMBLAGE 3 (3.2‰ – 3.6‰)

In A3, *C. constricta* form the largest thecamoebian component (35%), with foraminifera emerging as the dominant taxonomic grouping in the assemblage. Plate 1 illustrates the separation of species C. *aculeata* and C. *constricta* based on the geometric angle between the aperture face and test wall (After Fig. 10 in Medioli and Scott, 1983; see Plate 1). In A3, the test of *C. constricta* is consistently autogenous; the strain without spines dominates the assemblage (*C. constricta* "aerophila", 20%); and they have an overall dwarfed size (45 – 63 µm). As such, these *C. constricta* "aerophila" are interpreted as the most euryhaline of the thecamoebians, persisting at the ecological boundary of the group. This taxonomic observation was similarly noted in Gehrels and others (2001). Again, we interpret the lack of spines in *C. constricta* "aerophila" as physiologically advantageous over the morphotype with spines.

The increased salinity at the sites of samples from A3 is becoming more suitable for foraminifera, with increased abundance of *J. macrescens* (29%) and *T. fusca* (3%). *Ammonia tepida* and *E. gunteri* are also present, and the presence of small numbers of aberrant tests indicates some minor ecological stress on the populations. Longer-term salinity data in the cenote indicate very minor shifts over time (Beddows, unpublished data), however, the shallower average depth for A3 (~2.5 m) introduces possible micro-environmental control on the distributions from the formation of seasonally-controlled lower salinity fresh-cap layers in the cenote. The fresher surface waters during rainy seasons would negatively affect the health of hyaline foraminifera, but positively affect thecamoebians (especially opportunist taxa such as Centropyxid taxa). In contrast, evaporation during the dry season would slightly increase salinity creating conditions that are more favorable for foraminifera. This probably explains the smaller amounts of *C. aculeata* in the assemblage and large intra-sample variability in the relative abundance *E. gunteri* and *T. fusca* in A3. This assemblage represents the ecological boundary of thecamoebians in the cenotes.

2.6.5. ASSEMBLAGE 4 (3.3‰ – 4.1‰)

The most saline assemblage is A4 (mean 3.7‰), located at deeper depths relative to the water table at EE than A3. Foraminifera dominate A4 with minor contribution from *C. aculeata* (mean <10%) to the assemblage. Since this cenote is a popular tourist area (snorkeling, SCUBA diving) and flow rates are higher (3 cm/s), the possibility of thecamoebian tests being disturbed through recreational activity (post-mortem re-suspension) and transported to another deeper local cannot be ignored. Since the biocoenosis was not quantified directly, the reworking and transport of tests from shallower depths is a likely explanation of variable thecamoebian tests in A4, and further explains the high intra-sample variability (standard deviations) in the mean abundance of taxa. The foraminifer *A. tepida* (51%) dominates the assemblage, along with *T. fusca* (21%), and *E. gunteri* (11%). The general trend in the cenotes of decreasing *J. macrescens* and increasing abundance of other halophiles is consistent with general foraminiferal ecology (Scott and Medioli, 1978, 1980).

Aberrant tests in foraminifera have been widely observed in response to anthropogenic influences such as pollution and natural variability in temperature, salinity, dissolved oxygen, pH, nutrition, and other factors at the ecological boundary of a species (Geslin and others, 2000; Yanko and others, 1994; Boltovskoy and others, 1991). In the cenotes, dissolved oxygen, pH, and temperature are all within the ecological requirements for healthy foraminiferal growth. The occurrence of test aberrancy is not as great in the cenotes as in other settings (e.g., Wennrich and others, 2007), thereby suggesting only minor ecologic stress on the foraminiferal populations. However, the marginal salinities are interpreted as being the dominant ecological stress causing the observed test aberrancy.

2.7. CONCLUSIONS

Standing populations of foraminifera and thecamoebians exist in the cenotes on the Yucatan Peninsula, Mexico, and these populations are responding to environmental controls in this low latitude setting similarly to other temperate high latitude systems. The anchialine (and hyposaline) nature of cenotes makes them another ideal setting to study thecamoebian and foraminiferan ecology. In contrast to other coastal systems suitable for concurrently studying thecamoebians and foraminifera, sheltered cenotes remain permanently aquatic habitats with consistent longer-term hydrology that are sheltered from coastal perturbations (e.g. wave action or hurricanes). This intrinsic characteristic makes standing rhizopod populations dominantly influenced by aquatic conditions (not percent moisture) and less susceptible to taphonomic processes from wave activity or storms (i.e., hurricanes).

The thecamoebian morphotypes without spines dominated over the morphotypes with spines as salinity increased in the cenotes. The environmental influence on the presence or absence of spines is additional evidence for the morphological fluidity in individual thecamoebian species. An important transition from thecamoebian-dominated populations to foraminiferan-dominated populations occurs at a salinity of ~3.5‰. This is the first paper to report such an ecological boundary for thecamoebians, which needs further evaluation in other environments. However, this evidence further supports salt marsh research that documents thecamoebians surviving in saline conditions more elevated than previously thought. In the cenotes, dwarf (45 – 63 μm) autogenous *C. constricta* "aerophila" persisted as the most euryhaline thecamoebian. This ecophenotype is interpreted as the most favorable Centropyxid morphology under stressed conditions imposed through elevated salinity.

Finally, there are few environments where thecamoebians and foraminifera can ecologically co-exist in the same populations. Since foraminifera and thecamoebians play important roles in the nutrient transfer from detritus, primary productivity, and prokaryotes to higher trophic levels (Finlay and Estaban, 1998; Lipps and Valentine 1970), their interspecific interactions within the same ecological niche warrants further investigation. These results indicate that anchialine cenotes are another paralic environment in addition to the salt marsh where the collective ecology of thecamoebians and foraminifera can be studied. In these unique settings, the ecological interactions between fauna in the total rhizopod population—thecamoebians and foraminifera—are currently unknown.

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2.10. CHAPTER 2 FIGURES

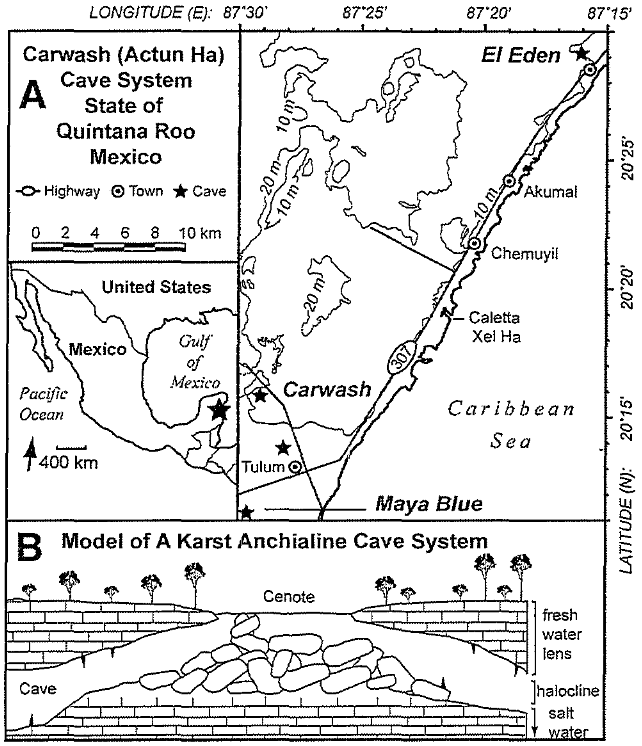

FIGURE 2.1. A: Map of Quintana Roo and location of cenotes. **B:** Idealized model of a karst anchialine cave system on the Yucatan Peninsula, Mexico.

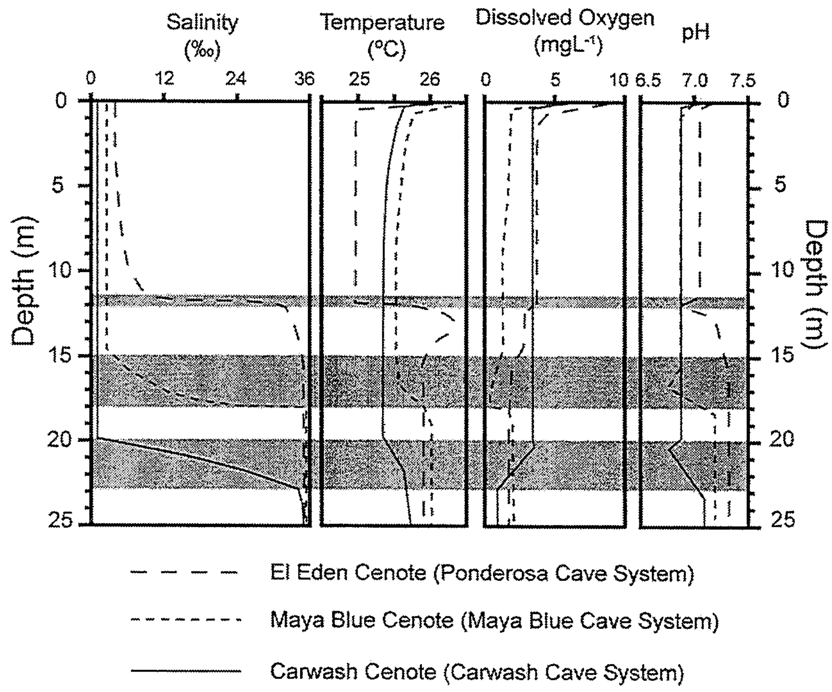

FIGURE 2.2. Depth profiles of hydrologic variables at each cenote. The shaded region represents the location of the halocline at each site.

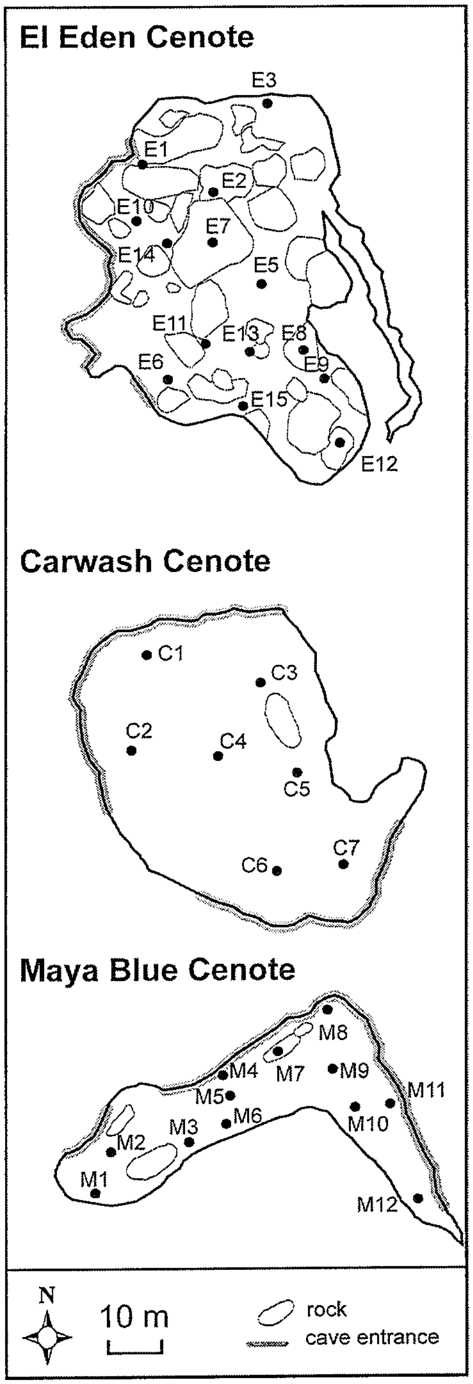

FIGURE 2.3. Map of cenotes with surface sample locations.

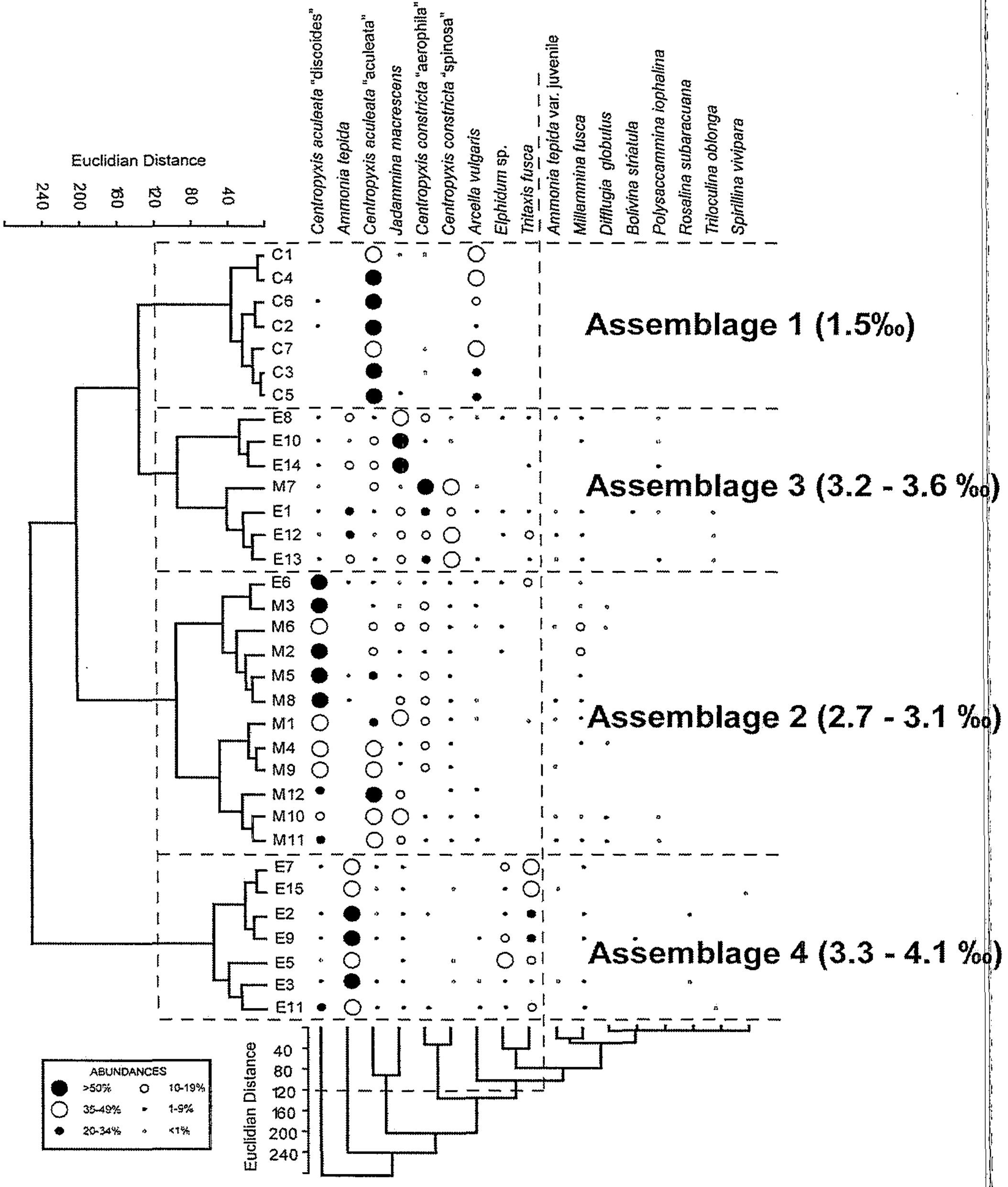

FIGURE 2.4. R-mode versus Q-mode dendrogram that indicates four distinct assemblages. The mean salinity in a 1 standard deviation range for each assemblage are provided, and is interpreted as the dominant control the faunal distributions. Sample labels follow Figure 3.3.

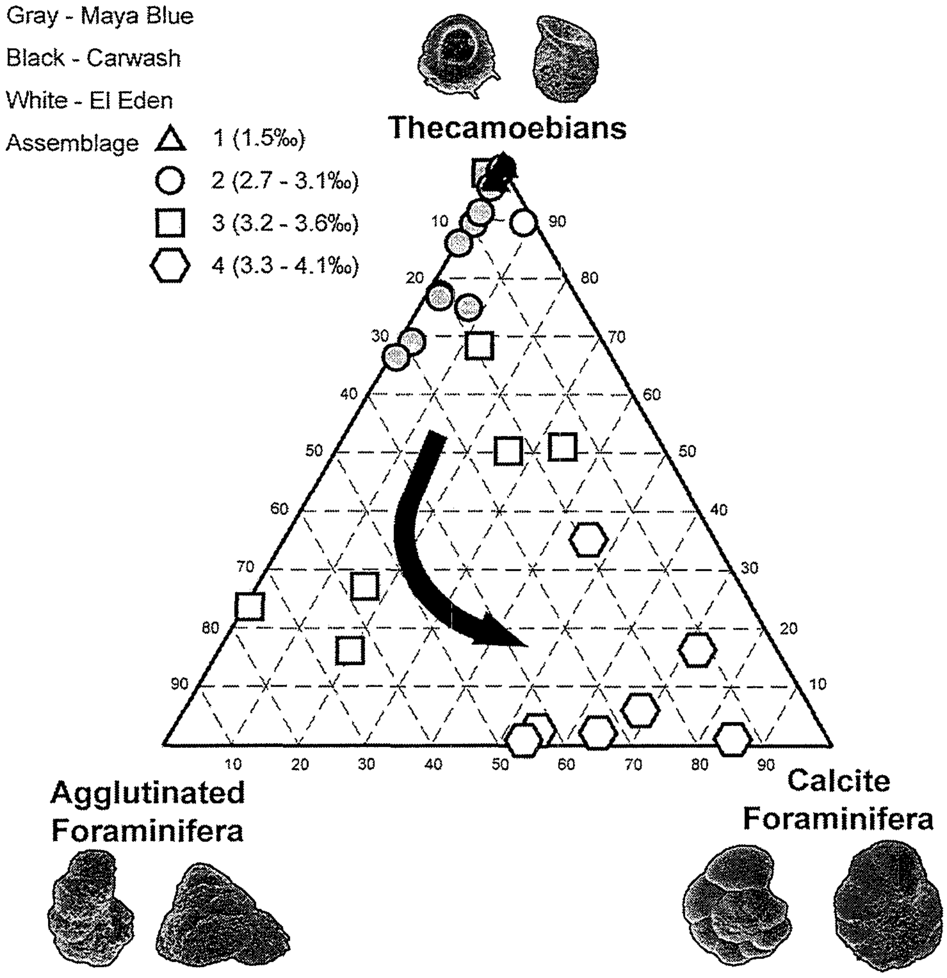

FIGURE 2.5. A low-salinity (<5‰) ternary diagram that includes both thecamoebians and foraminifera. Note the relative location of samples in the four assemblages relative to the black arrow, which is indicating the expected faunal trends with increasing salinity.

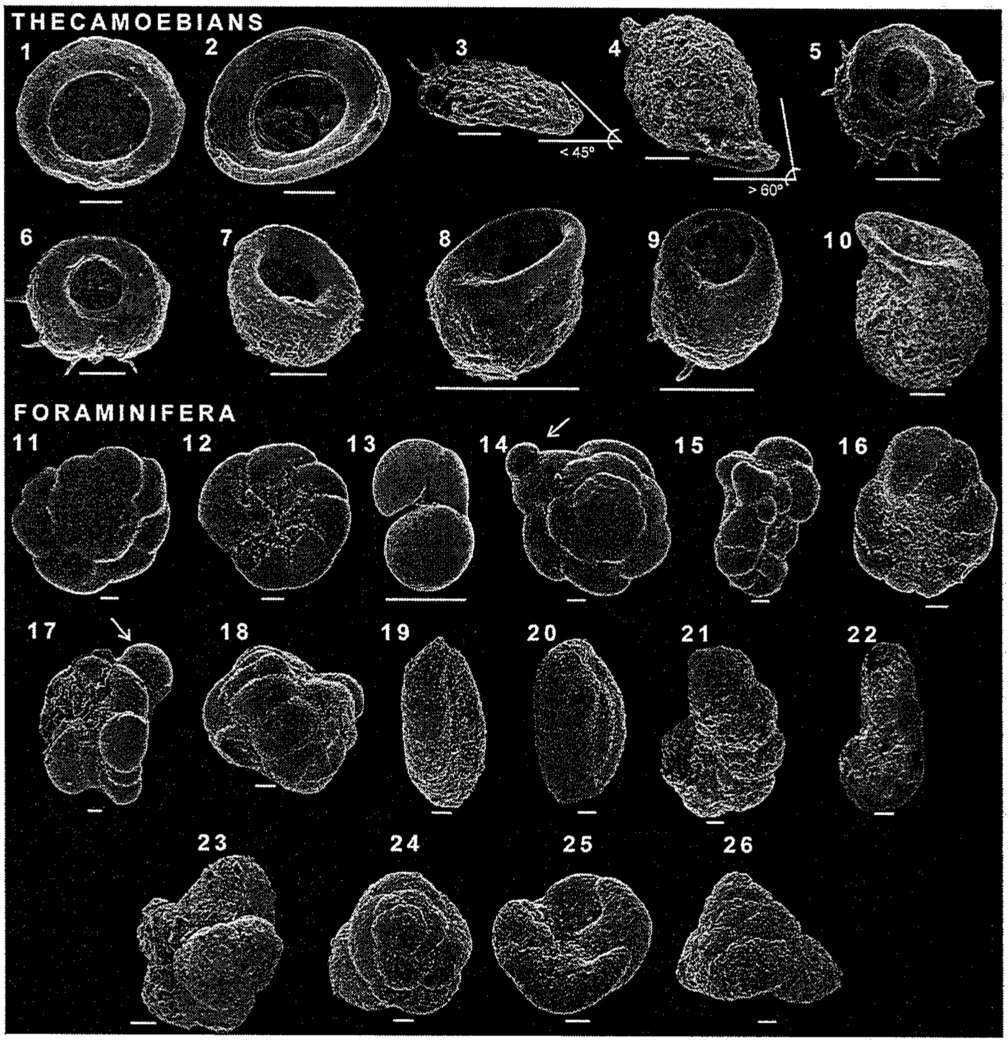
THECAMOEBIANS
1
2
3
4
5
< 45°
> 60°
6
7
8
9
10
FORAMINIFERA
11
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PLATE 2.1. ***(PRECEDING PAGE)*** **Thecamoebians:** 1-10. 1. *Arcella vulgaris* – autogenous test (x341). 2. *A. vulgaris* (x341). 3. *Centropyxis aculeata* – xenogenous test (x406). Note the taxonomic criterion for angle between aperture face and body wall to be < 45° (Medioli and Scott, 1983; x600). 4. *C. constricta* specimen with spines on xenogenous test (x300). Note the taxonomic criterion for angle between aperture face and body wall to be > 60°, (Medioli and Scott, 1983; x600). 5. *C. aculeata* "aculeata" (x462). 6. *C. aculeata* "aculeata" (x388). 7. *C. aculeata* "discoides" (x462). 8. Impoverished and dwarfed *C. constricta* "aerophilia" with autogenous test (x425). 9. *C. constricta* "spinosa" – autogenous test (x300). 10. *C. constricta* "aerophilia" – xenogenous test (x425). **Foraminifera:** 11-34. 11. *Ammonia tepida*, spiral side (x388). 12 *A. tepida*, umbilical side (x462). 13. *A. tepida* var. juvenile. This juvenile form is just the proloculus and first two chambers, a consistent morphology (x775). 14 – 15. *Ammonia tepida* – aberrant test shapes (x150). 14. Arrow points to abnormal additional chamber. 15. Complex form. 16. *Elphidium gunteri* (x150). 17 – 18. *E.* gunteri - aberrant test shapes (x150). 17. Arrow points to abnormal additional chamber. 18. Complex form. 19 – 20. *Millammina fusca* (x150). 21. *Jadammina macrescens* (x150). 22. *J. macrescens* - aperture view (x203). 23. *J. macrescens* – aberrant test displaying a twinned tests (x186). 24. *Tritaxis fusca* – spiral view (x170). 25. *T. fusca* – umbilical view (x231). 26. *T. fusca* – side view (x156). See Figure 4 of Geslin and others (2000) for categorization of difference morphological abnormalities.

2.11. CHAPTER 2 TABLES

TABLE 2.1. Average Shannon Diversity Index, salinity, and relative abundance for each taxon in the four assemblages.

	Biofacies	1	2	3	4
	Shannon Diversity Index (*H*)	1.034	1.367	1.509	1.284
	Salinity (Mean ‰)	1.5	2.9 ± 0.2	3.3 ± 0.2	3.7 ± 0.4
THECAMOEBIANS	*Arcella vulgaris*	**21 ± 12**	2 ± 3	<1	<1
	Centropyxis aculeata "aculeata"	**53 ± 8**	**27 ± 18**	8 ± 5	3 ± 3
	Centropyxis aculeata "discoides"	21 ± 6	**41 ± 19**	3 ± 2	5 ± 9
	Centropyxis constricta "spinosa"	<1	3 ±1	15 ±15	<1
	Centropyxis constricta "aerophila"	<1	9 ± 6	**20 ± 17**	<1
FORAMINIFERA	*Jadammina macrescens*	2 ± 4	3 ± 4	**29 ± 28**	3 ±2
	Tritaxis fusca	0	<1	3 ± 4	**21 ± 13**
	Ammonia tepida	0	<1	13 ±10	**51 ± 9**
	Elphidium gunteri	0	<1	<1	11 ± 12

2.12. ONLINE SUPPLEMENTARY MATERIAL

TABLE 2.S1. (Following Pages) Percent abundance, standard error, depth, salinity for each sample.

Sample	**E1**	**E2**	**E3**	**E5**	**E6**	**E7**	**E8**	**E9**	**E10**
Depth (m)	3.4	6.4	1.8	5	3	4	2.1	5.5	2.4
Salinity (‰)	3.5	4.2	3.4	3.7	3.5	3.6	3.4	4	3.5
Sum	331	179	327	496	259	394	1075	462	219
Individuals/cc	26	24	131	50	104	79	430	185	88
Shannon Diversity Index (*H*)	1.965	1.187	1.28	1.078	0.997	1.232	1.529	1.41	1.143
Arcella vulgaris	1.81	-	0.61	-	1.93	-	0.28	1.52	-
standard error (±)	1.44	-	0.85	-	1.68	-	0.32	1.11	-
Buliminella indicata	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-
Centropyxis aculeata "aculeata"	3.63	0.56	9.17	-	4.63	1.02	3.91	2.81	12.79
standard error (±)	2.01	1.09	3.13	-	2.56	0.99	1.16	1.51	4.42
Centropyxis aculeata "discoides"	1.21	1.12	6.12	0.81	76.45	1.52	3.26	1.73	6.39
standard error (±)	1.18	1.54	2.60	0.79	5.17	1.21	1.06	1.19	3.24
Centropyxis cconstricta "aerophila"	29.91	0.56	-	-	5.02	-	19.72	-	3.65
standard error (±)	4.93	1.09	-	-	2.66	-	2.38	-	2.48
Centropyxis constricta "constricta"	14.80	-	0.31	0.20	2.32	-	0.09	-	0.91
standard error (±)	3.83	-	0.60	0.39	1.83	-	0.18	-	1.26
Difflugia globulus	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-
Difflugia oblonga	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-
Lagenodifflugia vas	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-
Ammonia tepida var. juvenile	0.30	-	0.61	-	-	-	0.84	-	-
standard error (±)	0.59	-	0.85	-	-	-	0.54	-	-
Ammonia tepida	25.68	58.66	66.06	48.59	5.41	44.42	13.77	51.95	0.46
standard error (±)	4.71	7.21	5.13	4.40	2.75	4.91	2.06	4.56	0.89
Bolivina striatula	3.63	-	-	-	-	-	-	1.30	-
standard error (±)	2.01	-	-	-	-	-	-	1.03	-
Bolivina variabilis	0.30	-	-	-	-	-	-	-	-
standard error (±)	0.59	-	-	-	-	-	-	-	-
Elphidum sp.	3.02	2.23	4.28	36.69	2.70	10.66	1.30	14.94	-
standard error (±)	1.84	2.17	2.19	4.24	1.97	3.05	0.68	3.25	-
Millammina fusca	1.21	3.91	7.65	-	0.39	2.79	5.30	2.60	9.13
standard error (±)	1.18	2.84	2.88	-	0.76	1.63	1.34	1.45	3.82
Polysaccammina iophalina	0.30	-	-	-	-	-	0.09	-	0.46
standard error (±)	0.59	-	-	-	-	-	0.18	-	0.89
Rosalina subaracuana	-	2.79	0.61	-	-	-	-	-	-
standard error (±)	-	2.41	0.85	-	-	-	-	-	-
Spirillina vivipara	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-
Textularia earlandi	0.60	-	-	-	-	-	-	-	-
standard error (±)	0.83	-	-	-	-	-	-	-	-
Triloculina oblonga	0.60	-	-	-	-	-	-	-	-
standard error (±)	0.83	-	-	-	-	-	-	-	-
Tritaxis fusca	2.72	27.37	2.14	12.70	0.77	38.32	1.67	21.43	-
standard error (±)	1.75	6.53	1.57	2.93	1.07	4.80	0.77	3.74	-
Jadammina macrescens	10.27	2.79	2.45	1.01	0.39	1.27	49.77	1.73	66.21
standard error (±)	3.27	2.41	1.67	0.88	0.76	1.11	2.99	1.19	6.26

Sample	**E11**	**E12**	**E13**	**E14**	**E15**	**M1**	**M2**	**M3**	**M4**	**M5**
Depth (m)	1.8	3.4	2.7	2.1	6.7	1.8	4.9	1.8	5.49	2.7
Salinity (‰)	3.4	3.5	3.5	3.4	4.4	2.9	2.9	2.9	2.9	2.9
Sum	193	296	215	93	242	382	234	233	328	162
Individuals/cc	25	24	29	37	24	306	187	186	328	162
Shannon Diversity Index (*H*)	1.666	1.779	1.977	1.124	1.139	1.625	1.242	1.146	1.298	1.277
Arcella vulgaris	3.63	-	2.33	-	-	0.52	-	9.01	-	-
standard error (±)	2.64	-	2.01	-	-	0.72	-	3.68	-	-
Buliminella indicata	-	-	-	-	-	0.52	-	-	-	-
standard error (±)	-	-	-	-	-	0.72	-	-	-	-
Centropyxis aculeata "aculeata"	4.15	0.68	8.84	12.90	0.41	25.39	12.82	4.72	40.85	20.37
standard error (±)	2.81	0.93	3.79	6.81	0.81	4.36	4.28	2.72	5.32	6.20
Centropyxis aculeata "discoides"	26.42	0.68	5.58	3.23	-	35.60	59.83	64.38	38.11	55.56
standard error (±)	6.22	0.93	3.07	3.59	-	4.80	6.28	6.15	5.26	7.65
Centropyxis cconstricta "aerophila"	1.04	18.92	20.93	-	-	19.37	3.42	16.31	13.41	14.20
standard error (±)	1.43	4.46	5.44	-	-	3.96	2.33	4.74	3.69	5.37
Centropyxis constricta "constricta"	-	30.41	31.16	-	0.41	8.64	1.71	4.29	3.35	1.85
standard error (±)	-	5.24	6.19	-	0.81	2.82	1.66	2.60	1.95	2.08
Difflugia globulus	-	-	-	-	-	-	-	0.43	0.30	-
standard error (±)	-	-	-	-	-	-	-	0.84	0.60	-
Difflugia oblonga	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Lagenodifflugia vas	-	-	-	-	-	-	-	-	0.30	-
standard error (±)	-	-	-	-	-	-	-	-	0.60	-
Ammonia tepida var. juvenile	-	1.35	0.47	-	0.41	0.52	-	-	-	-
standard error (±)	-	1.32	0.91	-	0.81	0.72	-	-	-	-
Ammonia tepida	38.86	22.64	11.16	19.35	49.59	-	-	-	-	0.62
standard error (±)	6.88	4.77	4.21	8.03	6.30	-	-	-	-	1.21
Bolivina striatula	-	0.68	-	-	-	-	-	-	-	-
standard error (±)	-	0.93	-	-	-	-	-	-	-	-
Bolivina variabilis	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Elphidum sp.	6.22	0.68	-	-	2.48	-	1.71	-	-	-
standard error (±)	3.41	0.93	-	-	1.96	-	1.66	-	-	-
Millammina fusca	1.04	1.69	1.86	-	-	4.97	17.09	0.43	2.13	3.70
standard error (±)	1.43	1.47	1.81	-	-	2.18	4.82	0.84	1.56	2.91
Polysaccammina iophalina	-	-	2.33	2.15	-	-	-	-	-	-
standard error (±)	-	-	2.01	2.95	-	-	-	-	-	-
Rosalina subaracuana	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Spirillina vivipara	-	-	-	-	0.83	-	-	-	-	-
standard error (±)	-	-	-	-	1.14	-	-	-	-	-
Textularia earlandi	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Triloculina oblonga	0.52	0.34	0.47	-	-	-	-	-	-	-
standard error (±)	1.01	0.66	0.91	-	-	-	-	-	-	-
Tritaxis fusca	14.51	11.82	3.72	1.08	36.36	0.26	-	-	-	-
standard error (±)	4.97	3.68	2.53	2.10	6.06	0.51	-	-	-	-
Jadammina macrescens	3.63	10.14	11.16	61.29	9.50	4.19	3.42	0.43	1.52	3.70
standard error (±)	2.64	3.44	4.21	9.90	3.70	2.01	2.33	0.84	1.33	2.91

Sample	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**	**C1**	**C2**	**C3**
Depth (m)	4	1.8	5.2	3.6	2.4	4.6	2.4	5.5	4.9	4.6
Salinity (‰)	2.9	2.9	2.9	2.9	2.9	2.9	2.9	1.5	1.5	1.5
Sum	246	253	224	429	169	260	120	294	157	223
Individuals/cc	246	204	196	343	135	208	96	78	31	45
Shannon Diversity Index (*H*)	1.731	1.049	1.565	1.22	1.636	1.672	1.18	1.005	0.901	1.0379
Arcella vulgaris	0.41	0.40	0.45	-	4.14	2.31	5.83	38.78	5.73	21.08
standard error (±)	0.80	0.77	0.87	-	3.00	1.83	4.19	5.57	3.64	5.35
Buliminella indicata	-	-	0.45	-	-	0.38	-	-	-	-
standard error (±)	-	-	0.87	-	-	0.75	-	-	-	-
Centropyxis aculeata "aculeata"	13.82	13.83	8.93	34.27	35.50	40.00	56.67	48.98	63.69	52.91
standard error (±)	4.31	4.25	3.73	4.49	7.21	5.95	8.87	5.71	7.52	6.55
Centropyxis aculeata "discoides"	34.15	0.40	51.79	46.15	14.20	22.69	21.67	11.56	28.03	25.56
standard error (±)	5.93	0.77	6.54	4.72	5.26	5.09	7.37	11.56	28.03	25.56
Centropyxis cconstricta "aerophila"	17.48	50.99	11.61	12.82	5.33	5.00	0.00	-	2.55	-
standard error (±)	4.75	6.16	4.19	3.16	3.39	2.65	0.00	-	2.46	-
Centropyxis constricta "constricta"	3.25	33.99	2.23	4.43	2.96	5.38	2.50	0.34	-	0.45
standard error (±)	2.22	5.84	1.93	1.95	2.55	2.74	2.79	0.67	-	0.88
Difflugia globulus	0.41	-	-	-	4.73	1.54	-	-	-	-
standard error (±)	0.80	-	-	-	3.20	1.50	-	-	-	-
Difflugia oblonga	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Lagenodifflugia vas	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Ammonia tepida var. juvenile	0.41	-	4.91	0.23	0.59	1.92	-	-	-	-
standard error (±)	0.80	-	2.83	0.46	1.16	1.67	-	-	-	-
Ammonia tepida	-	-	2.23	-	-	-	-	-	-	-
standard error (±)	-	-	1.93	-	-	-	-	-	-	-
Bolivina striatula	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Bolivina variabilis	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Elphidum sp.	1.22	-	-	-	-	-	-	-	-	-
standard error (±)	1.37	-	-	-	-	-	-	-	-	-
Millammina fusca	17.07	-	3.57	-	0.59	1.54	-	-	-	-
standard error (±)	4.70	-	2.43	-	1.16	1.50	-	-	-	-
Polysaccammina iophalina	-	-	-	-	0.59	0.77	-	-	-	-
standard error (±)	-	-	-	-	1.16	1.06	-	-	-	-
Rosalina subaracuana	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Spirillina vivipara	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Textularia earlandi	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Triloculina oblonga	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Tritaxis fusca	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Jadammina macrescens	11.79	0.40	13.84	2.10	31.36	18.46	13.33	0.34	-	-
standard error (±)	4.03	0.77	4.52	1.36	7.00	4.72	6.08	0.67	-	-

Sample	**C4**	**C5**	**C6**	**C7**
Depth (m)	4.3	3	5.9	4
Salinity (‰)	1.5	1.5	1.5	1.5
Sum	90	286	106	113
Individuals/cc	29	57	84	90
Shannon Diversity Index (*H*)	0.924	1.174	1.037	1.177
Arcella vulgaris	31.11	20.98	14.15	32.74
standard error (±)	9.56	4.72	6.64	8.65
Buliminella indicata	-	-	-	-
standard error (±)	-	-	-	-
Centropyxis aculeata "aculeata"	57.78	52.45	62.26	36.28
standard error (±)	10.20	5.79	9.23	8.87
Centropyxis aculeata "discoides"	11.11	22.38	19.81	29.20
standard error (±)	11.11	22.38	19.81	29.20
Centropyxis cconstricta "aerophila"	-	-	2.83	-
standard error (±)	-	-	3.16	-
Centropyxis constricta "constricta"	-	-	-	0.88
standard error (±)	-	-	-	1.73
Difflugia globulus	-	-	-	-
standard error (±)	-	-	-	-
Difflugia oblonga	-	-	-	-
standard error (±)	-	-	-	-
Lagenodifflugia vas	-	0.70	0.94	-
standard error (±)	-	0.97	1.84	-
Ammonia tepida var. juvenile	-	1.05	-	0.88
standard error (±)	-	1.18	-	1.73
Ammonia tepida	-	-	-	-
standard error (±)	-	-	-	-
Bolivina striatula	-	-	-	-
standard error (±)	-	-	-	-
Bolivina variabilis	-	-	-	-
standard error (±)	-	-	-	-
Elphidum sp.	-	-	-	-
standard error (±)	-	-	-	-
Millammina fusca	-	-	-	-
standard error (±)	-	-	-	-
Polysaccammina iophalina	-	-	-	-
standard error (±)	-	-	-	-
Rosalina subaracuana	-	-	-	-
standard error (±)	-	-	-	-
Spirillina vivipara	-	-	-	-
standard error (±)	-	-	-	-
Textularia earlandi	-	-	-	-
standard error (±)	-	-	-	-
Triloculina oblonga	-	-	-	-
standard error (±)	-	-	-	-
Tritaxis fusca	-	-	-	-
standard error (±)	-	-	-	-
Jadammina macrescens	-	2.45	-	-
standard error (±)	-	1.79	-	-

CHAPTER 3

FORAMINIFERA AND TESTATE AMOEBAE (THECAMOEBIANS) IN AN ANCHIALINE CAVE

**P.J. van Hengstum, E.G. Reinhardt, Beddows, P.A.,
H.P. Schwarcz and J.J. Gabriel**

This manuscript is currently in review at Geology (submitted Feb 2008) and generally follows the style required by the publisher of this journal.

3.1. ABSTRACT

This study presents the first systematic documentation of testate amoebae and foraminifera from a phreatic cave environment. Seventy-five surface samples were collected in the anchialine cave system Carwash (Actun Ha) located in Quintana Roo, Mexico. The assemblages divide into two biofacies which have distinct isotopic signatures. The Freshwater Biofacies is dominated by living *Centropyxis aculeata* (≈38%), *Arcella vulgaris* (≈10%), and *Ammonia tepida* var. juvenile (≈35%) with a mean $\delta^{13}C$= -10.8‰ and $\delta^{18}O$= -4.9‰ as measured on *Cytheridella ilosvayi* ostracods. This biofacies is located proximal to nearby sinkholes (<75 m upstream, <150 m downstream) and in black to brown colored sediment (mean TOC = 17.5%). The Marine Biofacies is dominated by foraminifera *Bolivina* sp. (≈73%) and *Elphidium* sp. (≈11%) with a mean $\delta^{13}C$= -5.5‰ and $\delta^{18}O$= -2.7‰ measured on foraminifera. This biofacies is located in areas distal from sinkholes in yellow-orange colored sediment (mean TOC = 3.6%). The Marine Biofacies is aged to at least the Sangamon interglacial (MIS 5e) based on overlying flowstone; whereas, taxa of the Freshwater Biofacies are living in the modern cave. No new endemic taxa (stygobites) were found, although the results do indicate that foraminifera and testate amoebae live in aquatic cave systems and the documented species can be considered stygophiles. These results show that foraminifera and testate amoebae can be used as proxies to reconstruct cave environments similarly to other coastal environments.

Keywords: Foraminifera, testate amoebae, thecamoebians, anchialine, cave, cenote

2.2. INTRODUCTION

Foraminifera and testate amoebae (thecamoebians) are found in most aquatic environments throughout the salinity continuum (Mitchell et al., 2008, Murray, 2007; Riveiros et al., 2007; Charman, 2001; Gehrels et al, 2001). They are abundant (>300 mL^{-1}), cosmopolitan, and environmentally sensitive which makes them particularly suited as paleoenvironmental indicators in coastal systems (e.g., sea level, influx of pollution, trophic status; Murray, 2007, Scott et al., 2001). However, even though they have a proven utility in coastal environments, they have not been examined in phreatic cave systems and there is no mention of the cave environment in micropaleontological texts (i.e., Murray, 2007; Scott et al., 2001; Martin, 2000; Sen Gupta, 1999; Ogden and Hedley, 1981). There is some knowledge of naked protists in underground aquifers (Novarino et al., 1997) and testate rhizopods in vadose cave systems (Barr, 1964), but only a brief mention of foraminifera in test samples from phreatic caves (Javaux and Scott, 2003). Previous geobiologic research in phreatic caves is focused more on prokaryotic ecology, food webs of larger taxa, but omits the protozoan community (Sánchez et al., 2002; Schmitter-Soto et al., 2002; Pohlman et al., 1998; Sarbu et al., 1996; Iliffe, 1992).

Here we present the first documentation of modern testate amoebae and foraminifera from the anchialine Carwash (Aktun Ha) Cave System, Quintana Roo, Mexico (Fig. 3.1). The micropaleontological evidence shows that benthic communities of testate amoebae and foraminifera exist in phreatic caves and are responding to physico-chemical conditions similarly to other coastal settings.

2.2. CARWASH CAVE SYSTEM

The tectonically-stable Yucatan karst landscape in Mexico contains ubiquitous anastomosing caves and sinkholes (cenotes) in Miocene to late Pleistocene reefal limestone (Weidie, 1985; Szabo et al., 1978; Fig. 3.1). The aquifer is characterized by a freshwater lens separated from intruding marine water below by a halocline, a chemical dissolution zone dominantly responsible for phreatic anchialine cave formation (Smart et al., 1998, 2006; Ward et al., 1985). Neogene eustatic sea level oscillations have shifted the relative elevation of the halocline resulting in polygenetic caves that transverse through both fresh and salt water sections of the aquifer.

Carwash Cave System is located 8.4 km inland with passages dominantly located in the fresh water mass (1.5 PSU, 25.5°C, 3.5 mgL^{-1} dissolved oxygen) with one cave chamber below the halocline (21 m; Chamber of the Ancients, 35 PSU, 25.7°C, ~1.0 mgL^{-1} dissolved oxygen, Fig. 3.S1). Organic matter and nutrients from aquatic and terrestrial sources around the cenotes are transported into the cave by hydrologic flow (1 cms^{-1}, Moore et al., 1992), and ambient light penetrates only decimeters into the cave. Cave sediment is organic-rich gyttja near the cenotes but is increasingly patchy and sparse in the deep cave. The non-organic sediments are characterized by larger calcite minerals (>250 μm in diameter), limestone fragments (>1 cm in diameter), and marl with goethite. These sediments, where present, were sampled for microfossil analysis (n=75) encompassing all cave sub-environments (i.e., light, salinity, depth).

2.3. FRESHWATER BIOFACIES

The Freshwater (FW) Biofacies is found in the main cenote (1.5 PSU) and into the shallow cave (<75 m upstream and <150 m downstream) ranging in depth from -3 m to -22 m. The contributing taxa were identified as living using rose bengal stain (Fig. 3.2A). *Centropyxis aculeata* (77%) and *Arcella vulgaris* (21%) dominate in the cenote open water (max. depth -6 m), which is associated with higher productivity (mesotrophia), extensive benthic algal mats, diverse aquatic communities (Sánchez et al., 2002; Schmitter-Soto et al., 2002), and organic-rich sediment (TOC=69.7%). These results are similar to other coastal and lacustrine studies where: (1) *Centropyxis* sp. is a known opportunist and the most euryhaline of the testate amoebae (i.e., Riveiros et al. 2007; Scott et al. 2001); and (2) *Arcella vulgaris* is an indicator of environmental stress and nutrient-loading (i.e., Roe and Patterson, 2006; Reinhardt et al., 2005; Patterson et al., 2002).

In the shallow cave, the testate amoebae diversity increases, an unexpected observation as lower diversities would be anticipated in light-limited (to dark) conditions. Xenogenous testate amoebae *Lagenodifflugia vas* (1.6%*), Difflugia globulus* (3.3%), and *Difflugia oblonga* (2.5%) diversify the assemblage along with the appearance of foraminifers *Ammonia tepida* var. juvenile (45 - 60 μm, 35.1%) and rare (<1%) textularids *Jadammina macrescens*, *Millammia fusca*, and *Polysaccammina iophalina*. Salinity remains 1.5 PSU and transported cenote and terrestrial (sticks, leaves) detritus contributes to black-brown sediment (TOC=11.2%). This cave assemblage is unique from that of the cenote, but we still consider it part of the FW Biofacies because it is found above the halocline. The taxa inhabiting the cave environment have not been transported from the cenotes since they are absent in the open water pool. It is possible that some tests of *Centropyxis aculeata* (38.1%) may be transported; however, they are also living in the cave as rose bengal stained individuals were found (~2 mL^{-1}).

Diverse communities of testate amoebae are typically found in healthy lacustrine settings with continual sediment supply and diverse organic matter (OM) pools (Scott et al., 2001). The increase in testate amoebae diversity in the cavern may be related to changing food and OM resource-partitioning; where there is a shift from the dominance of algal grazing in the cenotes to detrital and bacterial grazing in the cave. However, the continued dominance of *Centropyxis aculeata* still indicates a saline-stressed testate amoeba community. Since *Millammina fusca* and *J. macrescens* represent very euryhaline foraminifera taxa (typical range > 1.5 PSU; Murray 1991), the rare textularids recovered from the shallow cave are persisting at their ecologic fresh water boundary. Although *Lagenodifflugia vas* and *Difflugia oblonga* are typical of lake environments, these results are consistent with the salt marsh study of Riveiros et al. (2007) which has recently extended the salinity range of these taxa (0 to ~2 PSU). The freshwater ostracod *Cytheridella ilosvayi* from the sediments of the Freshwater Biofacies had an average $\delta^{13}C = -10.8‰$ and $\delta^{18}O = -4.9‰$ which indicates they were secreted in fresh to slightly brackish water as the $\delta^{13}C$ of the DIC above the halocline is -14‰ (Pohlman et al., 1997) and the $\delta^{18}O$ value of rainwater on the Yucatan Peninsula ranges from -4‰ to -6‰. Since testate amoebae do not have a calcium carbonate shell, they were not analyzed for carbonate isotopes directly. The foraminifera and testate amoebae trends in the shallow cave follow predictable trends based on ecological and environmental controls as seen in other coastal settings (Murray, 2007; Scott et al., 2001).

2.4. MARINE BIOFACIES

There is an abrupt transition from the FW Biofacies to the Marine Water (MW) Biofacies both upstream (~175 m) and downstream (~150 m) of the main cenote in the oligotrophic deep cave (Fig. 3.3). The dominant taxon is *Bolivina striatula* (54.2%), with smaller amounts of *Bolivina variabilis* (16.0%), *Elphidium* sp. (11.3%), and *Rosalina subaracuana* (4.3%). The sediment was yellow-orange with lower TOC (3.6%). Foraminifera eroding out of the limestone were easily identified as steinkerns (Fig. 3.S2) and rare non-stained tests of Centropyxids and Arcellids (<1%) are transported.

Taxa in the MW Biofacies are not tolerant of freshwater conditions but rather are typical bathyl fauna in the Gulf of Mexico (Poag, 1981). Biserially-dominated foraminifera assemblages (i.e. bolivinids) are typical in dysoxic-eutrophic marine environments (Jorissen, 1999) and the fresh water cave is currently oligotrophic and oxic (dissolved oxygen is 3.5 mg/L, Fig. 3.S3). The average isotopic values on foraminifera shells was $\delta^{13}C$ = -5.5‰ and $\delta^{18}O$ = -2.7‰ (Fig 2B). Considering the $\delta^{13}C_{DIC}$ = -3‰ below the modern halocline (Pohlman et al., 1997) and $\delta^{18}O$ close to seawater, the foraminifera shells must have been secreted in a more saline water mass than present. The hyaline foraminifera were not stained by rose bengal and found at a depth of 10.2 m—which is 12.8 m above the modern halocline in fresh water. The ecological tolerances, isotopic composition, and recovery of only dead taxa indicate that the MW Biofacies is a dead assemblage from relict saline conditions in the cave. Importantly, two samples were found sealed beneath vadose speleothem flowstone (S17 and S9; Fig. 3.S1).

2.5. DISCUSSION

No new species of foraminifera or testate amoebae stygobites were found, and taxa in the cenote and shallow cave follow expected trends based on ecological and environmental relationships found in other environments. Light does not seem to be an ecological control on the distribution of cave taxa, but the observed taxa can now be considered stygophiles. Cenote-derived nutrients and OM seem to be a stronger control on distributions, since the living FW Biofacies terminates where TOC sharply decreases (Fig. 3.3B). The one location where modern salinity remains suitable for marine foraminifera (Chamber of the Ancients) is barren because the thin (<2cm) and patchy veneer of calcite-mineral sediment does not receive OM due to the physiographic position of the chamber (Fig. 3.S1).

Chronologically, recovering marine foraminifera +8 m above the present halocline and beneath vadose flowstone indicates that the foraminifera were living in a previous phreatic phase during a prior sea level high stand. The sediments were then sealed in situ beneath flowstone in several locations in vadose conditions during a subsequent sea level low stand (Fig. 3.S1). This sequence of events last occurred after the Sangamon Interglacial high stand (MIS 5e, 124-119 ky BP). Mean sea level stood +4 to +6 m above present during MIS 5e, with many regional reports +8 to +9 m above present (Rohling et al., 2008; Thompson and Goldstein, 2005; Hearty and Neumann, 2001). During this time, dysoxic-tolerant Bolivinids (73.4%) and specialists in herbivory and bactivory *Rosalina* and *Elphidium* (16.4%; Murray, 2007) colonized the cave. The dysoxic-tolerant taxa (bolivinids) indicate that dysoxic water occupied the flooded cave and allochthonous OM and bacterial populations were the dominant food resources. Seeding of the cave environment with foraminifera may be related to bathyl foraminiferal propagules (Alve and Goldstein, 2003) transported with coastal upwelling and subsequently incorporated into geothermal saline convection cells (Moore et al., 1992) and/or sea level driven marine water circulation (Beddows et al., 2007) through the porous limestone. Subsequent sea level drop during the Late Pleistocene drained the cave causing: (1) oxidization of organic matter in the sediment resulting in the iron-oxide orange color, (2) death of the foraminiferan community recorded through the MW Biofacies, and (3) location-specific formation of vadose flowstone over exposed sediments. Early Holocene sea level rise re-flooded the cave with fresh water and initiated OM and nutrient transport mechanisms to create a suitable habitat for testate amoebae and brackish foraminifera in other parts of the cave where OM is sufficiently abundant.

2.6. CONCLUSIONS

Phreatic anchialine cave systems are common throughout neo-tropical carbonate coastlines and these are the first results demonstrating that testate amoebae and foraminifera colonize these systems through time. Geobiologically, their contribution to styglobitic food webs is still unknown, and warrants further research. Since testate amoeba and foraminifera follow predictable environmental trends in the cave, they may provide an unexplored proxy for: (1) phreatic cave geomorphology, (2) average benthic watermass conditions in phreatic caves, and (3) (paleo-) halocline migration and aquifer stratification. In cave systems with sediment accumulations, linkages with sea level, climate, and aquifer circulation could be examined through protist micropaleontology. Finally, where coastal freshwater resources are threatened by pollution and anthropogenic stress, testate amoebae and foraminifera in phreatic systems can provide a cost-effective aquifer-monitoring tool (Scott et al. 2001; Murray 2007).

2.7. ACKNOWLEDGEMENTS

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CHAPTER 3 FIGURES

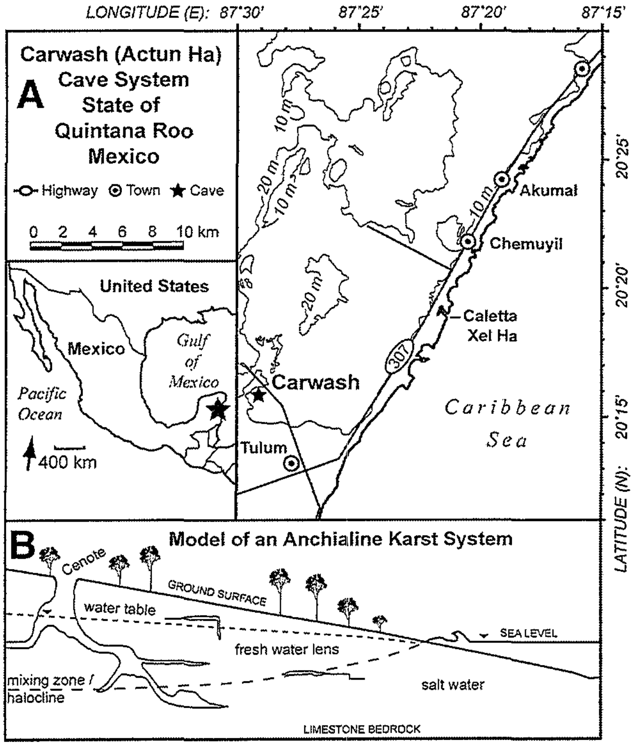


FIGURE 3.1. A: Location of Carwash Cave System along the eastern Yucatan coast. **B:** Model of an anchialine karst cave system.

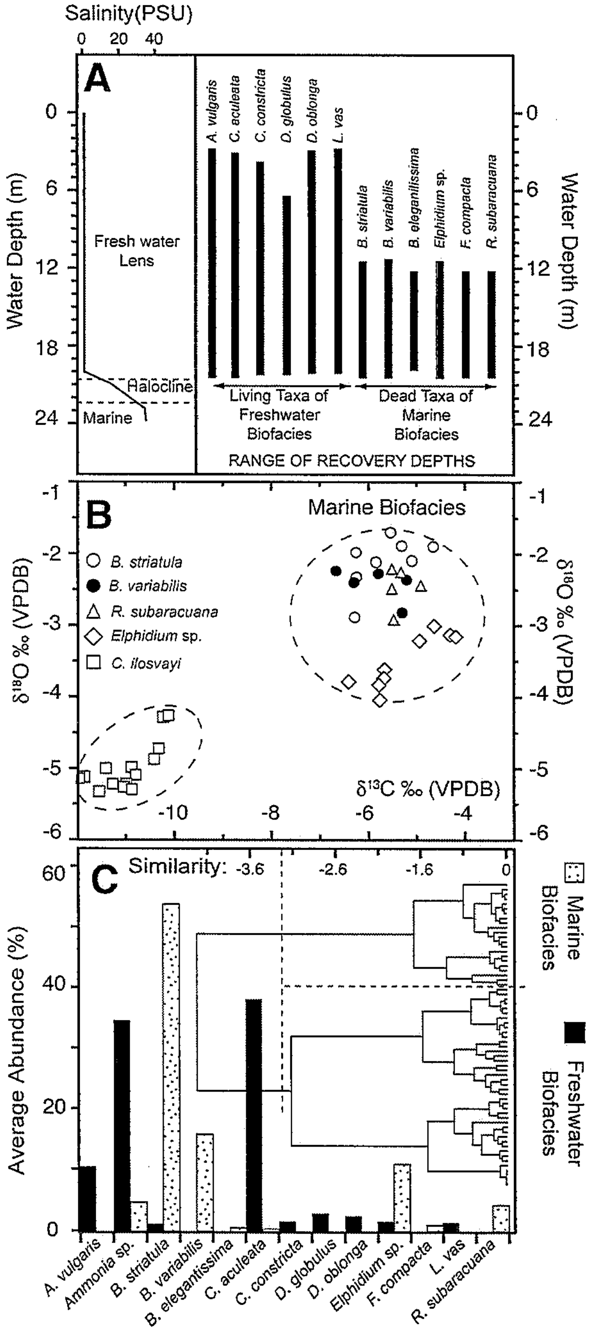


FIGURE 3.2. Biofacies classification (taxa >45 μm).

A: The aquifer salinity profile and range of recovery depths for different species according to rose bengal staining (Bernhard, 2000).

B: Isotopic analysis of freshwater ostracods and foraminifera. Analytical precision for $\delta^{13}C$ and $\delta^{18}O$ on replicate NBS-19 samples was better than ±0.1‰ on a VG Optima at McMaster University.

C: Dendrogram from Q-mode cluster analysis (Wards Method, Euclidean distance) of statistically significant samples (n = 64, Fishbein and Patterson, 1993). A three-cluster interpretation would be inconsistent with the measured environmental variables.

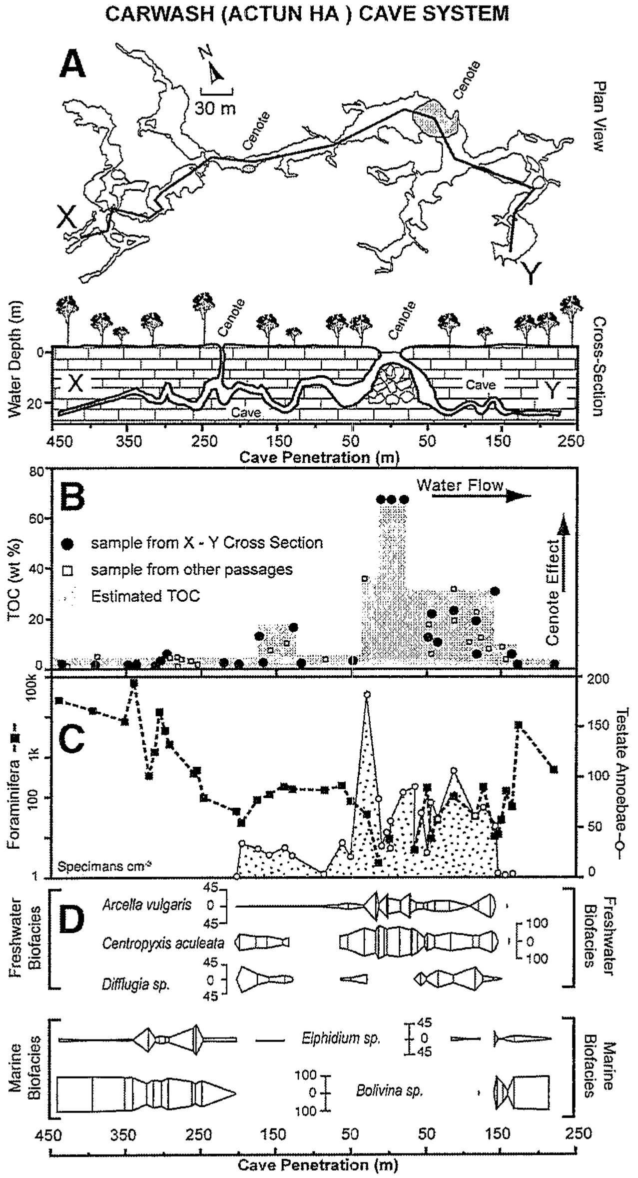


FIGURE 3.3. Biofacies along the horizontal transect X – Y. **A:** Scaled plan-view of passages and transect with cave cross-section. **B:** TOC (wt. %) obtained through standard LOI procedure (Heiri et al., 2000). **C:** Absolute abundance. **D:** Dominant taxa in the different biofacies.

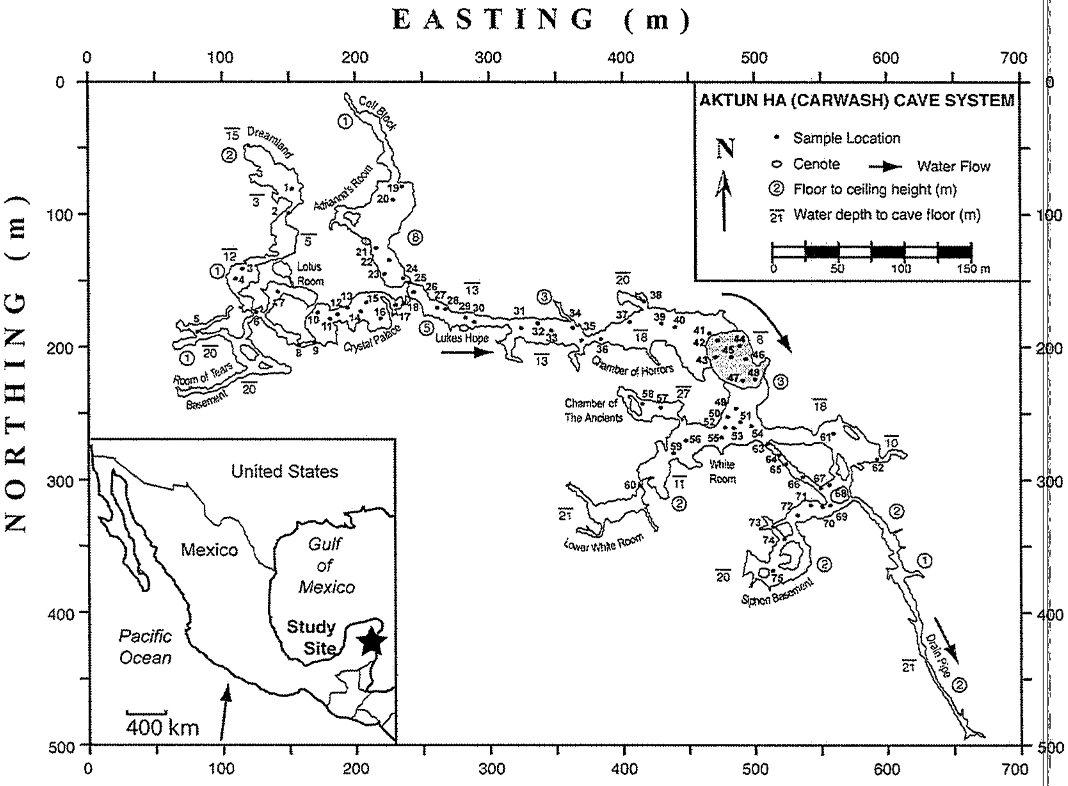


FIGURE 3.S1. Detailed location map that shows 75 surface sample locations and geomorphology in Carwash Cave System. Note that S9 and S17 were sealed beneath 2 cm of flowstone. The shallowest samples in the Marine Biofacies are S16 (depth 10.2 m) and S37 (depth 10.9 m).

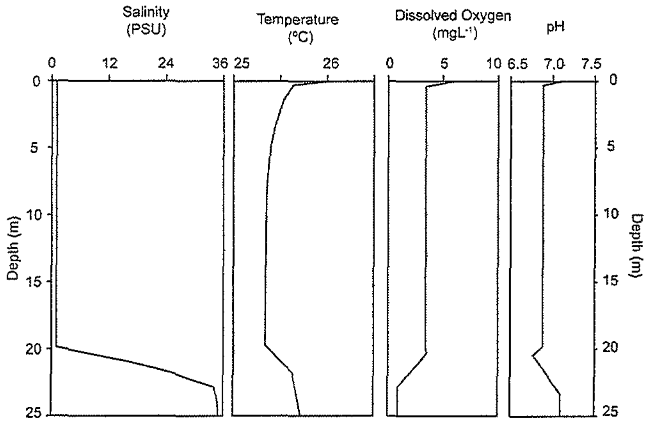
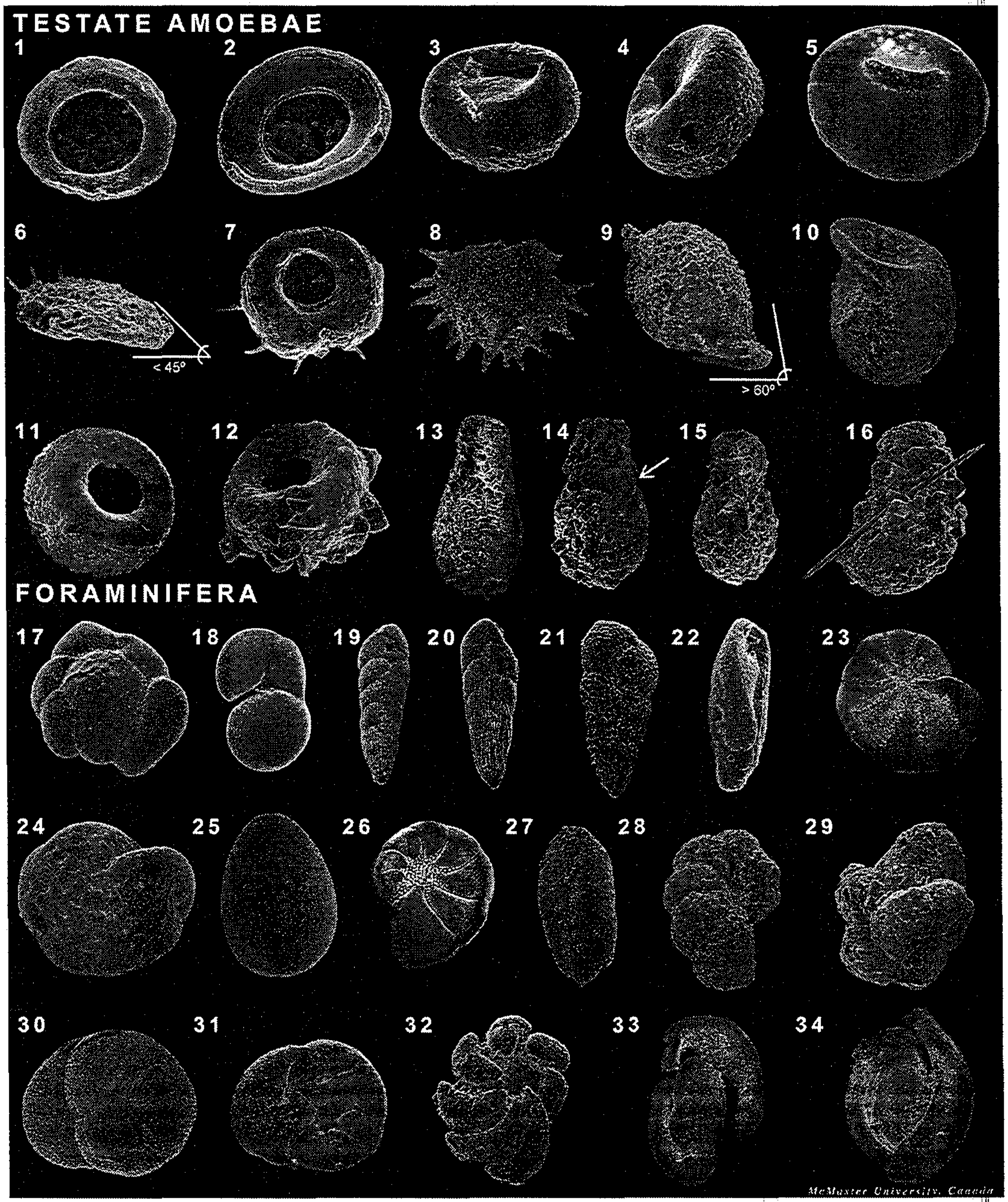


FIGURE 3.S2. A comparison of vertical profiles of pH, dissolved oxygen, salinity in the local aquifer at Carwash Cave. A total of 14 profiles collected over a 7-year period were averaged to provide greater representation of long-term conditions.

FIGURE 3.S3. ***(FOLLOWING PAGE)*** **Thecamoebians:** 1-16. 1. *Arcella vulgaris* (x341). 2. *A. vulgaris* (x341). 3. *Bulinularia indica* (x600). 4. *B. indica* (x600). 5. *B. indica* (x549). 6. *Centropyxis aculeata* (x406). Note the taxonomic criterion for angle between aperture face and body wall to be < 45°, Medioli and Scott, (1983). 7. *C. aculeata* (x388). 8. *C. aculeata* (x312). 9. *C. constricta* specimen with spines on fundus (x300). Note the taxonomic criterion for angle between aperture face and body wall to be > 60°, Medioli and Scott, (1983). 10. *C. constricta* specimen without spines (x300). 11. *Difflugia globulus* (x1150). 12. *D. globulus* with coarsely-agglutinated grains on the fundus/aboral end (x655). 13. *D. oblonga* (x263). 14. *Lagenodifflugia vas* (arrow points to restriction in neck, x300). 15. *L. vas* (x300). 16. *L. vas* (Note the agglutinated sponge spicule agglutinated to body wall, x252). **Foraminifera: 17-34.** 17. *Ammonia tepida* (x186). 18. *A. tepida* – Juvenile individual (x775). 19. *Bolivina striatula* (x150). 20. *Bolivina striatula* (x120). 21. *B. variabilis* (x300). 22. *Buliminella elegantissima* (x462). 23. *Elphidium* sp.1 (x115). 24. *Elphidium sp.2* (x252). 25. *Fissurina compacta* (x503). 26. *Haynesina germanica* (x341). 27. *Millamimina fusca* (x178). 28. *Jadammina macrescens* (x150). 29. *J. macrescens* (x186). Note irregular chamber arrangement suggesting growth during environmentally stressed conditions. 30. *Rosalina subaracuana* – spiral side (x300). *R. subaracuana* – umbilical side (x252). Foraminifera steinkerns (casts) eroding out of host limestone bedrock. 32-34. 32. Affinity to *Elphidium* (x203). 33. Affinity to *Quinqueloculina* (x212). 34. Affinity to *Quinqueloculina* (x163).


TESTATE AMOEBAE
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FORAMINIFERA
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McMaster University, Canada

SAMPLE	**S01**	**S02**	**S03**	**S04**	**S05**	**S06**	**S07**	**S08**	**S09**	**S10**
Elevation (m below SL)	-5.8	-10	-14	-3.6	-20.1	-17.3	-18.5	-14.6	-13.4	-14.2
Count	0	0	0	0	938	905	563	434	1596	181
Individuals per cm^{-3}	0	0	0	0	19210	9267	28826	4444	65372	579
TOC (% wt)	2.58	0.76	3.41	1.65	N/A	N/A	5.48	N/A	2.32	N/A
Biofacies	-	-	-	-	M	M	M	M	m	M
TESTATE AMOEBAE										
Arcella vulgaris	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Buliminella indicata	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Centropyxis aculeata	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Centropyxis constricta	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Difflugia globulus	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Difflugia oblonga	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Lagenadifflugia vas	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
FORAMINIFERA										
Ammodiscus tenuis	-	-	-	-	-	-	-	-	-	1.10
standard error (±)	-	-	-	-	-	-	-	-	-	1.52
Ammonia tepida var. juvenile	-	-	-	-	-	-	1.60	-	0.25	1.10
standard error (±)	-	-	-	-	-	-	1.04	-	0.25	1.52
Ammonia tepida	-	-	-	-	0.21	-	0.36	-	-	-
standard error (±)	-	-	-	-	0.30	-	0.49	-	-	-
Ammonia parkinsoniana	-	-	-	-	0.43	0.55	-	1.84	0.06	1.66
standard error (±)	-	-	-	-	0.42	0.48	-	1.27	0.12	1.86
Bolivina striatula	-	-	-	-	77.08	77.90	68.38	77.88	39.91	53.59
standard error (±)	-	-	-	-	2.69	2.70	3.84	3.90	2.40	7.27
Bolivina variabilis	-	-	-	-	17.16	17.24	16.34	11.52	49.25	26.52
standard error (±)	-	-	-	-	2.41	2.46	3.05	3.00	2.45	6.43
Buliminella elegantissima	-	-	-	-	0.00	-	-	0.69	-	-
standard error (±)	-	-	-	-	-	-	-	0.78	-	-
Elphidium sp.	-	-	-	-	3.73	0.66	6.75	2.53	2.26	10.50
standard error (±)	-	-	-	-	1.21	0.53	2.07	1.48	0.73	4.47
Fissurina compacta	-	-	-	-	0.53	-	2.13	0.46	0.38	-
standard error (±)	-	-	-	-	0.47	-	1.19	0.64	0.30	-
Haynesia depressula	-	-	-	-	0.21	0.22	1.60	-	0.06	-
standard error (±)	-	-	-	-	0.30	0.31	1.04	-	0.12	-
Helenina andersoni	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Millamina fusca	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Polysaccammina ipohalina	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Rosalina subaracuana	-	-	-	-	0.64	3.43	2.84	5.07	7.83	5.52
standard error (±)	-	-	-	-	0.51	1.19	1.37	2.06	1.32	3.33
Tritaxis fusca	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Jadammina macrescens	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-

SAMPLE	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**
Elevation (m below SL)	-15.5	-16.4	-16.6	-12.5	-15.2	-10.9	-18.3	-18.2	-14	-13.72
Count	253	329	332	348	352	0	373	752	0	0
Individuals per cm^{-3}	102	526	8499	2258	901	0	119.36	150	0	0
TOC (% wt)	N/A	1.11	3.18	6.15	N/A	N/A	1.24	N/A	3.41	0.48
Biofacies	M	M	M	M	M	-	M	M	-	-
TESTATE AMOEBAE										
Arcella vulgaris	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Buliminella indicata	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Centropyxis aculeata	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Centropyxis constricta	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Difflugia globulus	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Difflugia oblonga	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Lagenadifflugia vas	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
FORAMINIFERA										
Ammodiscus tenuis	-	-	0.60	3.74	-	-	-	-	-	-
standard error (±)	-	-	0.83	1.99	-	-	-	-	-	-
Ammonia tepida var. juvenile	-	-	0.60	-	-	-	-	-	-	-
standard error (±)	-	-	0.83	-	-	-	-	-	-	-
Ammonia tepida	-	-	-	-	-	-	-	0.27	-	-
standard error (±)	-	-	-	-	-	-	-	0.37	-	-
Ammonia parkinsoniana	0.40	2.43	6.33	0.29	1.42	-	-	-	-	-
standard error (±)	0.77	1.66	2.62	0.56	1.24	-	-	-	-	-
Bolivina striatula	37.15	59.57	57.23	52.87	71.31	-	47.45	36.04	-	-
standard error (±)	5.95	5.30	5.32	5.24	4.73	-	5.07	3.43	-	-
Bolivina variabilis	18.58	15.20	9.94	14.37	14.20	-	17.69	16.76	-	-
standard error (±)	4.79	3.88	3.22	3.69	3.65	-	3.87	2.67	-	-
Buliminella elegantissima	-	0.91	0.90	0.57	-	-	-	-	-	-
standard error (±)	-	1.03	1.02	0.79	-	-	-	-	-	-
Elphidium sp.	33.99	5.17	10.84	9.20	7.10	-	31.90	42.42	-	-
standard error (±)	5.84	2.39	3.34	3.04	2.68	-	4.73	3.53	-	-
Fissurina compacta	3.16	2.74	3.61	4.89	1.14	-	1.88	-	-	-
standard error (±)	2.16	1.76	2.01	2.26	1.11	-	1.38	-	-	-
Haynesia depressula	-	0.30	0.90	-	-	-	-	0.40	-	-
standard error (±)	-	0.59	1.02	-	-	-	-	0.45	-	-
Helenina andersoni	-	-	-	-	-	-	-	0.00	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Millamina fusca	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Polysaccammina ipohalina	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Rosalina subaracuana	6.72	13.68	9.04	14.08	4.83	-	1.07	4.12	-	-
standard error (±)	3.09	3.71	3.08	3.65	2.24	-	1.05	1.42	-	-
Tritaxis fusca	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Jadammina macrescens	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-

SAMPLE	**S21**	**S22**	**S23**	**S24**	**S25**	**S26**	**S27**	**S28**	**S29**	**S30**
Elevation (m below SL)	-2.14	-11.89	-13	-16.76	-13.8	-14.6	-11	-16.9	-11.58	-12.2
Count	7	113	23	384	0	385	96	147	54	192
Individuals per cm^{-3}	2	23	4	307	0	23	19	29	10	38
TOC (% wt)	4.90	5.27	4.39	3.85	2.30	N/A	N/A	2.47	1.97	N/A
Biofacies	F	F	F	F	M	M	M	M	F	F
TESTATE AMOEBAE										
Arcella vulgaris	-	-	-	1.30	-	-	-	-	-	1.04
standard error (±)	-	-	-	1.13	-	-	-	-	-	1.44
Buliminella indicata	-	-	-	0.78	-	-	-	-	-	-
standard error (±)	-	-	-	0.88	-	-	-	-	-	-
Centropyxis aculeata	-	-	-	2.08	-	-	-	-	5.56	45.83
standard error (±)	-	-	-	1.43	-	-	-	-	6.11	7.05
Centropyxis constricta	-	-	-	-	-	-	-	-	-	1.04
standard error (±)	-	-	-	-	-	-	-	-	-	1.44
Difflugia globulus	-	-	-	0.26	-	-	-	-	3.70	12.50
standard error (±)	-	-	-	0.51	-	-	-	-	5.04	4.68
Difflugia oblonga	28.57	-	-	-	-	-	-	-	-	26.04
standard error (±)	33.47	-	-	-	-	-	-	-	-	6.21
Lagenadifflugia vas	-	-	-	-	-	-	-	-	-	2.60
standard error (±)	-	-	-	-	-	-	-	-	-	2.25
FORAMINIFERA										
Ammodiscus tenuis	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Ammonia tepida var. juvenile	71.43	100.00	100.00	77.34	-	15.58	2.08	-	75.93	5.73
standard error (±)	33.47	0.00	0.00	4.19	-	0.18	2.86	-	11.40	3.29
Ammonia tepida	-	-	-	0.52	-	1.04	6.25	-	-	-
standard error (±)	-	-	-	0.72	-	0.05	4.84	-	-	-
Ammonia parkinsoniana	-	-	-	0.52	-	-	-	1.36	-	-
standard error (±)	-	-	-	0.72	-	-	-	1.87	-	-
Bolivina striatula	-	-	-	6.77	-	61.56	39.58	55.10	1.85	2.08
standard error (±)	-	-	-	2.51	-	0.25	9.78	8.04	3.60	2.02
Bolivina variabilis	-	-	-	4.69	-	9.09	10.42	8.16	1.85	-
standard error (±)	-	-	-	2.11	-	0.15	6.11	4.43	3.60	-
Buliminella elegantissima	-	-	-	-	-	0.26	-	-	-	-
standard error (±)	-	-	-	-	-	0.03	-	-	-	-
Elphidium sp.	-	-	-	3.91	-	4.42	32.29	28.57	5.56	-
standard error (±)	-	-	-	1.94	-	0.10	9.35	7.30	6.11	-
Fissurina compacta	-	-	-	1.04	-	0.78	2.08	-	-	-
standard error (±)	-	-	-	1.02	-	0.04	2.86	-	-	-
Haynesia depressula	-	-	-	0.52	-	4.16	5.21	-	5.56	-
standard error (±)	-	-	-	0.72	-	0.10	4.44	-	6.11	-
Helenina andersoni	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Millamina fusca	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Polysaccammina ipohalina	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Rosalina subaracuana	-	-	-	-	-	3.12	2.08	6.80	-	-
standard error (±)	-	-	-	-	-	0.09	2.86	4.07	-	-
Tritaxis fusca	-	-	-	-	-	-	-	-	-	1.04
standard error (±)	-	-	-	-	-	-	-	-	-	1.44
Jadammina macrescens	-	-	-	0.26	-	-	-	-	-	2.08
standard error (±)	-	-	-	0.51	-	-	-	-	-	2.02

SAMPLE	**S31**	**S32**	**S33**	**S34**	**S35**	**S36**	**S37**	**S38**	**S39**	**S40**
Elevation (m below SL)	-13.72	-13	-16.9	-19.2	-17.37	-11	-10.2	-18.2	-17.37	-17
Count	247	53	262	395	320	0	70	209	433	188
Individuals per cm^{-3}	49	10	52	79	64	0	5	42	87	38
TOC (% wt)	13.17	2.62	8.00	10.58	16.59	2.50	n/a	4.28	N/A	3.82
Biofacies	F	F	F	F	F	-	F	F	F	F
TESTATE AMOEBAE										
Arcella vulgaris	-	5.66	1.15	0.51	0.94	-	-	1.44	4.16	7.98
standard error (±)	-	6.22	1.29	0.70	1.06	-	-	1.61	1.88	3.87
Buliminella indicata	0.40	-	-	1.52	0.94	-	-	1.44	-	-
standard error (±)	0.79	-	-	1.21	1.06	-	-	1.61	-	-
Centropyxis aculeata	31.17	52.83	31.68	14.68	23.75	-	-	2.87	33.26	39.89
standard error (±)	5.78	13.44	5.63	3.49	4.66	-	-	2.26	4.44	7.00
Centropyxis constricta	7.29	1.89	3.05	5.82	2.81	-	-	-	-	0.53
standard error (±)	3.24	3.66	2.08	2.31	1.81	-	-	-	-	1.04
Difflugia globulus	11.34	13.21	6.11	7.34	5.31	-	-	1.91	1.62	2.13
standard error (±)	3.95	9.12	2.90	2.57	2.46	-	-	1.86	1.19	2.06
Difflugia oblonga	4.45	-	0.38	4.05	-	-	-	-	0.92	2.13
standard error (±)	2.57	-	0.75	1.94	-	-	-	-	0.90	2.06
Lagenadifflugia vas	4.05	5.66	1.15	4.05	0.31	-	-	-	-	-
standard error (±)	2.46	6.22	1.29	1.94	0.61	-	-	-	-	-
FORAMINIFERA										
Ammodiscus tenuis	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Ammonia tepida var. juvenile	36.84	13.21	50.00	55.95	64.69	-	51.43	89.95	59.12	39.89
standard error (±)	6.02	9.12	6.05	4.90	5.24	-	11.71	4.08	4.63	7.00
Ammonia tepida	-	-	-	-	-	-	1.43	-	-	-
standard error (±)	-	-	-	-	-	-	2.78	-	-	-
Ammonia parkinsoniana	-	-	-	0.25	-	-	-	-	-	-
standard error (±)	-	-	-	0.50	-	-	-	-	-	-
Bolivina striatula	1.21	-	1.91	0.51	-	-	4.29	-	-	5.85
standard error (±)	1.37	-	1.66	0.70	-	-	4.74	-	-	3.36
Bolivina variabilis	-	1.89	-	0.25	-	-	-	-	-	-
standard error (±)	-	3.66	-	0.50	-	-	-	-	-	-
Buliminella elegantissima	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Elphidium sp.	1.21	-	0.76	2.28	-	-	18.57	0.96	-	-
standard error (±)	1.37	-	1.05	1.47	-	-	9.11	1.32	-	-
Fissurina compacta	-	-	-	-	-	-	1.43	0.48	-	0.53
standard error (±)	-	-	-	-	-	-	2.78	0.94	-	1.04
Haynesia depressula	-	1.89	-	0.25	-	-	21.43	-	0.69	-
standard error (±)	-	3.66	-	0.50	-	-	9.61	-	0.78	-
Helenina andersoni	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Millamina fusca	0.40	-	-	-	0.31	-	-	-	-	-
standard error (±)	0.79	-	-	-	0.61	-	-	-	-	-
Polysaccammina ipohalina	-	-	-	0.25	-	-	-	-	-	-
standard error (±)	-	-	-	0.50	-	-	-	-	-	-
Rosalina subaracuana	-	3.77	0.76	0.51	-	-	1.43	-	-	0.53
standard error (±)	-	5.13	1.05	0.70	-	-	2.78	-	-	1.04
Tritaxis fusca	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Jadammina macrescens	1.62	-	3.05	1.77	0.94	-	-	0.96	0.23	0.53
standard error (±)	1.57	-	2.08	1.30	1.06	-	-	1.32	0.45	1.04

SAMPLE	**S41**	**S42**	**S43**	**S44**	**S45**	**S46**	**S47**	**S48**	**S49**	**S50**
Elevation (m below SL)	-13	-5.5	-4.9	-4.6	-4.3	-3	-5.9	-4	-6.7	-17.1
Count	235	294	157	223	90	286	106	113	213	215
Individuals per cm^{-3}	188	78	31	45	29	57	84	90	28	69
TOC (% wt)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Biofacies	F	F	F	F	F	F	F	F	F	F
TESTATE AMOEBAE										
Arcella vulgaris	2.55	38.78	5.73	21.08	31.11	20.98	14.15	32.74	17.84	8.84
standard error (±)	2.02	5.57	3.64	5.35	9.56	4.72	6.64	8.65	0.78	3.79
Buliminella indicata	0.43	-	-	-	-	-	-	-	-	-
standard error (±)	0.83	-	-	-	-	-	-	-	-	-
Centropyxis aculeata	77.45	60.54	91.72	78.48	68.89	74.83	82.08	65.49	76.53	57.67
standard error (±)	5.34	5.59	4.31	5.39	9.56	5.03	7.30	8.77	0.87	6.60
Centropyxis constricta	2.13	0.34	2.55	0.45	-	-	2.83	0.88	0.47	4.65
standard error (±)	1.85	0.67	2.46	0.88	-	-	3.16	1.73	0.14	2.81
Difflugia globulus	8.94	-	-	-	-	-	-	-	-	5.58
standard error (±)	3.65	-	-	-	-	-	-	-	-	3.07
Difflugia oblonga	4.68	-	-	-	-	0.70	0.94	-	0.47	14.88
standard error (±)	2.70	-	-	-	-	0.97	1.84	-	0.14	4.76
Lagenadifflugia vas	-	-	-	-	-	1.05	-	0.88	2.35	1.86
standard error (±)	-	-	-	-	-	1.18	-	1.73	0.31	1.81
FORAMINIFERA										
Ammodiscus tenuis	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Ammonia tepida var. juvenile	1.28	-	-	-	-	-	-	-	1.88	5.58
standard error (±)	1.44	-	-	-	-	-	-	-	0.28	3.07
Ammonia tepida	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Ammonia parkinsoniana	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Bolivina striatula	-	-	-	-	-	-	-	-	0.47	-
standard error (±)	-	-	-	-	-	-	-	-	0.14	-
Bolivina variabilis	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Buliminella elegantissima	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Elphidium sp.	-	-	-	-	-	-	-	-	-	0.47
standard error (±)	-	-	-	-	-	-	-	-	-	0.91
Fissurina compacta	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Haynesia depressula	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Helenina andersoni	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Millamina fusca	0.43	-	-	-	-	-	-	-	-	-
standard error (±)	0.83	-	-	-	-	-	-	-	-	-
Polysaccammina ipohalina	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Rosalina subaracuana	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Tritaxis fusca	0.85	-	-	-	-	-	-	-	-	-
standard error (±)	1.17	-	-	-	-	-	-	-	-	-
Jadammina macrescens	1.28	0.34	-	-	-	2.45	-	-	-	0.47
standard error (±)	1.44	0.67	-	-	-	1.79	-	-	-	0.91

SAMPLE	S51	S52	S53	S54	S55	S56	S57	S58	S59	S60
Elevation (m below SL)	-17.3	-17.4	-17.3	-16.3	-17.3	-22	-27.43	-27.43	-20.5	-15.9
Count	333	240	361	378	552	168	0	0	64	92
Individuals per cm^{-3}	69	38	72	76	110	33	0	0	13	18
TOC (% wt)	13.09	N/A	18.13	22.12	6.33	31.81	N/A	N/A	10.94	9.10
Biofacies	F	F	F	F	F	F	-	-	F	F
TESTATE AMOEBAE										
Arcella vulgaris	7.81	14.58	8.31	9.79	-	26.79	-	-	-	4.35
standard error (±)	2.88	4.47	2.85	3.00	-	6.70	-	-	-	4.17
Buliminella indicata	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Centropyxis aculeata	22.52	65.42	27.70	50.00	1.45	64.29	-	-	-	6.52
standard error (±)	4.49	6.02	4.62	5.04	1.00	7.25	-	-	-	5.05
Centropyxis constricta	0.60	3.75	0.83	2.91	-	-	-	-	-	2.17
standard error (±)	0.83	2.40	0.94	1.69	-	-	-	-	-	2.98
Difflugia globulus	3.30	5.00	4.16	3.44	-	0.60	-	-	-	2.17
standard error (±)	1.92	2.76	2.06	1.84	-	1.16	-	-	-	2.98
Difflugia oblonga	1.20	9.58	-	10.32	-	-	-	-	-	-
standard error (±)	1.17	3.72	-	3.07	-	-	-	-	-	-
Lagenadifflugia vas	-	0.42	3.05	21.69	-	-	-	-	-	-
standard error (±)	-	0.81	1.77	4.16	-	-	-	-	-	-
FORAMINIFERA										
Ammodiscus tenuis	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Ammonia tepida var. juvenile	62.76	0.83	55.40	-	98.55	3.57	-	-	100.00	79.35
standard error (±)	5.19	1.15	5.13	-	1.00	2.81	-	-	0.00	8.27
Ammonia tepida	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Ammonia parkinsoniana	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Bolivina striatula	-	-	-	-	-	1.79	-	-	-	1.09
standard error (±)	-	-	-	-	-	2.00	-	-	-	2.12
Bolivina variabilis	-	-	-	-	-	-	-	-	-	1.09
standard error (±)	-	-	-	-	-	-	-	-	-	2.12
Buliminella elegantissima	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Elphidium sp.	1.50	-	-	-	-	-	-	-	-	2.17
standard error (±)	1.31	-	-	-	-	-	-	-	-	2.98
Fissurina compacta	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Haynesia depressula	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Helenina andersoni	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Millamina fusca	-	-	-	0.53	-	0.60	-	-	-	-
standard error (±)	-	-	-	0.73	-	1.16	-	-	-	-
Polysaccammina ipohalina	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Rosalina subaracuana	-	-	-	-	-	-	-	-	-	1.09
standard error (±)	-	-	-	-	-	-	-	-	-	2.12
Tritaxis fusca	-	0.42	-	-	-	-	-	-	-	-
standard error (±)	-	0.81	-	-	-	-	-	-	-	-
Jadammina macrescens	0.30	-	0.55	1.32	-	2.38	-	-	-	-
standard error (±)	0.59	-	0.77	1.15	-	2.31	-	-	-	-

SAMPLE	**S61**	**S62**	**S63**	**S64**	**S65**	**S66**	**S67**	**S68**	**S69**	**S70**
Elevation (m below SL)	-15.58	-7.01	-20.4	-20.4	-16.76	-20.4	-20.4	-17.2	-15.24	-20.1
Count	312	38	313	397	332	237	299	407	260	60
Individuals per cm^{-3}	62	8	63	132	66	47	118	81	52	6
TOC (% wt)	12.84	4.25	10.92	22.95	19.40	6.25	N/A	8.18	31.01	N/A
Biofacies	F	F	F	F	F	F	F	M	F	F
TESTATE AMOEBAE										
Arcella vulgaris	17.63	26.32	13.74	14.36	0.30	6.75	13.38	1.97	31.92	21.67
standard error (±)	4.23	14.00	3.81	3.45	0.59	3.19	3.86	1.35	5.67	10.42
Buliminella indicata	-	-	-	0.25	0.30	-	-	-	-	-
standard error (±)	-	-	-	0.49	0.59	-	-	-	-	-
Centropyxis aculeata	35.26	44.74	31.95	49.37	32.83	14.35	33.44	7.37	58.85	43.33
standard error (±)	5.30	15.81	5.17	4.92	5.05	4.46	5.35	2.54	5.98	12.54
Centropyxis constricta	3.21	-	6.07	2.52	7.83	4.22	2.01	0.25	2.31	-
standard error (±)	1.95	-	2.65	1.54	2.89	2.56	1.59	0.48	1.83	-
Difflugia globulus	0.64	-	17.57	8.06	10.84	0.84	4.68	0.49	1.92	-
standard error (±)	0.89	-	4.22	2.68	3.34	1.16	2.39	0.68	1.67	-
Difflugia oblonga	-	-	7.99	2.77	24.40	1.27	5.02	-	1.92	3.33
standard error (±)	-	-	3.00	1.61	4.62	1.42	2.47	-	1.67	4.54
Lagenadifflugia vas	0.96	-	13.42	3.02	13.86	-	-	-	-	-
standard error (±)	1.08	-	3.78	1.68	3.72	-	-	-	-	-
FORAMINIFERA										
Ammodiscus tenuis	0.32	-	-	-	-	-	0.33	-	-	3.33
standard error (±)	0.63	-	-	-	-	-	0.65	-	-	4.54
Ammonia tepida var. juvenile	29.17	28.95	6.07	4.79	3.01	61.60	15.38	33.91	2.69	-
standard error (±)	5.04	14.42	2.65	2.10	1.84	6.19	4.09	4.60	1.97	-
Ammonia tepida	0.64	-	-	-	-	-	8.36	1.23	-	-
standard error (±)	0.89	-	-	-	-	-	3.14	1.07	-	-
Ammonia parkinsoniana	-	-	-	1.01	-	0.42	-	0.98	-	-
standard error (±)	-	-	-	0.98	-	0.83	-	0.96	-	-
Bolivina striatula	0.32	-	-	2.52	-	4.22	9.03	36.36	-	6.67
standard error (±)	0.63	-	-	1.54	-	2.56	3.25	4.67	-	6.31
Bolivina variabilis	-	-	-	-	-	-	2.68	9.09	-	-
standard error (±)	-	-	-	-	-	-	1.83	2.79	-	-
Buliminella elegantissima	-	-	-	-	-	-	-	-	-	-
standard error (±)	-	-	-	-	-	-	-	-	-	-
Elphidium sp.	2.88	-	0.64	4.53	-	5.06	2.34	2.70	-	18.33
standard error (±)	1.86	-	0.88	2.05	-	2.79	1.71	1.58	-	9.79
Fissurina compacta	-	-	-	0.50	-	0.42	-	0.74	-	-
standard error (±)	-	-	-	0.70	-	0.83	-	0.83	-	-
Haynesia depressula	-	-	0.64	2.27	-	-	-	0.98	-	3.33
standard error (±)	-	-	0.88	1.46	-	-	-	0.96	-	4.54
Helenina andersoni	-	-	-	-	-	-	1.67	-	-	-
standard error (±)	-	-	-	-	-	-	1.45	-	-	-
Millamina fusca	2.56	-	-	0.50	5.42	-	1.34	0.49	0.38	-
standard error (±)	1.75	-	-	0.70	2.44	-	1.30	0.68	0.75	-
Polysaccammina ipohalina	-	-	-	0.50	0.60	-	-	0.25	-	-
standard error (±)	-	-	-	0.70	0.83	-	-	0.48	-	-
Rosalina subaracuana	1.92	-	-	1.01	-	-	-	2.70	-	-
standard error (±)	1.52	-	-	0.98	-	-	-	1.58	-	-
Tritaxis fusca	-	-	-	-	-	-	0.33	-	-	-
standard error (±)	-	-	-	-	-	-	0.65	-	-	-
Jadammina macrescens	4.49	-	1.92	2.02	0.60	0.84	-	0.49	-	-
standard error (±)	2.30	-	1.52	1.38	0.83	1.16	-	0.68	-	-

SAMPLE	**S71**	**S72**	**S73**	**S74**	**S75**
Elevation (m below SL)	-20.7	-20.7	-20.5	-20.1	-19.8
Count	68	206	80	2603	1543
Individuals per cm^{-3}	5	41	16	3332	154
TOC (% wt)	N/A	N/A	5.86	N/A	N/A
Biofacies	M	M	F	M	M
TESTATE AMOEBAE					
Arcella vulgaris	-	0.49	5.00	-	0.19
standard error (±)	-	0.95	4.78	-	0.22
Buliminella indicata	-	-	-	-	-
standard error (±)	-	-	-	-	-
Centropyxis aculeata	-	5.83	16.25	-	-
standard error (±)	-	3.20	8.08	-	-
Centropyxis constricta	-	-	1.25	-	-
standard error (±)	-	-	2.43	-	-
Difflugia globulus	-	-	1.25	-	-
standard error (±)	-	-	2.43	-	-
Difflugia oblonga	-	-	-	-	-
standard error (±)	-	-	-	-	-
Lagenadifflugia vas	-	-	-	-	-
standard error (±)	-	-	-	-	-
FORAMINIFERA					
Ammodiscus tenuis	-	-	-	-	-
standard error (±)	-	-	-	-	-
Ammonia tepida var. juvenile	51.47	-	62.50	0.08	-
standard error (±)	11.88	-	10.61	0.11	-
Ammonia tepida	-	0.49	-	-	0.45
standard error (±)	-	0.95	-	-	0.34
Ammonia parkinsoniana	-	-	-	0.35	1.43
standard error (±)	-	-	-	0.23	0.59
Bolivina striatula	36.76	72.82	8.75	67.69	66.04
standard error (±)	11.46	6.08	6.19	1.80	2.36
Bolivina variabilis	10.29	16.50	2.50	17.02	25.34
standard error (±)	7.22	5.07	3.42	1.44	2.17
Buliminella elegantissima	-	-	-	0.61	0.39
standard error (±)	-	-	-	0.30	0.31
Elphidium sp.	1.47	0.97	-	9.41	1.43
standard error (±)	2.86	1.34	-	1.12	0.59
Fissurina compacta	-	0.49	-	0.92	0.45
standard error (±)	-	0.95	-	0.37	0.34
Haynesia depressula	-	0.97	-	0.92	0.71
standard error (±)	-	1.34	-	0.37	0.42
Helenina andersoni	-	-	-	2.46	3.50
standard error (±)	-	-	-	0.59	0.92
Millamina fusca	-	-	-	-	-
standard error (±)	-	-	-	-	-
Polysaccammina ipohalina	-	-	-	-	-
standard error (±)	-	-	-	-	-
Rosalina subaracuana	-	0.97	1.25	0.54	0.06
standard error (±)	-	1.34	2.43	0.28	0.13
Tritaxis fusca	-	-	-	-	-
standard error (±)	-	-	-	-	-
Jadammina macrescens	-	0.49	1.25	-	-
standard error (±)	-	0.95	2.43	-	-

Sample	**Taxa**	**$\delta^{13}C_{carb}$ (‰ VPDB)**	**$\delta^{18}O_{carb}$ (‰ VPDB)**
S09	*B. striatula*	-5.1	-2.1
S07	*B. striatula*	-5.5	-1.7
S06	*B. striatula*	-5.9	-2.1
S75	*B. striatula*	-6.3	-2.3
S05	*B. striatula*	-5.3	-1.9
S09	*B. striatula*	-4.7	-1.9
S74	*B. striatula*	-6.3	-2.0
S17	*B. striatula*	-6.3	-2.9
S74	*B. variabilis*	-6.7	-2.2
S09	*B. variabilis*	-5.2	-2.4
S75	*B. variabilis*	-5.8	-2.3
S06	*B. variabilis*	-6.3	-2.4
S09	*B. variabilis*	-5.3	-2.8
S11	*Elphidium sp.*	-5.7	-3.7
S07	*Elphidium sp.*	-4.3	-3.1
S17	*Elphidium sp.*	-5.8	-3.8
S17	*Elphidium sp.*	-4.9	-3.2
S75	*Elphidium sp.*	-4.2	-3.2
S74	*Elphidium sp.*	-6.4	-3.8
S27	*Elphidium sp.*	-5.7	-3.6
S17	*Elphidium sp.*	-5.8	-4.0
S09	*R. subaracuana*	-4.6	-3.0
S06	*R. subaracuana*	-5.5	-2.5
S74	*R. subaracuana*	-5.5	-2.9
S09	*R. subaracuana*	-4.9	-2.5
S05	*R. subaracuana*	-5.3	-2.3
S07	*R. subaracuana*	-5.5	-2.2
S41	*C .ilosvayi*	-10.9	-5.3
S39	*C .ilosvayi*	-11.3	-5.2
S45	*C .ilosvayi*	-11.9	-5.1
S52	*C .ilosvayi*	-10.9	-5.0
S34	*C .ilosvayi*	-10.4	-4.9
S49	*C .ilosvayi*	-10.8	-5.1
S50	*C .ilosvayi*	-10.3	-4.7
S74	*C .ilosvayi*	-10.3	-4.3
S07	*C .ilosvayi*	-10.1	-4.3
S30	*C .ilosvayi*	-11.0	-5.2
S45	*C .ilosvayi*	-11.4	-5.0
S67	*C .ilosvayi*	-11.0	-5.3
S37	*C .ilosvayi*	-12.0	-5.2
S29	*C .ilosvayi*	-11.6	-5.3
S51	Host Limestone	-2.1	-6.0

CHAPTER 4

MID-LATE HOLOCENE ENVIRONMENTAL RECONSTRUCTION OF A MEXICAN PHREATIC ANCHIALINE CAVE SYSTEM USING TESTATE AMOEBAE (THECAMOEBIANS), FORAMINIFERA, AND STABLE ORGANIC ISOTOPES (δ^{13}C and δ^{15}N)

P.J. van Hengstum, E.G. Reinhardt,
P.A. Beddows, and J.J. Gabriel

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4.1. ABSTRACT

A mid-late Holocene paleoenvironmental reconstruction was completed using cores spanning the last 6.5 ka from the phreatic and anchialine Carwash (Aktun Ha) Cave System, Mexico. Three paleoenvironmental phases occur in the cave, isolated through changes in testate amoebae, foraminifera, stable organic isotopes (δ^{13}C and δ^{15}N), total nitrogen, total organic carbon, and particle size analysis. Chronological control was obtained through ten AMS radiocarbon ages. Phase 1 (6.5 – 4.3 ka) is characterized by elevated salinity (>3.5 ppt), as determined through larger foraminiferal populations, fine-grained particle sizes (<16 μm), lower C/N ratio (min of 14). A mangrove marsh system at the top of the sinkhole-breakdown pile was drowned by sea level rise at 6.5 ka, with deeper open water conditions developed in the sinkhole by 4.3 ka. Elevated salinity in the freshwater aquifer is attributed to increased turbulent flow from increased precipitation and aquifer discharge at the coast. Phase 2 (4.3 – 2.67 ka) is characterized by the colonization of euryhaline testate amoebae (salinity ~2 - 3 ppt) and foraminifera, higher C/N ratios with decreased TN, increased sedimentation rates with poorly-sorted sediment. Continual coastal progradation is interpreted as increasing hydrogeologic impedance for aquifer discharge at the coast, causing a concomitant decrease in the salinity of the aquifer. Increased El Nino-related storm activity is interpreted as contributing to enriched δ^{15}N

and minor centennial scale salinity shifts (<1 ppt) in the freshwater lens. Phase 3 (2.67 ka – present) represents modern environmental conditions in the cave and sinkhole. The colonization of the cave by testate amoebae *Lagenodifflugia vas* and *Difflugia oblonga* indicate modern salinity conditions (1.5 ppt) and decreased C/N ratio and increased TN indicated developed primary productivity in the cenote. These results indicate that the testate amoebae and organic material in phreatic caves are an archive of paleoenvironmental evolution in the cave system. More broadly, Carwash Cave is responding to regional climatic changes, suggesting that phreatic cave systems can be more widely used for paleoclimate research.

4.2. INTRODUCTION

Anchialine phreatic cave systems are meromictic coastal aquatic environments where basal saline water is stratified from superior freshwater (Fig. 4.1). These systems have a global extent, including the Caribbean region, along Mediterranean coastlines, into the Middle East, and on many islands in the eastern and south Pacific Ocean. Over the last 30 years, the phreatic cave environment has been the focus of many interesting studies in the disciplines of cave biology, karst geology and hydrogeology, archaeology, and Quaternary sea level change. In contrast to the abundance of literature regarding other coastal systems, there are no published examples of paleoenvironmental reconstructions in currently flooded caves.

4.2.1. OBJECTIVES

Here we employ a multi-proxy approach to reconstruct the mid-late Holocene paleoenvironmental history in Carwash (Aktun Ha) Cave, an anchialine system near Tulum, Quintana Roo, Mexico. Several questions are central to this investigation. (1) Can testate amoebae and/or foraminifera be used as a proxy for paleohydrology? (2) Do bulk organic isotopes ($\delta^{13}C$ and $\delta^{15}N$) record temporal changes in cenote productivity and/or changes in the terrestrial vegetation? (3) Do broader teleconnections exist between the cave environment, hydrogeology, and mid-late Holocene climate? (4) Are there any linkages between aquifer dynamics and local human populations?

4.2.2. TESTATE AMOEBAE AND FORAMINIFERA

Testate amoebae (benthic, fresh water to brackish protists) and benthic foraminifera (brackish to saline protists) are shelled amoebae that are readily preserved in sediments. Foraminifera have a developed reputation in coastal science as environmental proxies of dissolved oxygen, pH, depth, temperature, sea level, etc. (Murray, 2007). Recently, foraminifera have been found in surface samples from a Mexican phreatic cave. The recovered fauna were responding to environmental conditions in the flooded cave similarly to other coastal settings (van Hengstum et al., 2008a), indicating equal potential for their application for cave paleoenvironmental research.

Testate amoebae are a lesser-known group of protist microfossils that have predominantly received attention in wetland research (Ogden and Hedley, 1981). In wetlands, testate amoebae are reliable proxies of percent moisture and pH changes, which can be related to water table elevation (Mitchell et al., 2007; Charman et al., 2000). In the last 20 years more research attention has been focused on testate amoebae (thecamoebians) in lacustrine and coastal settings (Escobar et al., 2008; Holcová, 2007; Patterson et al., 2002; Scott et al., 2001). Taxa have been used to indicate: (1) a lake's trophic state and nutrient loading - *Cucurbitella tricuspis* and *Centropyxis aculeata*; (2) hydrologic flow and flooding - *Pontigulasia compressa*; (3) heavy metal contamination – ecophenotypes of *Difflugia* sp.; (4) and salinity changes (Holcová; 2007; Patterson and Kumar, 2002; Reinhardt et al., 1998).

Of the various abiotic factors, salinity is a major factor controlling the presence/absence of foraminifera and testate amoebae in aquatic environments. The most euryhaline foraminiferal taxa typically require salinity >1 ppt, and include many salt marsh taxa including *Jadammina macrescens*, *Ammonia tepida*, and *Millammina fusca* (Murray, 2007). In contrast, only recently have testate amoebae been demonstrated to persist beyond 1 ppt in coastal settings (Riverios et al., 2007, Gehrels et al., 2001). When studying surface samples from different sinkholes (cenotes) in Mexico, van Hengstum et al. (2008b) found ~3.5 ppt as the maximum salinity habitable to the most euryhaline testate amoebae (centropyxid taxa). When collectively using foraminifera and testate amoebae, salinity can be reconstructed in very low salinity ranges using the ecological tolerances of species: testate amoebae *Difflugia oblonga* and *Lagenodifflugia vas* from ~0-1 ppt; (2) *Centropyxis aculeata* and *Jadammina macrescens* from ~1-3.5 ppt; (3) *Centropyxis constricta* and *Ammonia tepida* (with other marsh foraminifera taxa) from ~2.5 - 4.5 ppt; (4) other low-salinity tolerant foraminifera >4.0 ppt (van Hengstum et al., 2008b). Although neither foraminifera nor testate amoebae have been used in a phreatic cave paleoenvironmental reconstruction, these faunal transitions allow for the inference of quantitative values for paleosalinity.

4.2.3. PLANT CARBON ISOTOPES, NITROGEN ISOTOPES, AND C/N RATIOS

Isotopic analysis of bulk organic carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) has been used as a paleoenvironmental proxy in aquatic research (i.e., Diefendorf et al., 2008; Lamb et al., 2006). Different plants and plant tissues have variable $\delta^{13}C$ and $\delta^{15}N$ values dependant upon a variety of physiological (i.e., photosynthetic pathway employed), and environmental (precipitation, salinity, atmospheric isotopic ratios, nutrient sources, etc.) variables (Lamb et al., 2006; Leng et al., 2006; France, 1995). To date, no studies have investigated the paleoenvironmental potential of $\delta^{13}C$ and $\delta^{15}N$ as a proxy in a phreatic cave. However, organic isotopes in vadose cave sediments have proven to be a useful proxy for changes to terrestrial vegetation in the cave vicinity (Polk et al., 2007; Panno et al., 2004).

In a phreatic cave, equal consideration needs to be given to aquatic and terrestrial sources of organic matter (OM). The majority of OM is dominantly allochthonous, as the lack of light prevents primary productivity in all areas but passages near the cave entrance. Surface runoff will transport terrestrial detritus and OM into the sinkhole, which is host to diverse aquatic communities (Sánchez et al. 2002; Schmitter-Soto et al. 2002). Continual hydrologic flow in the freshwater lens of the aquifer will systematically transport terrestrial and aquatic OM from the cenote and into the phreatic cave, where it becomes part of the sediment record. Due to the homogenized nature of the OM in a phreatic cave, determining the OM source will be important when interpreting final isotopic signatures.

The $\delta^{13}C$ value of organic matter derived from terrestrial plants is dependent upon different factors than aquatic plants. In terrestrial plants, carbon is fractionated differently depending upon the photosynthetic pathway employed. Most trees, shrubs, and herbaceous plants discriminate strongly against atmospheric ^{13}C through the C_3 photosynthetic pathway, resulting in a mean $\delta^{13}C = -26.7$ (n=370; Cerling et al., 1999). In contrast, most grasses (warm-season) and a select few dicots employ the C_4 photosynthetic pathway, resulting in $\delta^{13}C = -12.5$ (n=455; Ehleringer et al., 1997). The isotopic signal of tissues from plants utilizing the Crassulacean Acid Metabolic pathway is midway between C_3 and C_4 plants, but these species are considerably less abundant. In contrast, freshwater aquatic plants exhibit considerable isotopic variability with values of $\delta^{13}C$ ranging from -11‰ to -50‰. This variability arises from several factors, including: (1) the carbon species used by the aquatic plants (HCO_3- vs. CO_2); (2) variability in $\delta^{13}C$ of the source carbon; (3) the inorganic carbon species used by the plant photosynthetic pathway; and (4) the natural resistance of atmospheric diffusion of CO_2 into an aquatic environment (Keeley and Sandquist, 1992; Osmond, et al., 1981). As a result, understanding carbon isotope profiles in a cave may be challenging due to the high potential for variability and the homogenization of OM.

The isotopic ratio of $^{15}N/^{14}N$ in cave sediment will also reflect a combined signal from terrestrial and aquatic sources. The majority of terrestrial plants (except legumes with bacterial symbiosis), aquatic macrophytes, and phytoplankton do not fix inorganic nitrogen directly. Instead, they are reliant on residual pools of aqueous NH_4+ (ammonium) or NO_3- (nitrate) in soil or the water column for incorporation into cellular tissues (Stevens and Hedges, 2004; Talbot, 2001). Through the systematic removal of ^{14}N in terrestrial systems, land plants and soil organic matter (SOM) become progressively enriched (land plants $\delta^{15}N$ = +2 to +10; SOM = +5%; Talbot. 2001). Aquatic macrophytes discriminate in favor of the lighter isotope ($\delta^{15}N$ range of -10 to >0), causing successive enrichment of the residual nitrogen pools and the phytoplankton that use these pools (Talbot, 2001). Similarly to carbon, the nitrogen isotopic profiles will be homogenized, but aquatic versus terrestrial OM may be easier to discern.

Finally, the C/N ratio of organic matter can be a useful secondary way of discriminating the source of organic matter when used in conjunction with $\delta^{13}C$ and $\delta^{15}N$. Aquatic plants (marine and lacustrine) and phytoplankton have high nitrogen levels from protein-rich tissues when compared to terrestrial plants, which are lignin and cellulose-rich (Mackie et al., 2005). As a result, the C/N ratio of aquatic OM is lower (4-10) when compared to terrestrial OM (>20) and biplots between $\delta^{13}C$, $\delta^{15}N$, and the C/N ratio are frequently used to characterize the source organic matter (i.e., Fig. 2 in Lamb et al., 2006; Fig. 3 in Mackie et al., 2005; Fig. 8 in Cloern et al., 2002.).

4.2.4. THE ANCIENT MAYA AND FRESH WATER

In chronological summary, cultural evolution of the ancient Maya is generally divided into 3 periods: the Pre-Classic (4000 - 2000 yrs BP), Classic (1900 - 1000 yrs BP), and the Post-Classic (1000 – 400 yrs BP). Significant research attention has been focused on climatic/environmental and cultural linkages that have contributed to different phases of Maya collapse (Hodell et al., 2005; Haug, et al., 2003; Santley et al., 1986). Growing scientific evidence correlates intervals of drought and aridity to agricultural breakdown and population collapse during the Classic Periods (Hodell et al., 2001). However, there has been less research focused on the environmental framework for the Pre-Classic rise of the Maya, especially in the Northern Lowlands.

The spatial extent of the Mayan civilization is divided into three regions, each with different climatic and geologic conditions that provided the environmental context for different social, cultural, and political growth. These regions are: The Pacific Plain and highlands (southern Guatemala, Chiapas state of Mexico, El Salvador), Southern and Central Lowlands (northern Guatemala, western Honduras, Belize) and the Northern Lowlands (the Yucatan Peninsula of Mexico; see Sharer, 1994). The Northern Lowlands receive the least mean annual rainfall and experience the highest mean annual temperatures of all the areas. Since there are no freshwater rivers and only a few lakes in the Northern Lowlands, freshwater resources would have been central to any ancient people living in this area, a concern that prevails even today.

Many large Maya population centers emerged in areas without rivers or lakes, largely due to the ability of the people to manipulate natural freshwater resources (Hansen et al., 2002; Adams, 1991). Approximately 70% of the ancient Maya were situated above karst terrain (Veni, 1990), an important factor considering precipitation readily percolates into the local freshwater lens through the porous limestone. To help contain volumes of freshwater, the ancient Maya developed seasonal swamps (*bajos*) and man-made reservoirs (*aguadas*) for precipiation storage. The physical control of fresh water resources is argued as a major source of political power, especially in the Classic period (Johnston, 2004; Hansen et al., 2002, Lucero, 1999).

Sinkholes are ubiquitous in the Yucatan region, known as cenotes from the Yucatec Mayan word *dz'onot*, and routinely intersect the water table and freshwater lens. In the Northern Lowlands, cenotes would have been an important source of fresh water for Mayan settlements. Cenotes are common near large population centers, such as the Sacred Cenote of Sacrifice and Cenote Xtoloc at Chichen Itza (see Fig. 1 in Dahlin, 2002). However, regional aquifer conditions will have a strong control on the water quality in the cenotes, which may or may not have changed through the mid-late Holocene. A better understanding is needed of mid-late Holocene aquifer evolution to better understand the potential of cenotes as a freshwater resource to the ancient Maya through time.

4.3. STUDY SITE AND PHYSIOGRAPHY

The tectonically-stable karst landscape of the Yucatan Penninsula, Mexico, contains ubiquitous anastomosing caves and sinkholes (cenotes) in Miocene to late Pleistocene reefal limestone (Weidie, 1985; Szabo et al., 1978; Fig. 4.1). The local aquifer is divided into a superior freshwater lens separated from intruding marine water below by a halocline. The halocline (or mixing zone) is an area of chemical dissolution dominantly responsible for phreatic anchialine cave formation (Smart et al., 2006, 1998; Ward et al., 1985). Neogene eustatic sea level oscillations have shifted the relative elevation of the halocline resulting in polygenetic caves that transverse through both fresh and saltwater sections of the aquifer.

Carwash (Aktun Ha) Cave System is located 8.5 km west of the Caribbean coast with passages dominantly located in the fresh water mass (1.5 PSU, 25.5°C, 3.5 mgL^{-1} dissolved oxygen) above the halocline at 21 m (Alcocer et al., 1998). A main sinkhole provides physical access into the cave, which has a general elliptical morphology with a mean length of 46 m, width of 15 m, and depth of 4.5 m. In the cenote and shallow cave, dissolved oxygen is above the ecological requirements of most aerobic foraminfera (>1 mg/L), but since salinity is 1.5‰, only the most euryhaline foraminifera and testate amoebae can colonize the substrate.

Sediment in the cave is not homogeneous with distance from the central cenote. The organic matter and nutrients from aquatic cenote and surrounding terrestrial environment are systematically transported into the cave by hydrologic flow (1 cms^{-1}, Moore et al., 1992). Cave sediment is organic-rich gyttja near the cenotes, which reaches significant accumulations in some locations (>3 m), but is increasingly patchy and sparse in the deep cave. This pattern of sediment accumulation has been previously documented in a Mediterranean flooded cave (Fichez, 1990).

Recently, Gabriel et al. (2008) documented Holocene sea level and the flooding history of the main cenote in Carwash Cave System. Using a core from the center of the cenote (C1, Fig. 4.1), a significant accumulation of mangrove peat (dominated by *Rhizophora* pollen) transitioned to a condensed horizon containing characteristic fresh water microfossils (dominated by ostracods and testate amoebae). Using plant macrofossils, the environmental transition from a mangrove swamp to a flooded cenote was dated to ~6580 Cal yrs BP. Since the mangrove-peat radiocarbon ages and a previous radiocarbon age on charcoal (Coke et al., 1991) fit well on the sea level curve of Toscano and McIntyre (2003), the minimum age for the flooding of the cenote and breakdown pile was estimated at ~6580 Cal yrs BP. Although not the focus of this research, the radiocarbon ages and core lithology are repeated here to facilitate interpretation of the cave sedimentary record.

4.4. METHODS

4.4.1. SEDIMENT CORING

Four push cores were obtained for the present study using 3" diameter polyvinyl carbonate tubing in August 2006 and July 2007. Locations downstream from the cenote were selected as there was a lack of sediment accumulation in the upstream cave passages. The core locations are: (1) C1 — point of 0 m in the center of the cenote at a depth of 4.5 m; (2) C2 — 39 m downstream from the cenote in the cave at 16.8 m depth; (3) C3 — 46 m downstream from the cenote at 20.2 m depth, (4) C4 — 60 m downstream from the cenote at 20.2 m depth. To first find a suitable coring location, sediment probing was completed with a small 1 cm polycarbonate rod. Since the limestone cave floor is not a uniform surface, not all areas contain equal potential for sediment accumulation (Fig. 4.2A). Sediment cores were then obtained using a simple percussion device. An underwater lift bag, inflated with a diver's breathing gas, was used to provide upward force to extract the core (Fig. 4.2B). Immediately upon extraction, the cores were capped, sealed, and transported out of the cave system using Diver Propulsion Vehicles. On land, the core was manually extruded, logged, sampled at 1 cm intervals in separate re-sealable bags, and transported back to the laboratory for further analysis.

4.4.2. LABORATORY ANALYSIS

To establish a chronology for the sediment cores, six additional bulk organics samples (1 cm interval) from C2 and C4 were selected for measurement of ^{14}C through Accelerator Mass Spectrometry (AMS). There were no plant macrofossils (seeds, twigs) suitable for dating and bulk organic matter was favored over carbonates to avoid potential hard water effects. Organic samples were treated with the standard acid/alkali/acid pretreatment to remove carbonates prior to analysis. Radiocarbon measurement was completed by Beta Analytic Inc. AMS facility and the resulting conventional radiocarbon ages were calibrated to calendar years through INTCAL98 (Stuiver et al., 1998; Table 4.1).

For microfossil analysis, samples (1 cm intervals) were wet sieved on 45 μm screens to retain testate amoeba and foraminifera tests every 2.5 cm to 5 cm intervals down each core. Taxonomy followed Medioli and Scott (1983) and Poag (1981). Testate amoebae and foraminifera were considered collectively in this study to calculate fractional abundance and standard error (2σ) was calculated according to Patterson and Fishbein (1989). Most individual foraminifera were statistically insignificant after standard error calculations—and were amalgamated into three categories representing similar ecologic information: (1) the dominant *A. tepida* var. juvenile; (2) hyaline taxa (*Bolivina striatula, Spirilina vivipara*), and (3) agglutinated taxa (Textularia, species *J. macrescens, M. fusca*). *Ammonia tepida* var. juvenile was not combined with the rest of the hyaline foraminifera because it was the most dominant and key taxon in this study. The eight new taxonomic groupings (5 thecamoebian and 3 foraminifera) were used for Q-mode cluster analysis using constrained incremental sum of squares (CONISS) with a Euclidian Distance to identify biofacies (or zones) in the cores (Grimm, 1987).

Isotopes measured on bulk organic matter ($\delta^{13}C$ and $\delta^{15}N$), total organic carbon (TOC), and total nitrogen (TN) analysis was performed on finely ground samples that were treated with 10% HCl for 12 hours to remove carbonates. Following the acid digestion, samples were rinsed to neutrality, dried and re-ground to a homogenous powder. Isotopic analysis was performed on a sub-sample (~0.4 µg) with a Costech elemental analyzer connected to a Thermo-Finnigan DeltaPlus XP mass spectrometer at McMaster University. Carbon-isotope ratios were measured against several internal and international standards (NBS-21, USGS-24, ANU-Sucrose, Urea, Spar calcite, with reproducibility on replicates equal to ±0.2‰) and expressed in the standard delta (δ) notation in per mil (‰) against Vienna PeeDee Belemnite (VPDB). Replicate samples for organic and carbonate isotope analysis was ±0.2‰ and ±0.1‰, respectively.

Bulk sediment was directly loaded into a Beckman-Coulter LS 230 for particle size analysis at 1 cm intervals in the cores from the cave. Sediment digestions were not completed as the organic and carbonate fractions are the only sedimentary components. The standard Fraunhofer optical model was used for mathematical computations on each sample. Down core particle size distributions (PSD) were log transformed and interpolated using a triangular irregular network (Sambridge et al., 1995) and plotted as a color surface plot using Geosoft Oasis™ software (van Hengstum et al., 2007; Beierle et al., 2002).

4.5. RESULTS

4.5.1. CHRONOLOGY

The radiocarbon ages on bulk organic matter from C2 and C4 are in chronological order, with no ^{14}C inversions, suggesting they provide a reliable chronology for the cave sediments. On coeval OM and gastropods in C1, a minor radiocarbon offset was interpreted as a minor hard water effect (Gabriel et al., 2008), and the remaining dates were also in chronological order (Table 4.1, Fig. 4.3).

Sedimentation rates in the cave system are not uniform through space and time. In the open water at C1, rapid accumulation of peat occurs over 300 yrs, but this abruptly terminates at ~6580 ^{14}C yr. The sedimentation at C2 is not linear throughout the core, as different sedimentation rates exist above and below 21.5 cm. From 50 - 21.5 cm in C2, the sedimentation rate is higher (~0.03 cm yr^{-1}), which decreases (~0.006 cm yr^{-1}) above 21.5 cm. The sedimentation rate in C4 is ~0.05 cm yr^{-1} throughout the core, although the upper ~7 cm is interpreted as a concentrated horizon. Based on the chronological control, C2, C3, and C4 were further analyzed for micropaleontology, isotope geochemistry, and particle size analysis.

4.5.2. BIOLOGIC PROXIES

Foraminifera and testate amoebae shells generally had very good preservation. No evidence of calcium carbonate dissolution or significant breakage was observed in the foraminifera tests, suggesting little taphonomic influence on the final assemblages. Xenogeous and autogenous thecamoebians were intact and contained little breakage of test appendages, also suggesting little taphonomic reworking. The only core with statistically insignificant recovery of fauna was in C3 from 40.5 – 50 cm. The lack of recovered soil thecamoebians (i.e., *Bulinularia)* indicates that the contribution of soil-derived tests transported into the final assemblage is minimal, permitting interpretation of the testate amoeba assemblages as aquatic-based.

A total of six testate amoebae and thirteen foraminifera taxa units (three agglutinated and ten hyaline) were identified in the cores (Fig. 4.5). The testate amoebae *Centropyxis aculeata* and the foraminifer *Ammonia tepida* var. juvenile were the most dominant taxa in all cores. Using the CONISS clustering and the ecological tolerances of the dominant taxa in each grouping, three primary biofacies were identified: a Saline-Biofacies, Brackish-Freshwater Biofacies, and a Fresh Biofacies.

The Saline-Brackish (SB) biofacies is only present at the base of C4, from 49.5 – 60 cm. Here *A. tepida* var. juvenile averages 78.5% of the taxa and testate amoebae are 9.2%, and remaining hyaline foraminifera contribute 12%. In the most basal sample (57.5 cm), hyaline foraminifera taxa (e.g., *Bolivina striatula*, *Elphidium* sp.) represent 41.9% of the total assemblage.

The Freshwater-Brackish (FB) biofacies is located from 21.5 – 40.5 cm in C2, 24.5 – 49.5 cm in C3, and 7.5 – 49.5 cm in C4. Total testate amoebae represent 56% of the assemblage and *A. tepida* var. juvenile represent 42% (Table 2). *Centropyxis aculeata* are more dominant in the FB biofacies (mean 34%) as compared to the SB biofacies. Within the FB biofacies, there are oscillations in abundance of the *Centropyxis aculeata* and *A. tepida* var. juvenile (Fig. 4.4). This observation is noteworthy considering these taxa represent alternate salinity conditions, fresh vs. more saline respectively. Using linear regression analysis, a negative correlation was determined between these two taxa downcore ($r^2 = 0.67$, $p < 0.0001$; Fig. 4.6A), an expected relationship based on the ecological differences between these two taxa.

Finally, the Freshwater (F) biofacies is located in the upper most sediment of C1 (<23.5 cm), C3 (< 20.5 cm), and C4 (<5.5 cm). Testate amoebae occupy a mean of 90% of the assemblage with *Lagenodifflugia vas* becoming an important new taxon in the assemblages (mean 12%). Foraminiferal textularid taxa are also present, but are a minor contribution to the assemblage (mean 2%).

4.5.3. SEDIMENTARY CHARACTERISTICS

Cave core sediment was dominantly comprised of gyttja with varying amounts of carbonate minerals. Lighter-colored sediment horizons (i.e., C3 3-9 cm) are present at different stratigraphic depths, which is due to increased carbonate mineral content as observed during microfossil analysis (Figs. 4.3 and 4.4). C2 was dominated by gyttja, but regularly contained larger (>250 μm) organic matter fragments compared with the other cores.

The particle size distributions (PSDs) in C2 are quite peaked and dominantly unimodal from 63 – 250 μm. The FB biofacies contains multiple events of less-peaked PSDs (more poorly-sorted sediment) as compared with the F biofacies (19.5 - 25.5 cm, 36.5 - 44.5 cm), and several shifts to larger particle sizes >250 μm. The F biofacies is dominantly unimodal except the interval from 6.5 – 9.5 cm, where the introduction of coarser calcite minerals contributes to multi-modal intervals.

Using the PSDs, C3 can also be broadly divided into two sections that grossly correspond with transitions in the biologic proxies. The base of C3 (24.5 – 50 cm, FB biofacies) has alternations between coarser, unimodal PSDs, and less-peaked PSDs. The upper section of C3 (0 – 24.5 cm, F biofacies) is strongly unimodal in the 63-250 μm size range, with no alternations in the particle size mode.

The PSDs in C4 are more dynamic than the other cores. Coeval with the SB biofacies, the basal core (55.5 - 59.5 cm) is dominated by very fine particle sizes (<16 μm) that shift to less-peaked PSDs (51.5 – 55.5 cm). Throughout the FB biofacies in C4 (7.5 – 50.5 cm), continual alternations exist between less-peaked to more-peaked intervals, indicating shifts in the degree of sediment sorting. Above 25 cm, there are multiple events of multli-modal PSDs, due to increases in carbonate minerals. Although the top ~7 cm are condensed in C4, the F biofacies appears to be dominated by unimodal PSDs in the 63-250 μm size range, similarly as C2 and C3.

4.5.4. ORGANIC CARBON AND NITROGEN GEOCHEMISTRY

When interpreting organic isotopic data and C/N rations, one must first consider the role of taphonomy and diagenesis on final organic geochemical profiles. Factors such as particle size, selective degradation of plant compounds, and biologic activity can possibly affect the final absolute values (Freudenthal et al., 2001; Meyers, 1994; Benner et al., 1987). In sediment cores where TOC is >20%, organic matter may be subjected to selective degradation of organic matter compounds, by up to 2‰ in $\delta^{13}C$ (Meyers, 1994). Since the carbon isotopic alterations in the cave sediment are within 2-3 ‰ range, care is given not to over interpret the absolute value of the carbon isotope ratio. Although the cave sediment is a homogenized mixture of aquatic and terrestrial sources, previous work has demonstrated that there is no preferential preservation of one source over the other (Freudenthal et al., 2001). In addition, Meyers (1994) concluded that $\delta^{13}C$ and C/N preserve paleoenvironmental information even over millions of years. As such, although selective degradation of OM may have subtly altered the absolute isotopic values, these processes are assumed to be constant over the mid-late Holocene study interval. Therefore, the overall geochemical trends are interpreted as still preserving their original geochemical signals.

On a broad level, the organic data from C2 and C3 are most similar, with C4 having the most different geochemical trends. A natural division in the organic data, which is temporally synchronous with the F and FB biofacies transition, occurs at 23.5 cm in C3 and 20.5 cm in C2. In C3, mean C/N shifts from 18 ± 2 in the lower section to 14.0 ± 0.7 after the transition. Total nitrogen also shifts at this horizon from 2.4 ± 0.2% to 3.1 ± 0.3%. TOC and $\delta^{13}C$ are relatively stable throughout the core, although both proxies reach a minimum at the transition of 36% and 32.3‰ respectively. Throughout the interval, $\delta^{15}N$ oscillates around 2.6‰, although not as distinctively as in C2. In C2, mean C/N decreases at 20.5 cm from 26 ± 2 to 20 ± 2 and there is general increase in TN from 1.9 ± 0.1% to 2.5 ± 0.3%. Throughout the core, TOC oscillates around 48 ± 2% and $\delta^{13}C$ has <1‰ oscillations every ~2 cm. Notably in C2, $\delta^{15}N$ has 10 repeated and distinctive oscillations with an amplitude of ~0.5‰ every ~5 cm down core. In contrast to C2 and C3, the organic geochemical signals at C4 are significantly less variable than the other sites, although noticeable minor transitions occur at 8.5 cm and 50.5 cm that correlate to the biofacies transitions (Fig. 4.4). Synchronous with the SB – FB biofacies transition, shifts in TOC (mean 30 ± 13% to mean 45 ± 2%), TN (mean 1.7 ± 0.6% to 2.4 ± 0.1%), and $\delta^{15}N$ (mean 4.8 ± 0.8‰ to 3.9 ± 0.5‰) occur. At the FB to F biofacies in C4, notable shift in geochemical are shift in C/N ratio (mean 14 ± 1), TN (3.2 ±0.2 %), and $\delta^{15}N$ (mean 2.7 ± 0.5‰).

Cross plots between C/N, $\delta^{15}N$, and $\delta^{13}C$ indicate that in all the cores the organic matter is dominantly derived from freshwater vegetation sources, such as phytoplankton and macrophytes (Fig. 4.6). Of all the core sites, C4 is the most dominated by aquatic OM (AOM) sources and C2 has the largest component of terrestrial OM (TOM).

Regression analysis on total testate amoebae, *C. aculeata,* and *A. tepida* var. juvenile with $\delta^{15}N$ was completed to test the strength of relationship between oscillations in the proxies downcore. Only stratigraphic horizons that contained data from all proxies were used. Total testate amoebae are negatively correlated with $\delta^{15}N$ ($r^2=0.555$, $p<0.001$ at 95% significance), indicating that with increased salinity, $\delta^{15}N$ becomes more enriched (Fig. 4.6B). *Ammonia tepida* var. juvenile are positively correlated with $\delta^{15}N$ ($r^2=0.502$, $p<0.001$ at 95% significance), again indicating that with increased salinity $\delta^{15}N$ becomes more enriched (Fig. 4.6C). This relationship suggests that with increasing salinity there is a systematic addition of more enriched sources of organic matter into the cave system.

4.6. DISCUSSION

4.6.1. EVALUATION OF PHREATIC CAVE SEDIMENTS FOR PALEOENVIRONMENTAL RESEARCH

The sedimentary archive in Carwash Cave is interpreted as a complete temporal record of sedimentation, and not a temporally-homogenized sediment accumulation, based on several lines of evidence. (1) The radiocarbon ages are properly ordered, suggesting minimal sediment reworking with continual input of new OM into the phreatic cave through time. (2) There is no particle-size grading in the PSDs consistent with turbidites or slump facies. In contrast, the upper sections of both C2 and C3 indicate very peaked unimodal PSDs, suggesting continual sedimentary processes acting in the cave through time. (3) Considering the source of the OM, C2 is closer to the cave entrance, and contains proportionately more TOM than coeval OM sedimentation in C3. If homogenization of the sedimentary record had occurred, there would be a loss in this spatial relationship of OM deposition. (4) The trend to lower C/N values concurrent with the colonization of *Lagenodifflugia vas* at similar core depths in C2 and C3 suggests a uniform change occurring in the paleoenvironment at ~2760 Cal yrs BP affecting both the OM and biologic systems. (5) The continual $\delta^{15}N$ oscillations observed in C3 and C4 are inconsistent with a hydrologic mechanism in the cave taphonomically reworking the OM. (6) The downcore linear regressions between the biologic and geochemical proxies would be systematically removed during secondary sediment mobilization. (7) Finally, the testate amoebae and foraminifera profiles are changing through both time and space in the cave. This suggests that although each core site may have micro-environmental influences, broader hydrological trends are recorded through concurrent faunal shifts in the cave, as opposed to sedimentary reworking. The collective and individual analysis of the proxies indicates that the sedimentary record in the cave is temporally cohesive and is a suitable archive of paleoenvironmental change.

4.6.2. INTERPRETATION OF MID-LATE HOLOCENE AQUIFER EVOLUTION IN CARWASH CAVE

4.6.2.1. PHASE 1: 6500 - 4300 CAL YRS BP

The mangrove peat from the top of the cenote (~4.5 m deep), dated to 6840 - 6580 Cal yr BP, indicates that sea level was at this elevation during the mid Holocene (Gabriel et al., 2008; Toscano and McIntyre, 2003). This is coincident with the Holocene Thermal Maximum (HTM) and a more northerly extent of the Intertropical Convergence Zone (ITCZ, Haug et al., 2001), leading to increased annual precipitation on the

Yucatan Penninsula. With the stabilization of sea level during the mid-Holocene, coastal progradation and sediment accumulation along the Caribbean coastline was in its infancy.

Since total sediment along the coastline during the mid-Holocene would have been less than at present, the hydrogeologic impedance for aquifer discharge at the coastline would also have been less (~8.5 km away). Increased net annual precipitation during the HTM coupled with decreased hydrogeologic impedance at the Caribbean coast would lead to higher flow rates in the aquifer compared to modern conditions. With increasing hydrologic flow, there is a concomitant increase in turbulent flow along the boundary of immiscible fluids – the halocline – leading to increased entrainment of saline water into the freshwater lens. This physical mechanism would have sustained a more brackish 'freshwater lens' compared to present conditions (Fig. 4.8).

Biologically, a mangrove ecotope at the top of the cenote breakdown pile indicates elevated salinity conditions. Although continual sea level rise (1.46 mmyr^{-1}, Toscano and McIntyre, 2003) drowned the mangrove system at 6840 Cal yrs BP, salinity remained elevated until ~4200 Cal yrs BP, based on foraminifera at the base of C4 (SB biofacies, 51.5 – 60 cm). Based on the ecological tolerance of the recovered foraminiferan species (Murray 2007) and low (to absent) recovery of testate amoebae (van Hengstum et al., 2008), salinity in the freshwater lens must have been greater than ~3.5 ppt.

During this period, increased productivity in the shallow open water cenote is indicated by the increased fine marl deposition (C4: 55.5 – 60 cm) from loss of CO2 in the water column, decreased C/N values, and increased TN. Although there is lack of organic accumulation from this time interval, the lack of material at the coring sites may possibly be related to: (1) high biologic turnover ultimately contributing to low TOC values (to <10%) in the deep cave, (2) organic sedimentation in the shallow cave (<30 m penetration) where we have not cored, or (3) the sediment record is at a greater depth than recovered in the coring survey.

4.6.2.2. PHASE 2: 4300 - 2760 CAL YRS BP

The regional climate during Phase 2 is different than during Phase 1. At the beginning of Phase 2, the ITCZ began to migrate southward causing a regional decrease in net annual precipitation (Haug et al., 2001). Strong El Nino conditions prevailed from 4.3 – 3.8 ka (beginning of Phase 2) that trended into significant annual- and centennial-scale variability in El

Nino Southern Oscillation (ENSO) from 2.8 to 3.8 ka (end of Phase 2). In a spectral analysis of storm deposits from an alpine lake in Ecuador, the greatest El Nino frequencies (and storm activity) occur between ~3.5 to 2.6 ka (Rodbell et al., 1999). So, within a framework of decreased net annual precipitation, the regional storm activity accompanied with torrential rainfall began to increase at 4.3 ka and reach a maximum from 3.5 – 2.6 ka. This time interval is recorded in the three cave cores: C2 and C3 (3260 Cal yrs BP – present), and C4 (~4200 Cal yrs BP - ~3300 Cal yrs BP).

Sedimentation in the cave is influenced by both background hydrogeological flow and precipitation. In all the cores, the OM exhibits a consistent pattern of TOM deposited proximally to the cenote with AOM transported deeper into the cave. This pattern is consistent from 4.2 ka to 3.3 ka, where AOM is deposited in the deeper cave (C4) and relatively more TOM is deposited closer to the cenote (C2 and C3). During this interval at C4, the PSDs are rapidly fluctuating between peaked, unimodal PSDs, poorly-sorted broad PSDs, and mulit-modal PSDs. The consistently changing sedimentary pattern in C4 suggests an external influence on sedimentation beyond background hydrogeological flow. At the base of C2 and C3 (coeval with C4), PSDs are broad and less-peaked, indicating poorly sorted sediment from multiple sources.

The changing sedimentation patterns in the cave are interpreted as recording the influence of increasing storm activity in the region. With large volumes of rain at one time, increased erosion of the terrestrial surface and aquatic environment (due to increased recharge) would transport relatively more OM into the cave. Additionally, sedimentation rates in all cave cores are the highest during Phase 2 (0.05 - 0.06 cm yr^{-1}) further suggesting a storm-related increase in sediment inputs. At ~3.3 ka, sedimentation at C4 is almost shut-off compared to the other core locales, which is coincident with the onset of maximum EN Niño frequency (Rodbell et al., 1999). This may be related to several factors. First, elevation changes to the cave floor alter accommodation space, which then may be favoring sedimentation more proximal to the cenote. Second, there is a minor shift to larger particle sizes, suggesting the suspended load in the water column is deposited in the cave earlier. Third, the $\delta^{13}C$ at C4 suggests dominantly waxes and lignin-based, nitrogen rich OM (phytoplankton origin and easily retained in the suspended load), as opposed to more diversified, nitrogen-poor OM types in C3 and C2 (bark, stems, etc., decreased ability to remain in suspended load).

Similarly to the sedimentation patterns, the faunal community in the phreatic cave are influenced through background hydrogeological boundary conditions with subtle shifts in salinity superimposed. Biologically, there is shift from the SB biofacies to the FB biofacies from Phase 1 to Phase 2. Testate amoebae become an important contribution to the faunal assemblages in Phase 2, with foraminifera decreasing in their role. *A. tepida* var. juvenile is initially the dominant taxon (>80%) at the base of Phase 3, although an overall subtle desalinization trend is observed as abundances of *A. tepida* var. juvenile decrease (~80% to ~40%) and *C. aculeata* increase (~20% to ~40%) through the FB biofacies. Based on the ecological tolerances of these two species (van Hengstum et al., 2008b), the salinity in the aquifer during Phase 2 trends from ~3 ppt to ~2 ppt through the time interval. The decreasing salinity from Phase 3 to Phase 2 is explained by increased coastal progradation causing a net increase in hydrogeologic impedance for aquifer discharge. Increased impedance causes a concomitant decrease in hydrologic flow rates, in turn decreasing turbulent flow and overall salinity in the freshwater lens of the aquifer. The long-term desalinization trend is further evidence that continual coastal progradation (hydrologeologic impedance) caused longer-term alterations to the aquifer flow regime, and in turn the salinity in the fresh water lens at Carwash Cave.

Within the broad faunal trends, there are alternations in the abundance of *C. aculeata* and *A. tepida* var. juvenile in the FB biofacies. Through the linear regression, a negative correlation between these two fauna ($r^2 = 0.67$) indicates subtle salinity alternations. This relationship is expected as with increasing salinity, testate amoebae approach their ecological boundary and become increasingly stressed. However, the salinity fluctuations are interpreted to be minor (<1 ppt), as one taxonomic group never completely disappears from the FB biofacies. With increased storm activity during the FB biofacies (Phase 2), sudden precipitation events causing increased recharge would lead to increased hydrologic flow/turbulence in turn causing a minor increase in the salinity of the freshwater lens (and increased abundance of *A. tepida* var. juvenile). As dryer conditions return, the fresh water lens becomes less saline due to decreased turbulent flow and conditions more favorable for testate amoebae return. Amoebae are highly sensitive organisms, and populations can rapidly respond to changing environmental conditions due to a short generation time.

The climatic influences on the phreatic cave can be further evaluated through the linkage of separate paleoenvironmental proxies. Based on the OM sedimentation patterns, climate-induced precipitation is interpreted as causing increased terrestrial erosion and sedimentation in the cave. Based on alternations in the abundances of the biological proxies, climate-induced precipitation is interpreted as causing minor salinity shifts (<1 ppt) during Phase 2 and the FB biofacies. Using linear regression, total testate amoebae and $\delta^{15}N$ are negatively correlated (r^2 = 0.555), indicating that with increased salinity, there is increase in enriched OM deposited into the cave. Similarly, *A. tepida* var. juvenile and $\delta^{15}N$ values are positively correlated, indicating that with increased salinity there is an increase in enriched OM sources deposited into the cave. Increased precipitation can account for both increased salinity in the cave and increased deposition of enriched nitrogen sources of OM. With increased precipitation, there is increased erosion of TOM (soil and vegetation, $\delta^{15}N$ +2‰ to +10‰), which is deposited into the cave sedimentary record (Talbot, 2001). Additionally, continual transport of aquatic OM (phytoplankton, more depleted in $\delta^{15}N$) would be a positive feedback mechanism for continual enrichment in the residual nitrogen pools in the cenote pool, leading to more enriched plant tissues over time.

4.6.2.3. PHASE 3: 2760 CAL YRS BP - PRESENT

At 2760 Cal yrs BP, paleoenvironmental conditions in the cave begin to reflect the modern environment. The appearance of *Lagenodifflugia vas and Difflugia oblonga* (in C2, C3, and C4) is concurrent with the significant decrease in *A. tepida* var. juvenile indicate a final salinity shift in the freshwater lens. Modern salinity in the freshwater lens at Carwash is 1.5 ppt and the same fauna was found living at the sediment-water interface in Carwash Cave (van Hengstum et al., 2008a). As such, the faunal transition at 2760 Cal yrs BP is interpreted as representing the transition to modern hydrologic conditions in the freshwater lens of the aquifer.

During Phase 1, there is decreased climatic variability as compared to Phase 2 (Haug et al., 2001). Although cycles of drought and precipitation continue (Haug et al., 2001), the amplitude of the climate cycles are not as great, which is interpreted as creating more stabilized sedimentation patterns in the cave. Environmental stability creates more favorable conditions for increased aquatic primary productivity, as suggested by decreased C/N and increased TN. The region received decreased net annual precipitation and aquifer recharge during Phase 1, subsequently causing decreased hydrologic and turbulent flow rates, ultimately causing an increase in aquifer stratification and longer-term freshening of the freshwater lens. This physical mechanism explains the decreased OM sedimentation rates during Phase 1 from 0.03 – 0.05 cm yr^{-1} (C3) to 0.006 cm yr^{-1} (C2 and C3). Within the amoebae profiles (Fig. 4.4), there are intervals where there is an increase in foraminifera abundances and $\delta^{15}N$

oscillations of ~1‰ to 1.5‰ (and thereby salinity). Since intervals of drought and precipitation are still occurring throughout this time interval, these oscillations are attributed to the same physical climate-linked processes as in Phase 2. However, the resolution of this study does not allow for accurate association of these peaks to more specific drought events in the last 2000 years (see Hodell et al., 2003). The lack of drought-related $\delta^{13}C$ oscillations is a relationship also noted by Clark et al. (2002) in the plains of the USA.

4.6.3. IMPLICATIONS OF AQUIFER EVOLUTION ON THE MAYA

The Yucatan Peninsula has a known record of human habitation throughout the Holocene, with many skeletons and archaeological remains dated to the early Holocene (i.e., Dalton, 2005, Coke, 1994). However, the emergence of the Maya civilization did not occur until ~4000 yrs BP. According to Sharer (1994), at 3000 yrs BP Early Preclassic Mayan communities began emerging in the Southern and Central Lowland areas along rivers and near surface sources of freshwater. By 2750 yrs BP, Maya communities began settling in Southern and Central Lowland in non-riverine areas and by 2500 yrs BP, initial Maya architecture emerged with evidence of defined socio-economic and political structures in the Northern Lowlands (See Table 2.1. in Sharer, 1994). In a palynology-based paleoenvironmental investigation in Lake Cobá (within the archaeological site of the Cobá city), Leyden et al. (1998) detected initial land clearance at ~3650 Cal yrs BP. However, the onset of maximum land disturbance and the first maize pollen occur at ~2850 Cal yrs BP. These lines of evidence suggest that although humans were occupying the area before ~2850 Cal yrs BP, after this point, deforestation agriculture, and human activities significantly increased the area.

Using the paleoenvironmental information from Carwash Cave, a salinity shift of >2 ppt to 1.5 ppt in the freshwater lens of the aquifer is anthropologically important because the human physiological limit for the consumption of brackish water is 1.5-1.8 ppt salinity. After 2760 ± 40 Cal yrs BP, the freshwater lens at Carwash Cave (~35 km from Cobá) indicates a shift to potable water, suggesting widespread water availability in the eastern Northern Maya Lowlands and temporally coinciding with rapid Mayan settlement away from surface sources of freshwater in the Central Lowlands and initial architectural development in the Northern Lowlands. Although a salinity of 1.5 ppt is drinkable, the salt content would be noticeable and tasted, and presumably the social elite in Mayan society would have still preferred drinking meteoric water over cenote-based water by society's elite. However, with widespread availability of potable water located all over the Yucatan platform in cenotes, this would potentially have facilitated population expansion and migration and irrigation needs in the Lowlands. Additional research is needed to further investigate the scope of environmental / hydrogeological and social / cultural linkages between the aquifer and early development of the Maya civilization during the Early Preclassic in the Northern Lowlands.

4.7. CONCLUSIONS

Using a multi-proxy approach, this study presents the first paleoenvironmental reconstruction of a phreatic cave system. At Carwash Cave, a notable salinity shift (from >2 to 1.5 ppt) occurred in the freshwater lens at ~2760 ± 40 Cal yrs BP, which coincides with the onset of maximum land disturbance at the ancient Maya city of Cobá. Although possible cultural linkages have been presented regarding the implication of this salinity shift on the ancient Maya, additional research is needed to assess the spatial extent and further constrain the timing of this transition. This work further demonstrates the wider applicability of testate amoebae and foraminifera as hydrological proxies in the phreatic cave environment and that OM sediments in the cave are a valuable archive of environmental information. Any additional research should increase the sampling resolution (<1 cm) to better isolate the onset and duration of any $\delta^{15}N$ shifts, and their further linkage to more regional climatic patterns through the mid-late Holocene.

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4.10. CHAPTER 4 FIGURES

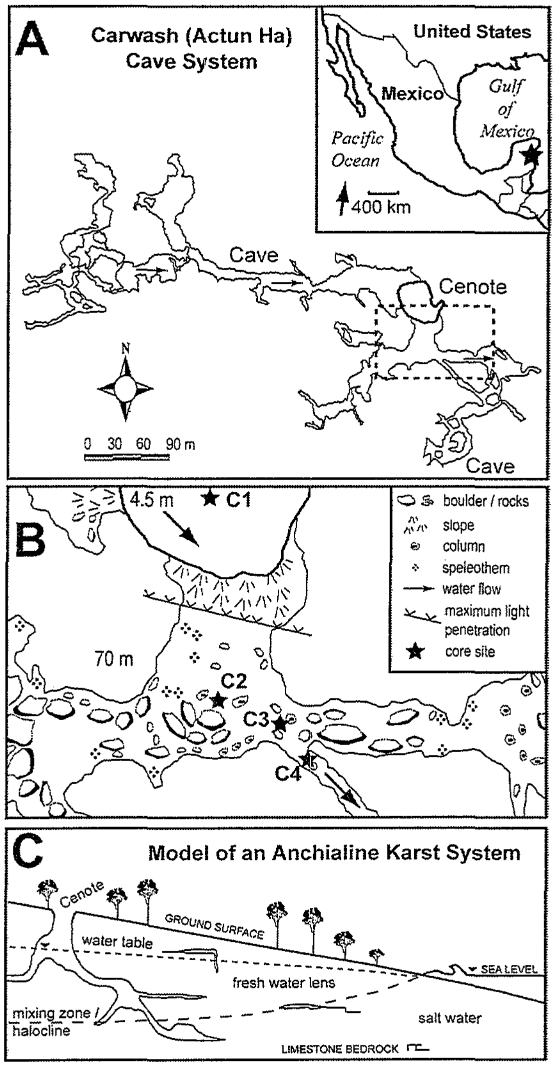

FIGURE 4.1. A: Plan view map of the passages in Carwash Cave System. The dashed square is the location for core survey relative to the rest of the cave. **B:** Magnification of coring area in the southeastern cave passages. **C:** Idealized model of an anchialine karst system.

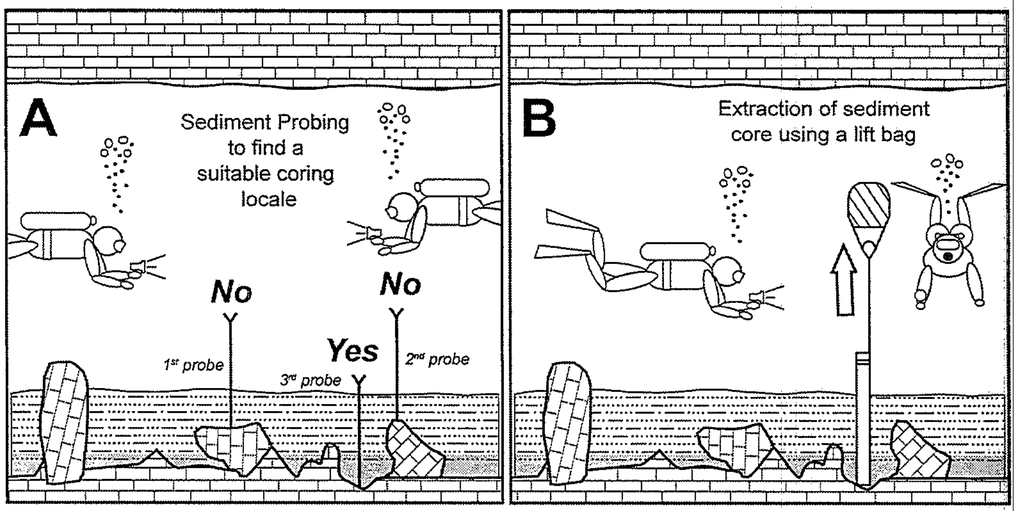

Figure 4.2. The process of coring in a phreatic cave. **A:** Divers conducting sediment probing to find a location that contains sufficient sediment accumulation, as the cave floor is not a uniform surface. **B:** Divers taking a sediment core using the buoyant force of a lift bag to for core extraction.

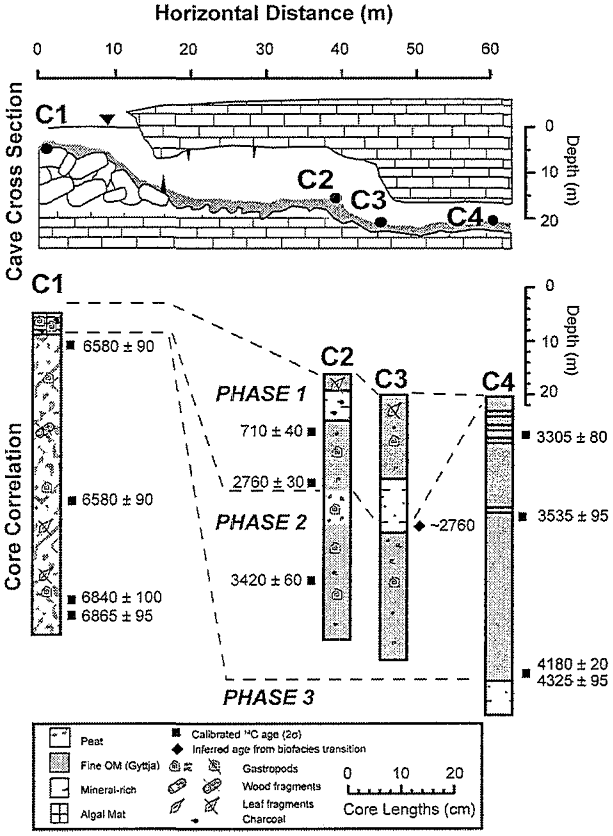

FIGURE 4.3. A stratigraphic correlation diagram using radiocarbon dates and biofacies to chronologically divide the cores into three separate phases. Note that none of the cores bottomed on bedrock, so actual sediment accumulation may be greater than illustrated.

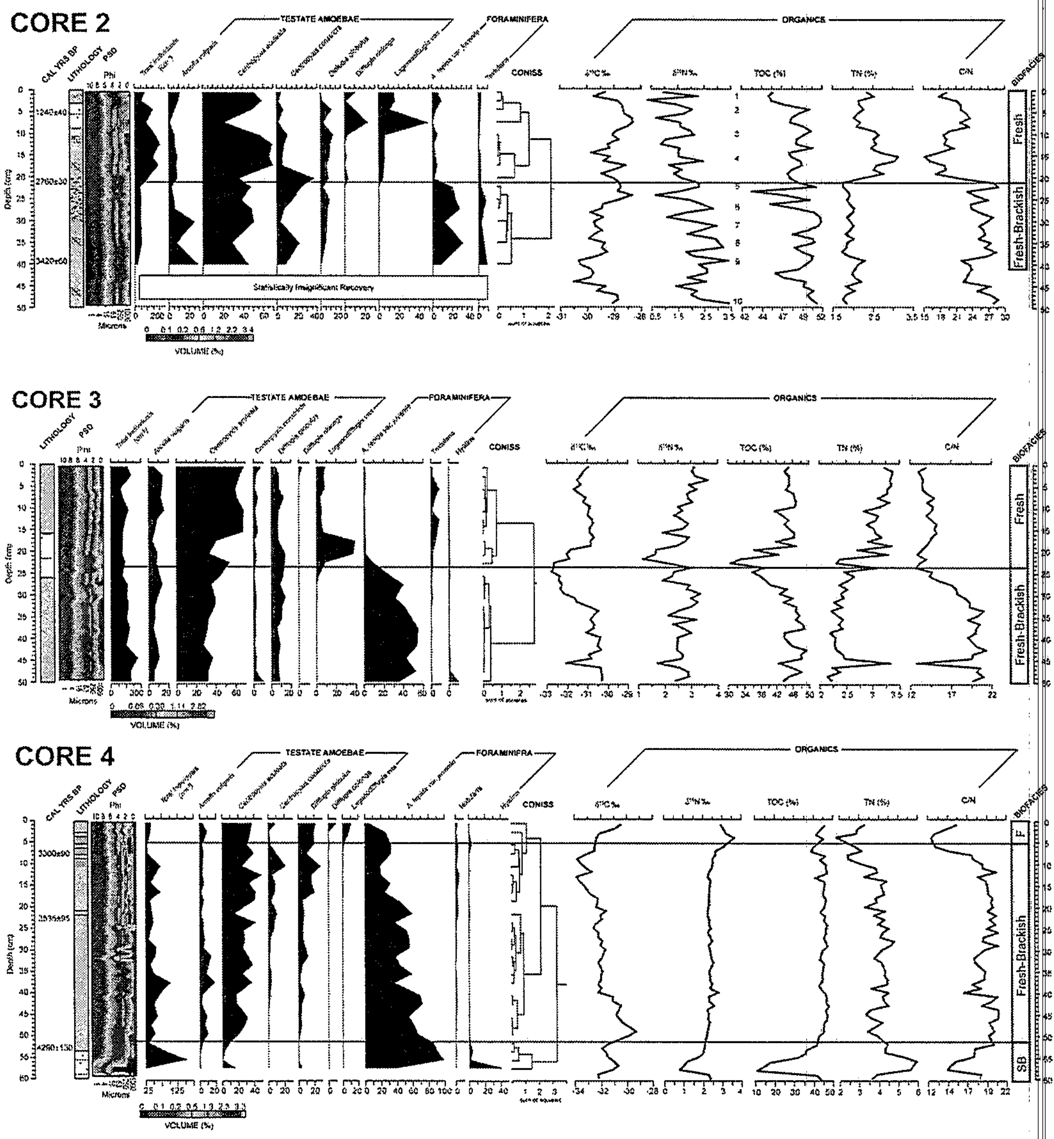

FIGURE 4.4. Down core profiles of micropaleontological and geochemical proxies. SB: Saline-brackish biofacies. F: Freshwater biofacies.

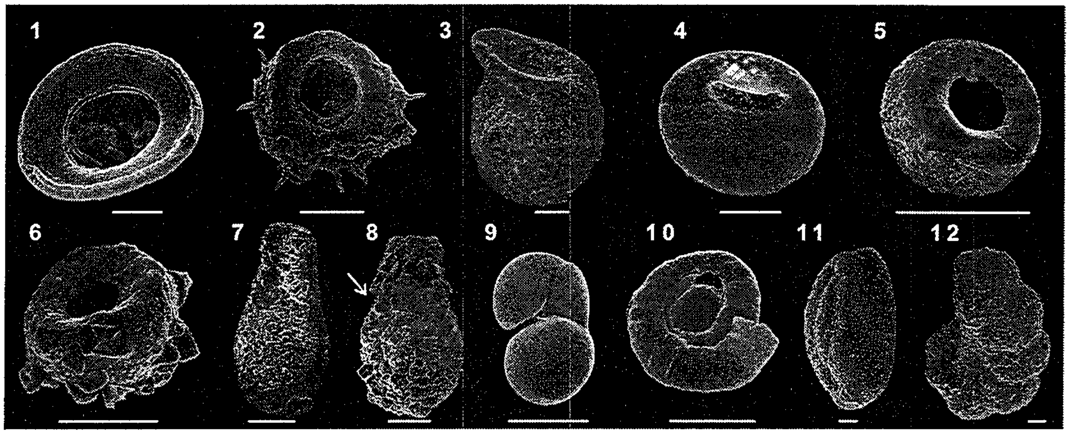

FIGURE 5.5. Scanning Electron Micrographs of representative taxa. 1. *Arcella vulgaris* (x341). 2. *Centropyxis aculeata* (x406). 3. *C. constricta* (x425). 4. *Buliminella indica* (x600). 5. *Difflugia globulus* (x1150) 6. *D. globulus* (x655). 7. *D. oblonga* (x263). 8. *Lagenodifflugia vas* (x300), arrow points to diagnostic restriction around the neck. 9. *Ammonia tepida* var. juvenile (x775). 10. *Spirillina vivipara* (x710). 11. *Millimmina fusca* (x150). 12. *Jadammina macrescens* (x150). All specimens were gold sputter-coated and imaged with a Philips 515 SEM at McMaster University. Scale bar represents 50 μm.

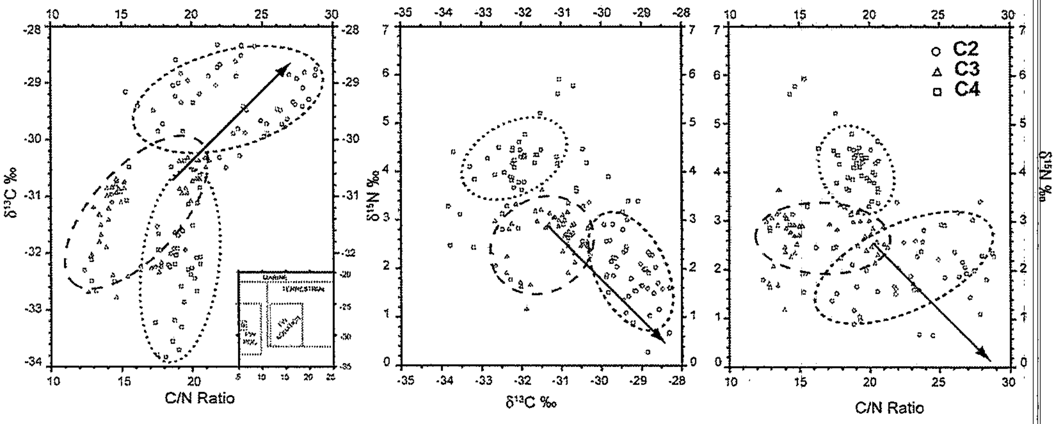

FIGURE 5.6. Cross-plots between different organic geochemical proxies to distinguish different organic matter inputs into the cave system. Arrow indicates the expected geochemical trend with increasing terrestrial organic matter input within each separate plot.

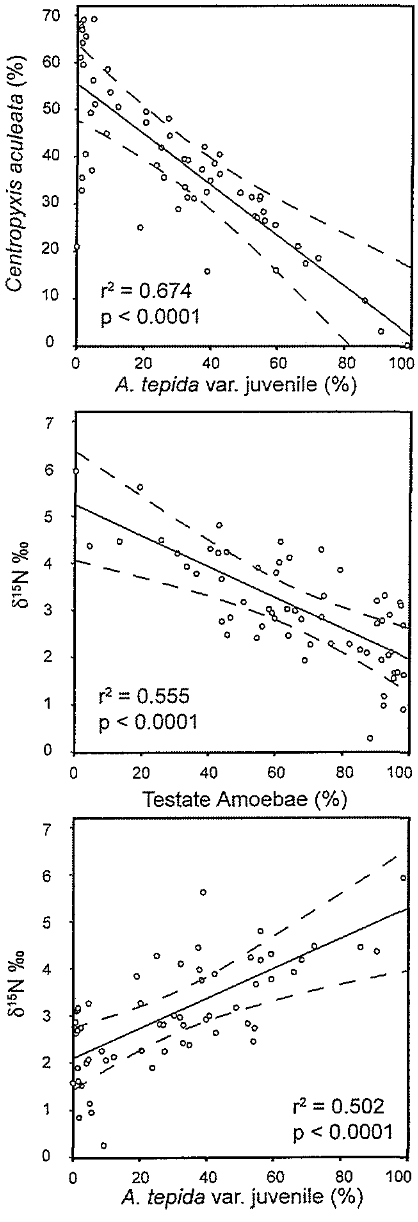

FIGURE 5.7. Linear regression between isotopic data and biological proxies. The 95% confidence limit on the regression model is denoted through the dashed line.

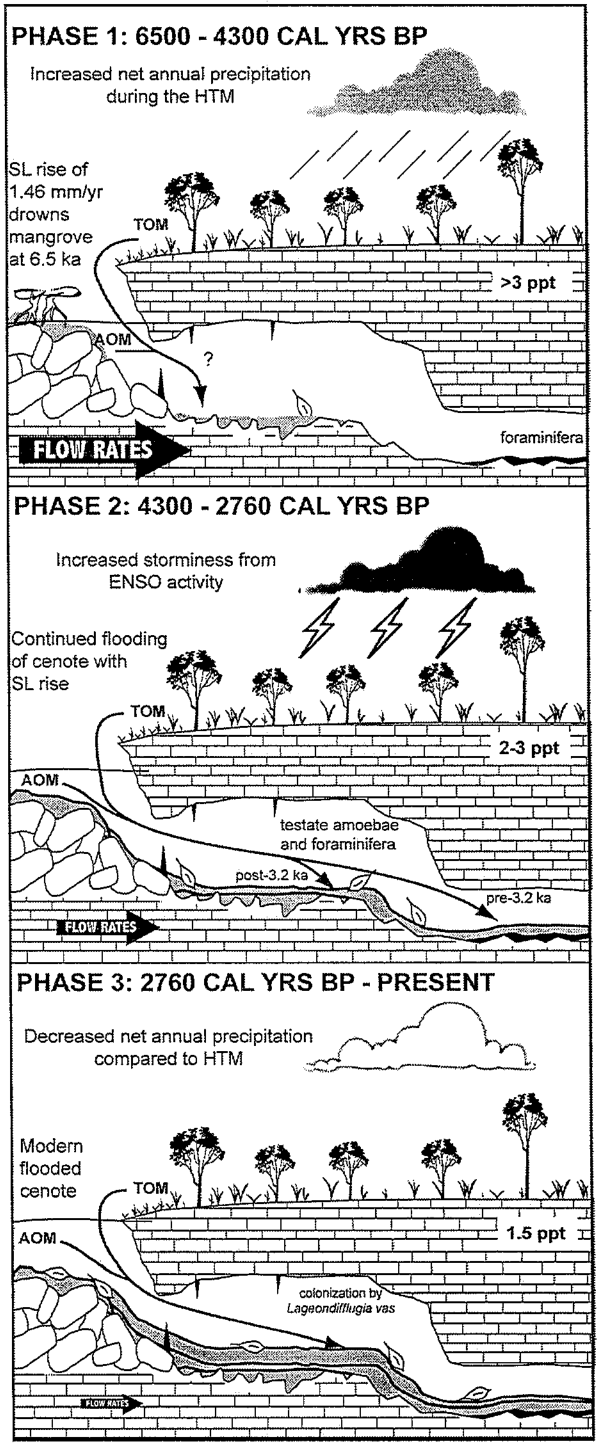

FIGURE 5.8. Interpretative diagram illustrating environmental evolution in the cave system.

4.11. CHAPTER 4 TABLES

Core	Depth in core (cm)	Conventional radiocarbon age (^{14}C yr BP)	δ13C (VPDB)	Material dated	Laboratory Code	Cal yr BP (2σ confidence interval)
C1*	7 - 8 cm	5790±40	-29.7‰	twig	Beta - 226967	6670 - 6490
C1*	22 - 23 cm	5780±40	-29.3‰	wood fragments	Beta - 226968	6670 - 6480
C1*	54 - 55 cm	5990±40	-29.3‰	wood fragments	Beta - 226969	6940 - 6740
C1*	55 - 57 cm	6390±40	-9.9 ‰	gastropods	Beta - 235036	6960 - 6770
C2	10 - 11 cm	820±40	-27.6‰	bulk organics	Beta - 239981	750 - 670
C2	21 - 22 cm	2760±40	-26.7‰	bulk organics	Beta - 239982	2790 - 2730
C2	39 - 40 cm	3260±40	-27.9 ‰	bulk organics	Beta - 239983	3480 - 3360
C4	7 - 8 cm	3090±40	-28.8‰	bulk organics	Beta - 237361	3390 - 3220
C4	22 - 23 cm	3300±40	-31.7‰	bulk organics	Beta - 237362	3630 - 3440
C4	52 - 53 cm	3890±40	-32.2‰	bulk organics	Beta - 237363	4420 - 4230 & 4200 - 4160

TABLE 5.1. Radiocarbon dates and calibrated ages for the cores from Carwash Cave. Note that ages marked with asterisks are from Gabriel et al. (2008).

	SPECIES							TAXONOMIC GROUPINGS		
BIOFACIES	*Arcella vulgaris*	*Centropyxis aculeata*	*Centropyxis constricta*	*Difflugia globulus*	*Difflugia oblonga*	*Lageno-difflugia vas*	*A. tepida var.* juvenile	Textularia	Adult Hyaline Foraminifera	Testate Amoebae
F	8 ± 3	52 ± 15	5 ± 7	10 ± 6	3 ± 5	12 ± 12	6 ± 9	2 ± 2	0	90 ± 10
FB	8 ± 6	34 ± 9	6 ± 6	8 ± 7	0	0	42 ± 16	2 ± 2	0	56 ± 15
BS	1 ± 2	9 ± 7	0	0	0	0	79 ± 27	0	12 ± 19	9 ± 9

TABLE 5.2. Average percent abundance with standard deviation of dominant species in the three different biofacies.

4.12. SUPPORTING ONLINE MATERIALS

TABLE 4.S1. Relative abundance and standard error (2σ) on taxa from cave cores.

	CORE DEPTH (cm)	**0.5**	**2.5**	**4.5**	**7.5**	**10.5**	**12.5**	**15.5**	**17.5**
C2	**Total count**	408	292	383	246	430	513	375	478
C2	**Specimens per cc**	82	58	153	98	172	205	150	191
C2	**Biofacies**	F	F	F	F	F	F	F	F
C2	*Arcella vulgaris*	11.0	9.2	6.3	1.2	6.0	6.0	8.3	7.1
C2	Standard Error (±)	3.0	3.3	2.4	1.4	2.3	2.1	2.8	2.3
C2	*Centropyxis aculeata*	51.2	58.6	49.3	21.1	56.3	69.0	65.6	69.2
C2	Standard Error (±)	4.9	5.7	5.0	5.1	4.7	4.0	4.8	4.1
C2	*Centropyxis constricta*	4.2	1.4	6.5	1.6	10.5	7.6	5.3	5.0
C2	Standard Error (±)	1.9	1.3	2.5	1.6	2.9	2.3	2.3	2.0
C2	*Difflugia globulus*	-	1.7	11.0	2.4	12.6	9.4	9.1	5.6
C2	Standard Error (±)	-	1.5	3.1	1.9	3.1	2.5	2.9	2.1
C2	*Difflugia oblonga*	10.5	1.0	9.9	22.8	2.6	0.2	1.6	-
C2	Standard Error (±)	3.0	1.2	3.0	5.2	1.5	0.4	1.3	-
C2	*Lagenodifflugia vas*	15.4	16.4	11.0	49.2	6.5	6.0	5.6	5.4
C2	Standard Error (±)	3.5	4.3	3.1	6.2	2.3	2.1	2.3	2.0
C2	*Ammonia parkinsoniana*	-	-	-	-	-	-	-	0.2
C2	Standard Error (±)	-	-	-	-	-	-	-	0.4
C2	*Ammonia tepida* var. juvenile	5.1	8.9	3.9	-	4.7	1.8	2.4	4.8
C2	Standard Error (±)	2.1	3.3	1.9	-	2.0	1.1	1.5	1.9
C2	*Elphidium* sp.	-	-	-	-	-	-	-	0.6
C2	Standard Error (±)	-	-	-	-	-	-	-	0.7
C2	*Haynesina depressula*	-	0.3	-	-	-	-	-	-
C2	Standard Error (±)	-	0.7	-	-	-	-	-	-
C2	*Millammina fusca*	0.7	0.3	-	0.4	0.2	-	1.1	-
C2	Standard Error (±)	0.8	0.7	-	0.8	0.5	-	1.0	-
C2	*Polysaccammina iophalina*	-	-	0.5	0.4	-	-	-	-
C2	Standard Error (±)	-	-	0.7	0.8	-	-	-	-
C2	*Spirillina vivipara*	-	-	-	-	-	-	-	-
C2	Standard Error (±)	-	-	-	-	-	-	-	-
C2	*Jadammina macrescens*	1.7	2.1	1.6	0.8	0.7	-	1.1	1.9
C2	Standard Error (±)	1.3	1.6	1.2	1.1	0.8	-	1.0	1.2

Table 4.S1. Continued...

C2	**CORE DEPTH (cm)**	**20.5**	**22.5**	**25.5**	**27.5**	**30.5**	**35.5**	**40.5**
C2	**Total count**	270	108	254	233	231	294	107
C2	**Specimens per cc**	108	43	51	47	46	59	21
C2	**Biofacies**	**F**	**FB**	**FB**	**FB**	**FB**	**FB**	**FB**
C2	*Arcella vulgaris*	8.5	2.8	5.1	5.6	25.1	7.8	29.0
C2	Standard Error (±)	3.3	6.1	1.3	2.9	5.6	3.1	8.6
C2	*Centropyxis aculeata*	35.6	47.2	38.2	48.1	50.6	28.9	44.9
C2	Standard Error (±)	5.7	5.7	4.9	6.4	6.4	5.2	9.4
C2	*Centropyxis constricta*	37.0	24.1	15.7	7.3	3.0	22.1	8.4
C2	Standard Error (±)	5.8	9.1	3.7	3.3	2.2	4.7	5.3
C2	*Difflugia globulus*	7.8	2.8	9.1	6.9	6.5	4.8	-
C2	Standard Error (±)	3.2	7.7	1.3	3.2	3.2	2.4	-
C2	*Difflugia oblonga*	3.0	-	-	-	-	-	-
C2	Standard Error (±)	2.0	-	-	-	-	-	-
C2	*Lagenodifflugia vas*	4.4	-	0.8	-	-	-	-
C2	Standard Error (±)	2.5	2.5	-	-	-	-	-
C2	*Ammonia parkinsoniana*	-	-	-	-	-	-	-
C2	Standard Error (±)	-	-	-	-	-	-	-
C2	*Ammonia tepida* var. juvenile	1.5	20.4	23.6	27.0	12.1	30.3	8.4
C2	Standard Error (±)	1.4	9.4	3.5	5.7	4.2	5.3	5.3
C2	*Elphidium* sp.	-	-	-	-	-	-	-
C2	Standard Error (±)	-	-	-	-	-	-	-
C2	*Haynesina depressula*	-	-	-	-	-	-	-
C2	Standard Error (±)	-	-	-	-	-	-	-
C2	*Millammina fusca*	-	-	-	-	-	-	-
C2	Standard Error (±)	-	-	-	-	-	-	-
C2	*Polysaccammina iophalina*	-	-	-	-	-	-	-
C2	Standard Error (±)	-	-	-	-	-	-	-
C2	*Spirillina vivipara*	-	-	-	-	-	0.7	0.9
C2	Standard Error (±)	-	-	-	-	-	0.9	1.8
C2	*Jadammina macrescens*	2.2	2.8	7.5	5.2	2.6	5.4	8.4
C2	Standard Error (±)	1.8	7.2	1.3	2.8	2.1	2.6	5.3

C3	**Core Depth (cm)**	**0.5**	**2.5**	**4.5**	**7.5**	**10.5**	**12.5**	**15.5**	**17.5**
C3	**Total count**	408	292	383	246	430	513	375	478
C3	**Specimens per cc**	82	58	153	98	172	205	150	191
C3	**Biofacies**	F	F	F	F	F	F	F	F
C3	*Arcella vulgaris*	11.0	9.2	6.3	1.2	6.0	6.0	8.3	7.1
C3	Standard Error (±)	3.0	3.3	2.4	1.4	2.3	2.1	2.8	2.3
C3	*Centropyxis aculeata*	51.2	58.6	49.3	21.1	56.3	69.0	65.6	69.2
C3	Standard Error (±)	4.9	5.7	5.0	5.1	4.7	4.0	4.8	4.1
C3	*Centropyxis constricta*	4.2	1.4	6.5	1.6	10.5	7.6	5.3	5.0
C3	Standard Error (±)	1.9	1.3	2.5	1.6	2.9	2.3	2.3	2.0
C3	*Difflugia globulus*	-	1.7	11.0	2.4	12.6	9.4	9.1	5.6
C3	Standard Error (±)	-	1.5	3.1	1.9	3.1	2.5	2.9	2.1
C3	*Difflugia oblonga*	10.5	1.0	9.9	22.8	2.6	0.2	1.6	-
C3	Standard Error (±)	3.0	1.2	3.0	5.2	1.5	0.4	1.3	-
C3	*Lagenodifflugia vas*	15.4	16.4	11.0	49.2	6.5	6.0	5.6	5.4
C3	Standard Error (±)	3.5	4.3	3.1	6.2	2.3	2.1	2.3	2.0
C3	*Ammonia parkinsoniana*	-	-	-	-	-	-	-	0.2
C3	Standard Error (±)	-	-	-	-	-	-	-	0.4
C3	*Ammonia tepida* var. juvenile	5.1	8.9	3.9	-	4.7	1.8	2.4	4.8
C3	Standard Error (±)	2.1	3.3	1.9	-	2.0	1.1	1.5	1.9
C3	*Elphidium* sp.	-	-	-	-	-	-	-	0.6
C3	Standard Error (±)	-	-	-	-	-	-	-	0.7
C3	*Haynesina depressula*	-	0.3	-	-	-	-	-	-
C3	Standard Error (±)	-	0.7	-	-	-	-	-	-
C3	*Millammina fusca*	0.7	0.3	-	0.4	0.2	-	1.1	-
C3	Standard Error (±)	0.8	0.7	-	0.8	0.5	-	1.0	-
C3	*Polysaccammina iophalina*	-	-	0.5	0.4	-	-	-	-
C3	Standard Error (±)	-	-	0.7	0.8	-	-	-	-
C3	*Spirillina vivipara*	-	-	-	-	-	-	-	-
C3	Standard Error (±)	-	-	-	-	-	-	-	-
C3	*Jadammina macrescens*	1.7	2.1	1.6	0.8	0.7	-	1.1	1.9
C3	Standard Error (±)	1.3	1.6	1.2	1.1	0.8	-	1.0	1.2

Table 4.S1. Continued...

C3	**Core Depth (cm)**	**20.5**	**22.5**	**25.5**	**27.5**	**30.5**	**35.5**	**40.5**
C3	**Total count**	270	108	254	233	231	294	107
C3	**Specimens per cc**	108	43	51	47	46	59	21
C3	**Biofacies**	**F**	**FB**	**FB**	**FB**	**FB**	**FB**	**FB**
C3	*Arcella vulgaris*	8.5	2.8	5.1	5.6	25.1	7.8	29.0
C3	Standard Error (±)	3.3	6.1	1.3	2.9	5.6	3.1	8.6
C3	*Centropyxis aculeata*	35.6	47.2	38.2	48.1	50.6	28.9	44.9
C3	Standard Error (±)	5.7	5.7	4.9	6.4	6.4	5.2	9.4
C3	*Centropyxis constricta*	37.0	24.1	15.7	7.3	3.0	22.1	8.4
C3	Standard Error (±)	5.8	9.1	3.7	3.3	2.2	4.7	5.3
C3	*Difflugia globulus*	7.8	2.8	9.1	6.9	6.5	4.8	-
C3	Standard Error (±)	3.2	7.7	1.3	3.2	3.2	2.4	-
C3	*Difflugia oblonga*	3.0	-	-	-	-	-	-
C3	Standard Error (±)	2.0	-	-	-	-	-	-
C3	*Lagenodifflugia vas*	4.4	-	0.8	-	-	-	-
C3	Standard Error (±)	2.5	2.5	-	-	-	-	-
C3	*Ammonia parkinsoniana*	-	-	-	-	-	-	-
C3	Standard Error (±)	-	-	-	-	-	-	-
C3	*Ammonia tepida* var. juvenile	1.5	20.4	23.6	27.0	12.1	30.3	8.4
C3	Standard Error (±)	1.4	9.4	3.5	5.7	4.2	5.3	5.3
C3	*Elphidium* sp.	-	-	-	-	-	-	-
C3	Standard Error (±)	-	-	-	-	-	-	-
C3	*Haynesina depressula*	-	-	-	-	-	-	-
C3	Standard Error (±)	-	-	-	-	-	-	-
C3	*Millammina fusca*	-	-	-	-	-	-	-
C3	Standard Error (±)	-	-	-	-	-	-	-
C3	*Polysaccammina iophalina*	-	-	-	-	-	-	-
C3	Standard Error (±)	-	-	-	-	-	-	-
C3	*Spirillina vivipara*	-	-	-	-	-	0.7	0.9
C3	Standard Error (±)	-	-	-	-	-	0.9	1.8
C3	*Jadammina macrescens*	2.2	2.8	7.5	5.2	2.6	5.4	8.4
C3	Standard Error (±)	1.8	7.2	1.3	2.8	2.1	2.6	5.3

Table 4.S1. Continued...

C4	**Depth (cm)**	**0.5**	**2.5**	**5.5**	**7.5**	**10.5**	**12.5**	**14.5**	**16.5**
C4	**Total Count**	266	255	188	201	444	345	258	443
C4	**Specimens per cc**	106	102	75	80	178	138	103	177
C4	**Biofacies**	**F**	**F**	**F**	**F**	**FB**	**FB**	**FB**	**FB**
C4	*Arcella vulgaris*	3.38	9.41	3.19	4.48	3.60	3.19	5.04	2.26
C4	Standard Error (±)	2.17	3.58	2.51	2.86	1.73	1.85	2.67	1.38
C4	*Centropyxis aculeata*	37.22	35.69	31.38	39.30	25.00	49.57	33.72	41.99
C4	Standard Error (±)	5.81	5.88	6.63	6.75	4.03	5.28	5.77	4.60
C4	*Centropyxis constricta*	9.02	1.18	3.19	9.95	21.85	4.93	8.91	8.58
C4	Standard Error (±)	3.44	1.32	2.51	4.14	3.84	2.28	3.48	2.61
C4	*Difflugia globulus*	21.80	19.61	19.68	10.45	27.93	16.81	16.67	20.54
C4	Standard Error (±)	4.96	4.87	5.68	4.23	4.17	3.95	4.55	3.76
C4	*Difflugia oblonga*	9.77	-	1.06	-	0.45	-	-	-
C4	Standard Error (±)	3.57	-	1.47	-	0.62	-	-	-
C4	*Lagenodifflugia vas*	11.28	7.84	1.60	-	0.45	-	-	0.45
C4	Standard Error (±)	3.80	3.30	1.79	-	0.62	-	-	0.62
C4	*Ammonia parkinsoniana*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Ammonia tepida* "juvenile"	4.51	25.88	32.98	32.84	18.92	20.29	32.17	24.83
C4	Standard Error (±)	2.49	5.38	6.72	6.49	3.64	4.24	5.70	4.02
C4	*Ammonia tepida*	-	-	0.53	-	-	-	-	-
C4	Standard Error (±)	-	-	1.04	-	-	-	-	-
C4	*Bolivina striatula*	-	-	1.60	-	0.45	0.29	-	-
C4	Standard Error (±)	-	-	1.79	-	0.62	0.57	-	-
C4	*Bolivina variabilis*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Elphidium* sp.	0.38	-	0.53	0.50	-	-	-	-
C4	Standard Error (±)	0.74	-	1.04	0.97	-	-	-	-
C4	*Fissurina compacta*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Haynesina depressula*	-	-	1.06	-	0.23	-	-	-
C4	Standard Error (±)	-	-	1.47	-	0.44	-	-	-
C4	*Millammina fusca*	0.75	-	1.06	-	-	-	-	-
C4	Standard Error (±)	1.04	-	1.47	-	-	-	-	-
C4	*Polysaccammina iophalina*	0.75	-	-	-	-	-	0.39	-
C4	Standard Error (±)	1.04	-	-	-	-	-	0.76	-
C4	*Rosalina subaracuana*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Spirillina vivipara*	0.00	-	-	-	-	-	-	-
C4	Standard Error (±)	0.00	-	-	-	-	-	-	-
C4	*Jadammina macrescens*	1.13	0.39	2.13	2.49	1.13	4.64	3.10	1.35
C4	Standard Error (±)	1.27	0.77	2.06	2.15	0.98	2.22	2.12	1.08

Table 4.S1. Continued...

C4	**Depth (cm)**	**18.5**	**21.5**	**23.5**	**25.5**	**27.5**	**29.5**	**31.5**	**33.5**
C4	**Total Count**	244	281	313	254	215	362	350	250
C4	**Specimens per cc**	98	112	125	102	86	145	140	100
C4	**Biofacies**	**FB**	**FB**	**FB**	**FB**	**FB**	**FB**	**FB**	**FB**
C4	*Arcella vulgaris*	5.33	1.78	4.79	4.72	6.51	4.70	14.00	11.20
C4	Standard Error (±)	2.82	1.55	2.37	2.61	3.30	2.18	3.64	3.91
C4	*Centropyxis aculeata*	40.57	16.01	42.17	27.56	28.37	26.52	32.57	38.00
C4	Standard Error (±)	6.16	4.29	5.47	5.49	6.03	4.55	4.91	6.02
C4	*Centropyxis constricta*	3.69	11.03	7.67	7.09	1.86	2.49	1.71	1.60
C4	Standard Error (±)	2.36	3.66	2.95	3.16	1.81	1.60	1.36	1.56
C4	*Difflugia globulus*	5.33	7.47	6.39	5.91	6.51	9.12	12.00	5.20
C4	Standard Error (±)	2.82	3.07	2.71	2.90	3.30	2.97	3.40	2.75
C4	*Difflugia oblonga*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Lagenodifflugia vas*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Ammonia parkinsoniana*	-	-	-	0.39	-	-	-	-
C4	Standard Error (±)	-	-	-	0.77	-	-	-	-
C4	*Ammonia tepida* "juvenile"	42.21	59.43	37.70	53.15	55.81	56.08	38.57	44.00
C4	Standard Error (±)	6.20	5.74	5.37	6.14	6.64	5.11	5.10	6.15
C4	*Ammonia tepida*	-	0.71	-	-	-	-	-	-
C4	Standard Error (±)	-	0.98	-	-	-	-	-	-
C4	*Bolivina striatula*	-	0.36	-	-	-	-	-	-
C4	Standard Error (±)	-	0.70	-	-	-	-	-	-
C4	*Bolivina variabilis*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Elphidium* sp.	-	-	-	-	-	-	0.29	-
C4	Standard Error (±)	-	-	-	-	-	-	0.56	-
C4	*Fissurina compacta*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Haynesina depressula*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Millammina fusca*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Polysaccammina iophalina*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Rosalina subaracuana*	-	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-	-
C4	*Spirillina vivipara*	-	-	0.32	0.39	-	-	0.29	-
C4	Standard Error (±)	-	-	0.63	0.77	-	-	0.56	-
C4	*Jadammina macrescens*	2.87	3.20	0.96	0.79	0.93	1.10	0.57	-
C4	Standard Error (±)	2.09	2.06	1.08	1.09	1.28	1.08	0.79	-

Table 4.S1. Continued...

C4	**Depth (cm)**	**35.5**	**37.5**	**40.5**	**42.5**	**45.5**	**47.5**	**49.5**
C4	**Total Count**	326	637	432	322	392	263	340
C4	**Specimens per cc**	130	255	173	129	157	105	136
C4	**Biofacies**	**FB**	**FB**	**FB**	**FB**	**FB**	**FB**	**FB**
C4	*Arcella vulgaris*	8.28	18.52	3.01	2.17	8.93	6.08	10.00
C4	Standard Error (±)	2.99	3.02	1.61	1.59	2.82	2.89	3.19
C4	*Centropyxis aculeata*	25.77	37.36	17.36	18.63	31.63	28.52	21.18
C4	Standard Error (±)	4.75	3.76	3.57	4.25	4.60	5.46	4.34
C4	*Centropyxis constricta*	3.68	3.45	2.31	0.93	0.26	0.38	0.29
C4	Standard Error (±)	2.04	1.42	1.42	1.05	0.50	0.74	0.58
C4	*Difflugia globulus*	2.76	2.35	7.87	4.04	3.06	3.42	2.06
C4	Standard Error (±)	1.78	1.18	2.54	2.15	1.71	2.20	1.51
C4	*Difflugia oblonga*	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-
C4	*Lagenodifflugia vas*	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-
C4	*Ammonia parkinsoniana*	-	-	-	-	0.26	-	-
C4	Standard Error (±)	-	-	-	-	0.50	-	-
C4	*Ammonia tepida* "juvenile"	59.20	37.21	68.52	72.05	54.85	61.22	66.18
C4	Standard Error (±)	5.33	3.75	4.38	4.90	4.93	5.89	5.03
C4	*Ammonia tepida*	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-
C4	*Bolivina striatula*	-	0.16	-	-	-	-	-
C4	Standard Error (±)	-	0.31	-	-	-	-	-
C4	*Bolivina variabilis*	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-
C4	*Elphidium* sp.	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-
C4	*Fissurina compacta*	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-
C4	*Haynesina depressula*	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-
C4	*Millammina fusca*	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-
C4	*Polysaccammina iophalina*	-	-	-	-	-	-	-
C4	Standard Error (±)	-	-	-	-	-	-	-
C4	*Rosalina subaracuana*	-	-	-	0.31	-	-	-
C4	Standard Error (±)	-	-	-	0.61	-	-	-
C4	*Spirillina vivipara*	-	-	-	0.31	0.51	-	0.29
C4	Standard Error (±)	-	-	-	0.61	0.71	-	0.58
C4	*Jadammina macrescens*	0.31	0.94	0.93	1.55	0.51	0.38	-
C4	Standard Error (±)	0.60	0.75	0.90	1.35	0.71	0.74	-

Table 4.S1. Continued...

C4	**Depth (cm)**	**51.5**	**53.5**	**55.5**	**57.5**
C4	**Total Count**	218	528	959	203
C4	**Specimens per cc**	87	211	384	81
C4	**Biofacies**	**SB**	**SB**	**SB**	**SB**
C4	*Arcella vulgaris*	3.67	0.95	-	0.49
C4	Standard Error (±)	2.50	0.83	-	0.96
C4	*Centropyxis aculeata*	9.63	3.22	-	15.76
C4	Standard Error (±)	3.92	1.51	-	5.01
C4	*Centropyxis constricta*	-	-	-	-
C4	Standard Error (±)	-	-	-	-
C4	*Difflugia globulus*	-	0.19	-	2.96
C4	Standard Error (±)	-	0.37	-	2.33
C4	*Difflugia oblonga*	-	-	-	-
C4	Standard Error (±)	-	-	-	-
C4	*Lagenodifflugia vas*	-	-	-	-
C4	Standard Error (±)	-	-	-	-
C4	*Ammonia parkinsoniana*	-	0.19	-	3.45
C4	Standard Error (±)	-	0.37	-	2.51
C4	*Ammonia tepida* "juvenile"	85.78	90.72	98.64	38.92
C4	Standard Error (±)	4.64	2.47	0.73	6.71
C4	*Ammonia tepida*	-	-	-	5.42
C4	Standard Error (±)	-	-	-	3.11
C4	*Bolivina striatula*	-	2.08	0.21	0.99
C4	Standard Error (±)	-	1.22	0.29	1.36
C4	*Bolivina variabilis*	-	-	-	-
C4	Standard Error (±)	-	-	-	-
C4	*Elphidium* sp.	-	1.14	0.94	27.59
C4	Standard Error (±)	-	0.90	0.61	6.15
C4	*Fissurina compacta*	-	-	0.21	0.49
C4	Standard Error (±)	-	-	0.29	0.96
C4	*Haynesina depressula*	-	0.57	-	2.96
C4	Standard Error (±)	-	0.64	-	2.33
C4	*Millammina fusca*	-	-	-	-
C4	Standard Error (±)	-	-	-	-
C4	*Polysaccammina iophalina*	-	-	-	-
C4	Standard Error (±)	-	-	-	-
C4	*Rosalina subaracuana*	-	0.38	-	0.99
C4	Standard Error (±)	-	0.52	-	1.36
C4	*Spirillina vivipara*	0.92	-	-	-
C4	Standard Error (±)	1.27	-	-	-
C4	*Jadammina macrescens*	-	0.57	-	-
C4	Standard Error (±)	-	0.64	-	-

TABLE 4.S2. Geochemical data for cave cores C2, C3, and C4.

	Depth (cm)	$\delta^{13}C_{org}$	TOC (%)	$\delta^{15}N_{org}$	TN (%)	C/N
C2	0.5	-29.3	45.7	1.0	2.4	19.2
C2	1.5	-29.7	45.3	2.2	2.5	17.9
C2	2.5	-28.9	45.6	0.3	2.2	21.2
C2	3.5	-28.7	47.2	1.5	2.2	21.8
C2	4.5	-28.7	50.1	2.0	2.3	22.0
C2	5.5	-28.6	48.8	1.7	2.1	23.1
C2	6.5	-28.3	48.0	0.7	2.0	23.5
C2	7.5	-28.3	47.8	1.6	2.2	21.8
C2	8.5	-28.5	49.4	1.6	2.1	23.5
C2	9.5	-29.0	50.2	2.0	2.5	19.7
C2	10.5	-29.0	49.4	2.1	2.6	18.7
C2	11.5	-28.8	48.6	1.0	2.5	19.2
C2	12.5	-29.2	48.5	0.9	2.6	18.8
C2	13.5	-29.2	50.4	1.6	2.5	20.5
C2	14.5	-29.8	47.6	1.5	2.7	17.6
C2	15.5	-29.2	47.9	1.5	3.1	15.3
C2	16.5	-29.4	49.0	2.4	3.0	16.1
C2	17.5	-28.6	49.1	1.2	2.6	18.9
C2	18.5	-29.0	48.0	1.6	2.5	19.0
C2	19.5	-29.5	47.4	1.4	2.8	17.2
C2	20.5	-29.0	47.8	1.7	2.2	21.7
C2	21.5	-28.8	48.8	2.3	1.7	28.0
C2	22.5	-28.8	50.8	2.3	1.8	28.8
C2	23.5	-28.9	43.1	1.6	1.9	23.1
C2	24.5	-28.3	47.2	0.7	1.9	24.5
C2	25.5	-28.9	50.1	1.9	1.9	26.9
C2	26.5	-29.4	45.5	2.4	1.9	23.6
C2	27.5	-29.5	48.5	2.8	2.0	23.9
C2	28.5	-29.9	50.7	1.8	2.0	25.4
C2	29.5	-29.4	51.4	1.1	1.8	27.8
C2	30.5	-29.8	51.5	2.2	1.9	26.6
C2	31.5	-29.7	51.1	2.9	2.0	25.3
C2	32.5	-29.5	49.9	2.4	1.9	26.0
C2	33.5	-29.7	48.1	1.9	1.8	26.4
C2	34.5	-29.9	50.4	2.9	2.0	25.1
C2	35.5	-29.4	50.6	3.0	1.9	27.3
C2	36.5	-29.5	48.3	3.2	1.8	26.3
C2	37.5	-29.3	49.1	1.8	1.7	28.3
C2	38.5	-29.6	50.4	2.0	1.9	26.8

Table 4.S2. Continued…

	Depth (cm)	$\delta^{13}C_{org}$	TOC (%)	$\delta^{15}N_{org}$	TN (%)	C/N
C2	39.5	-30.3	50.2	3.4	2.3	21.9
C2	40.5	-30.3	49.6	2.3	2.1	23.4
C2	41.5	-29.9	48.8	2.6	2.1	23.8
C2	42.5	-29.8	46.0	2.6	2.0	23.3
C2	43.5	-29.9	46.9	1.9	2.0	22.9
C2	44.5	-30.5	49.5	2.6	2.2	22.4
C2	45.5	-29.3	49.3	1.4	1.8	27.1
C2	46.5	-29.7	50.5	2.2	2.0	24.9
C2	47.5	-28.9	49.8	2.0	1.8	27.4
C2	48.5	-28.9	50.3	2.3	1.8	28.7
C2	49.5	-29.1	50.9	3.4	1.8	27.9
C3	0.5	-31.0	44.9	3.1	3.2	14.0
C3	1.5	-31.2	44.0	3.1	3.4	13.0
C3	2.5	-31.3	45.0	3.2	3.4	13.4
C3	3.5	-31.5	45.1	3.6	3.3	13.5
C3	4.5	-31.3	45.1	2.9	3.4	13.5
C3	5.5	-31.7	44.4	3.2	3.3	13.6
C3	6.5	-31.4	46.1	2.8	3.3	14.1
C3	7.5	-31.1	46.5	2.9	3.1	15.0
C3	8.5	-31.2	46.8	3.1	3.3	14.1
C3	9.5	-30.8	46.4	2.5	3.1	14.8
C3	10.5	-30.9	41.6	2.7	2.8	14.7
C3	11.5	-31.0	43.4	2.4	3.1	14.0
C3	12.5	-30.8	44.9	2.7	3.1	14.6
C3	13.5	-30.7	44.0	2.9	2.9	15.2
C3	14.5	-30.9	45.2	2.7	3.0	15.0
C3	15.5	-30.8	46.3	1.9	3.0	15.2
C3	16.5	-30.7	46.3	2.9	3.2	14.6
C3	17.5	-30.8	41.9	2.8	2.9	14.3
C3	18.5	-30.8	46.8	2.5	3.4	14.0
C3	19.5	-31.8	36.5	1.7	2.7	13.4
C3	20.5	-32.0	42.4	1.6	3.3	12.9
C3	21.5	-31.9	33.1	1.2	2.4	13.9
C3	22.5	-32.7	30.5	2.1	2.3	13.1
C3	23.5	-32.7	38.9	3.0	3.1	12.7
C3	24.5	-32.8	36.0	2.3	2.5	14.7
C3	25.5	-32.4	38.1	2.3	2.7	14.4
C3	26.5	-32.4	39.2	1.9	2.5	15.4
C3	27.5	-32.3	40.5	2.9	2.4	17.1

Table 4.S2. Continued…

	Depth (cm)	$\delta^{13}C_{org}$	TOC (%)	$\delta_{15}N_{org}$	TN (%)	C/N
C3	28.5	-32.3	44.5	2.8	2.5	17.6
C3	29.5	-32.1	42.8	3.3	2.4	17.8
C3	30.5	-31.6	46.0	3.0	2.4	18.8
C3	31.5	-31.5	43.6	3.2	2.3	19.2
C3	32.5	-30.9	46.8	3.0	2.4	19.3
C3	33.5	-30.5	46.7	2.5	2.2	21.0
C3	34.5	-30.7	47.5	2.1	2.3	20.3
C3	35.5	-30.9	46.6	3.2	2.4	19.1
C3	36.5	-30.7	48.3	2.3	2.4	20.5
C3	37.5	-30.7	48.8	2.7	2.4	20.1
C3	38.5	-31.1	43.9	3.0	2.2	19.9
C3	39.5	-30.4	43.8	2.5	2.3	19.1
C3	41.5	-30.5	45.8	2.5	2.3	20.1
C3	42.5	-30.3	49.4	2.5	2.3	21.3
C3	43.5	-30.9	47.8	2.7	2.4	19.7
C3	44.5	-30.8	48.1	2.6	2.5	19.6
C3	45.5	-32.0	42.5	1.7	3.3	12.8
C3	46.5	-30.4	45.9	2.6	2.2	20.5
C3	47.5	-30.3	48.1	2.8	2.4	20.2
C3	48.5	-30.3	44.9	2.9	2.1	20.9
C3	49.5	-30.3	43.7	2.4	2.2	19.7
C4	0.5	-30.5	45.8	3.3	2.9	15.5
C4	1.5	-31.0	43.8	2.9	3.1	14.3
C4	2.5	-32.1	41.5	2.8	3.2	12.9
C4	3.5	-32.3	45.2	1.8	3.6	12.4
C4	5.5	-32.5	39.9	2.8	3.1	12.9
C4	6.5	-32.5	39.1	3.1	2.7	14.5
C4	7.5	-33.2	46.0	2.4	2.6	17.4
C4	8.5	-33.8	43.8	2.5	2.5	17.5
C4	9.5	-33.5	46.6	3.1	2.5	18.6
C4	10.5	-33.2	46.3	3.8	2.5	18.7
C4	11.5	-32.7	47.2	3.9	2.3	20.3
C4	12.5	-33.8	41.1	3.3	2.3	18.1
C4	13.5	-33.7	45.8	4.4	2.4	19.1
C4	14.5	-33.3	45.6	4.1	2.4	19.2
C4	16.5	-32.9	45.1	4.3	2.3	19.4
C4	17.5	-32.3	46.5	3.8	2.3	20.0
C4	18.5	-32.3	46.2	3.9	2.3	19.7

Table 4.S2. Continued...

	Depth (cm)	$\delta^{13}C_{org}$	TOC (%)	$\delta^{15}N_{org}$	TN (%)	C/N
C4	19.5	-32.4	45.5	3.3	2.3	19.9
C4	20.5	-31.6	43.8	4.4	2.3	19.3
C4	21.5	-32.1	46.3	3.8	2.3	20.3
C4	22.5	-31.9	45.5	4.3	2.3	19.5
C4	23.5	-32.4	47.1	4.0	2.3	20.2
C4	24.5	-32.6	46.6	4.5	2.4	19.2
C4	25.5	-32.2	46.4	4.2	2.3	20.6
C4	26.5	-32.2	45.5	4.1	2.4	18.8
C4	27.5	-31.9	46.5	4.8	2.5	18.7
C4	28.5	-32.1	47.0	4.6	2.3	20.6
C4	29.5	-31.7	44.9	4.2	2.4	19.0
C4	31.5	-31.9	43.2	3.8	2.5	17.6
C4	32.5	-32.2	45.3	4.2	2.5	17.8
C4	34.5	-31.9	44.6	4.3	2.4	19.0
C4	35.5	-32.0	44.7	4.3	2.4	18.9
C4	36.5	-32.0	44.8	4.5	2.4	18.9
C4	37.5	-32.2	42.9	4.4	2.5	17.4
C4	38.5	-32.0	43.9	3.6	2.4	18.5
C4	39.5	-32.2	46.0	4.5	2.8	16.4
C4	40.5	-31.1	46.7	4.2	2.3	20.4
C4	41.5	-31.1	46.4	4.3	2.3	19.8
C4	42.5	-30.5	48.0	4.5	2.6	18.5
C4	43.5	-30.6	46.1	3.6	2.2	20.6
C4	44.5	-30.8	47.3	3.4	2.3	20.6
C4	45.5	-30.6	46.8	3.7	2.3	20.5
C4	46.5	-30.4	45.4	4.2	2.3	19.5
C4	48.5	-29.3	45.1	3.4	2.2	20.1
C4	49.5	-29.8	42.7	3.9	2.2	19.4
C4	51.5	-31.5	41.7	4.5	2.1	20.0
C4	52.5	-31.9	36.4	4.4	2.1	17.7
C4	53.5	-31.6	35.7	4.4	2.0	17.6
C4	54.5	-31.5	31.6	5.2	1.8	17.5
C4	55.5	-31.1	17.8	5.9	1.2	15.4
C4	56.5	-30.7	14.6	5.8	1.0	14.7
C4	57.5	-31.1	11.1	5.6	0.8	14.3
C4	58.5	-32.2	41.5	3.9	2.3	18.4
C4	59.5	-32.2	43.5	3.7	2.4	18.3

CHAPTER 5

CONCLUSIONS

To answer the original premise that stimulated this research, thecamoebians and foraminifera can unequivocally colonize Yucatan anchialine cenotes and phreatic caves, and they can be used as paleoenvironmental proxies in these environments. Additionally, foraminifera and thecamoebians are responding to their local physico-chemial parameters, similarly to other coastal settings. No new stygobites were found in this study, readily allowing these taxa to be used for paleoenvironmental interpretations.

5.1. ADDRESSING THE CENTRAL RESEARCH QUESTIONS

The first question of this thesis was: "*What are the distributions and environmental controls on thecamoebians and foraminifera in the open water cenote environment?*"

Investigating distributions in a sinkhole environment represented an important starting point for this research—if taxa were to be found in the adjacent cave environment, then they would be easily found in the cenotes. To investigate specific faunal trends with increasing salinity, three cenotes were chosen in a landward transect to the coast. Of the hydrologic variables measured (pH, dissolved oxygen, temperature, salinity), salinity was determined to be the dominant factor influencing distributions based on the ecological boundaries of the taxa. Previous research in the salt marsh has determined thecamoebians living to a maximum of ~3.0 ppt salinity. However, the transition of thecamoebian-dominated to foraminiferan-dominated assemblages with increasing salinity has proven challenging to elucidate.

In the cenote investigation, typical low salinity taxa of foraminifera and thecamoebians were found living in the cenotes. Since the cenotes remain perennially aquatic, faunal changes can be attributed dominantly to water conditions, as opposed to percent moisture, rapid diurnal temperature fluctuations, or pH shifts such as in the salt marsh environment. An transition at ~3.5 ppt was determined as the maximum salinity tolerance of thecamoebians, with small, autogenous *Centropyxis constricta* "aerophila" determined as the most euryhaline of the thecamoebians. The faunal transitions recovered from the cenote are an important new contribution to micropaleontology, and foreshadow the future application of transfer functions to paleosalinity.

Although insightful, there are limitations on this analysis, which must be considered for future work. This study used only the thanatocoenosis recovered in the sediments, which is inevitably influenced through taphonomic processes. Although some authors argue that this assemblage is sufficient for determining longer-term 'average' conditions at the sample site, the reality is that true ecological information can only be obtained by using living taxa (the biocoenosis). This study also only investigated only thirty-three samples from three different sites. Any future study planning on refining the salinity relationships presented in chapter 2 should consider: (1) obtaining a larger database (>50 samples) from at least 5 sites with even subtler salinity gradations, and (2) use an accurate vital stain (such as 5-chloromethylfluorescein diacetate coupled with epiflourescence microscopy) to examine only the biocenosis.

The second question of this thesis was: *"Are the thecamoebians and foraminifera in an anchialine cave the expected fauna based on modern environmental conditions and the known ecology of the taxa?"*

The results from Carwash Cave indicate that foraminifera and thecamoebians do inhabit the cave environment, both presently and in the geologic past. The cave itself predates the late Pleistocene, and therefore the cave has experienced late Quaternary sea level changes. Due to differences in modern sedimentation rates throughout the cave, an ancient Marine Biofacies, dominated by bathyl foraminifera, was found in cave surface sediment samples. Since several samples were found sealed beneath vadose flowstone, the minimum age from the Marine Biofacies is the Sangamon Interglacial (MIS 5e). Interestingly, there were a few additional thecamoebian taxa living in the cave than in the cenote. This is intriguing as light is nearly absent in the cave, and suggests a limited influence of light on thecamoebian populations. Otherwise, thecamoebian faunal gradations generally followed expected trends based on research completed in lacustrine environments. The results indicate that foraminifera and testate amoebae adapt to the changing cave environment, which is controlled to larger boundary conditions such as sea level change.

The results of this chapter pose several additional research questions. Since foraminifera and thecamoebians live in the cave, further work needs to be dedicated to understanding the ecological contributions these taxa have on stygobitic ecosystems. Since Carwash Cave has limited cave passages that transgressing through the halocline, a cave sub-environment containing the halocline intersecting the cave floor warrants further examination for the presence of foraminifera. Future work should focus on other cave locales to: (1) further find evidence of the ancient Marine Biofacies with possible U-Th dating on calcite to better constrain the age, and (2) demonstrate wider foraminiferal occupation of salt water passages in the phreatic caves in Mexico.

The final question of this thesis was: *"Can down core assemblages of foraminifera and thecamoebians be used to reconstruct environmental evolution in the phreatic cave?"*

The small coring survey that was completed in Carwash Cave recovered thecamoebian and foraminifera faunal through the mid-late Holocene. Since the results of Chapters 1 and 2 established that thecamoebians and foraminifera are following ecological boundaries in the cave as in other coastal environments, their application as paleoenvironmental proxies in caves was hypothetically possible. Using the microfauna and stable isotopes to complete the paleoenvironmental reconstruction, there were several key conclusions from this approach. First, the sedimentary archive in cave sediments documents changes in the local aquifer and terrestrial environment. Both foraminifera and testate amoebae are responding to hydrological changes in the phreatic cave and warrant wider used as a paleohydrological proxy. Second, at 2760 Cal yrs BP, the aquifer became fresher and primary productivity increased in the cenote. This transition demarcated the onset of modern environmental conditions in the phreatic cave. Third, desalinisation of the aquifer coincided with Maya population expansion, maximum land disturbance at the ancient city of Cobá, and a transition of settlement behaviour from settling around surface rivers/lakes to settling at arbitrary positions in the jungle. These should give promise to similar reconstructions in phreatic caves at other global locales.

When conducting future studies, several factors should be considered. At Carwash, the sedimentary record at individual core locations was incomplete, but collectively, documented the environmental history in the cave. Clearly, selecting good coring locations is key for a complete reconstruction. Temporal resolution in this study was a strong limitation, as the arbitrary 1 cm sample resolution was temporally coarser than expected. A future project should consider a sub-centimetre sampling resolution, especially if crucial Mexican archaeological intervals are a research target. Although many studies have examined speleothem-trapped archives from phreatic caves, extracting sedimentary cores and completing typical sediment-based environmental reconstructions is a novel approach. It is anticipated that much can be learned about phreatic cave environments through this technique.

5.2. CONCLUDING REMARKS

Although this entire thesis has focused on Mexican localities, I leave the reader with knowledge that Mexico is not unique. Test surface samples from Oman, Florida, and the previously established Bermuda sites, all contain unique foraminiferal assemblages—further indicating the global potential of this research. There is still much to learn regarding foraminiferal and thecamoebian ecology and paleoecology, and the phreatic cave is clearly emerging as a new environment in which to study these admirable organisms.

APPENDIX 1

TAXONOMIC NOTES

The purpose of this appendix is to give some additional detail to some of the taxonomic subtleties that have arisen through this research. This section is not intended as a taxonomic review, or as a complete systematic paleontology section. Instead, this section provides additional details to some of the more complicated fauna within the text. Only problematic, tentative, or interesting designations/fauna appear here for additional information specific to this study. The comments here do not change the interpretation of the fauna in the chapters where they appear, but it is hoped further research will better resolve some of the issues here.

Ammonia tepida (Cushman, 1926)
Ammonia tepida var. Juvenile (This work, Gabriel et al. 2008)

Remarks: These taxa appear in Chapter 4 and the paper by Gabriel et al. (2008) focusing on a reconstruction of Carwash Cenote. Interestingly, it dominantly appears in the Carwash Cave System (cavern surface samples and in the cenote core at ~4-7 cm), but only rarely elsewhere (Maya Blue cenote and cavern, Yax Chen). Based on significant pore distribution on the test, this taxon is more likely to be a foraminiferan, as opposed to a testate amoebae, of which only 1 calcareous taxon has only ever been found (*Mississippiella mulitapertura*, only known from in the Mississippi Delta). Stained specimens were found in both Maya Blue and Carwash, so effort should be dedicated to collecting live specimens for: (1) examination of the pseudopods for taxonomic clarification, and (2) culture to ascertain if this is indeed a juvenile *Ammonia*, or in fact a new species of small foraminifera.

Centropyxis constricta (Ehrenberg, 1843)
Centropyxis constricta "spinosa" (This work, Chapter 2)

Remarks: An important contribution of Chapter 2 was the separation of Centropyxid strains based **only** on the presence or absence of spines. Centropyxis constricta was divided by Reinhardt et al. (1998) into strains based on the absolute number of spines, which has been followed by any users of the Dalby and Kumar (1998) arcellacean taxonomic key. However, the <1 vs. 1-3 vs. >3 is an arbitrary designation, and it was deemed unlikely that the environment would control the arbitrary secretion of 3 versus 4 spines. Therefore, we **redefined** the *C. constricta* "spinosa" strain to include any *C. constricta* that had spines on the aboral region.

Difflugia oblonga (Ehrenberg, 1832)

Remarks: This species was not subdivided further in Chapter 4. If it is relevant to a future reader, all the *D. oblonga* specimens observed were of the strain "tenuis", as defined in Kumar and Dalby (1998). The isolation of this strain is an interesting point – as this genus is so morphologically diverse. This observation was not further explored in this study.

Difflugia globulus (Ehrenberg, 1848)

Remarks: As the reader is aware of, a major characteristic to diagnose and separate thecamoebian species is based on the structure or pseudopods of the protist. The obvious problem that occurs when working with dead assemblages is the lack of soft tissues, which creates a bit of trouble when working with *D. globulus*. The shell morphology of *D. globulus* and *Cyclopyxis eurystoma* Deflandre 1929 (synonymous with *C. arcelloides* Penard 1902) is identical: A radially-symmetrical, slightly-flattened sphere with a central aperature (almost spherical). All of the specimens found were 45-60 mm in size. Combining all fossil shells with this type of morphology into a *D. globulus* (generally among lacustrine workers) or *C. arcelloides* (generally among peatland researchers) designation is common practise. Again, taking care to examine the psuedopods in more living specimens from the cavern in Carwash Cave would resolve this issue in this study.

A final notable point is that *D. globulus* has a confusing taxonomic history due to writers misspelling the name (e.g., Difflugia *globulosa* Dujardin 1837, *D. globularis* Wallich 1864, and *D. globula*, all of which are now recognized as synonymous among testate amoebae workers (see Charman et al., 2000 – p. 67 and/or Medioli and Scott, 1983 – p. 24).

Tritaxis fusca (Williamson, 1858)

Remarks: This designation is only tentative, as we failed to find a perfect match. After reading Brönnimann and Whittaker (1988), several additional features of the apertures need to be established to better place it within a textularid genus displaying high-conical test morphologies.

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