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MICROFOSSIL SAMPLE PREPARATION
AND TECHNIQUES

By

Ronald W. Hoenstine P.G. #57

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Florida Microfossil Sample Preparation Techniques

Ronald W. Hoenstine PG#57

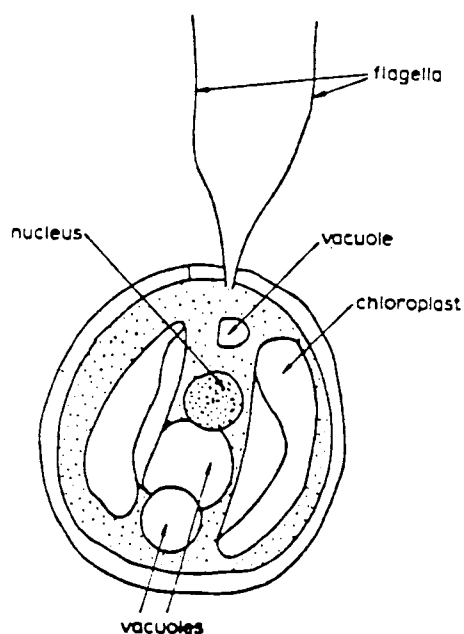
INTRODUCTION

A diversity of microscopic fossils, including calcareous nannoplankton (coccoliths), diatoms, dinoflagellates, foraminifera, micro-mollusks, ostracoda, pollen, silicoflagellates, radiolarians, dinoflagellates, as well as micro-vertebrates, broken macrofossil fragments, sponge spicules, and echinoid parts are present in Florida's subsurface sediments and rocks. Because of their abundance, small size and widespread geographic distribution, many of these microfossils represent a potentially significant tool in the study of Florida's past. Those of primary value to Florida studies in establishing regional stratigraphic correlations, age dating and comparisons, and reconstructing ancient depositional environments and ecosystems include coccoliths, diatoms, foraminifera, and ostracoda. The following information gives basic procedures and techniques for treating Florida's siliceous and calcareous sediments to facilitate the separation of these specific microfossil groups for investigation. The coccolith and foraminifera procedures can also be applied to discoaster and ostracods, respectively.

COCCOLITHS

Coccoliths (calcareous nannoplankton [nano (Greek) = dwarf] are small, unicellular, planktonic, marine, calcareous algae found throughout the photic zone (upper 0-450 feet) of the world's oceans. These calcareous algae, which have been part of the fossil record for the last 200 million years, are a common constituent of

many of Florida's carbonate sediments (Figure 1). Because of their susceptibility to dissolution and post-depositional disintegration, their use in the study of Florida's biostratigraphy has been limited. However, when present and identifiable to species level, they represent a valuable tool for correlating and determining the ages of Florida's stratigraphic sedimentary deposits.

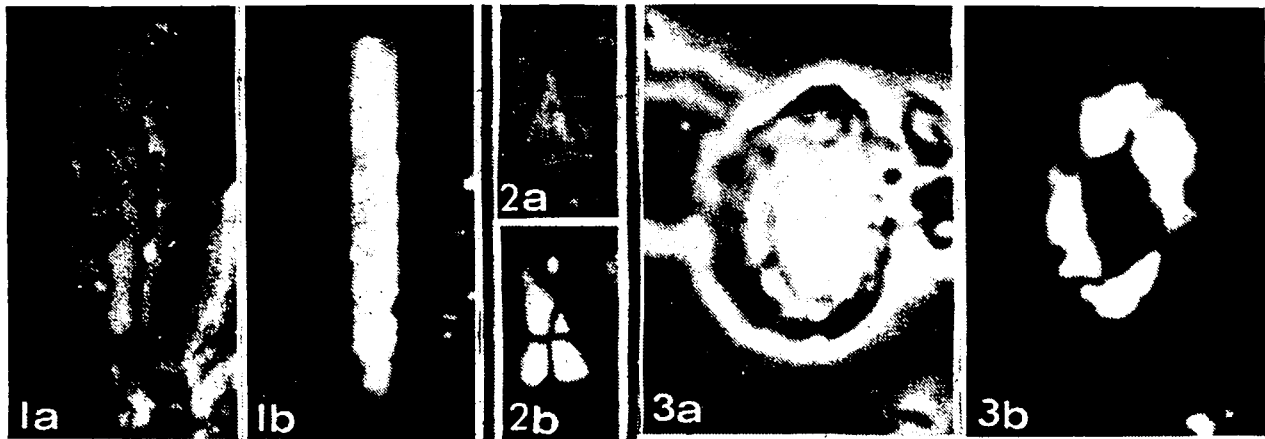


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Figure 1. Sketch of a living cell of *Cyclococcolithus leptoporus* (Murray and Blackman) Kamptner, showing flagellar apparatus (haptonema), vacuoles, chloroplasts and nucleus. X 2,000. (After Schiller, 1930).

Considered part of the plant kingdom, coccoliths are assigned to the Division Chrysophyta, Class Coccolithophyceae, Order Heliolithae, and Family Coccolithaceae. The living coccolith cell secretes a skeleton of numerous, small, calcareous, elliptical to circular shaped shields which, upon death, comes to rest on the ocean bottom. Subsequent post-depositional processes cause disarticulation and separation of these shields. The shields are commonly incorporated in the bottom sediments, becoming part of the fossil record. Fossil coccolith taxonomy is based primarily on the morphology of these shields. The shields, which range in size from 1 to 15 nanometers (nm), are extremely susceptible to dissolution in low pH water and mechanical abrasion and are often difficult to identify.

Coccoliths are commonly viewed with a transmitted light microscope, having both crossed nichols and a phase contrast condenser and objectives (Figure 2). A rotating mechanical stage is also required. Some species show distinct interference patterns in polarized light. Others are best seen in phase contrast. Many species may be identified at a magnification of 400x, but the smaller forms typically require at least 1000x for identification. Samples are often viewed on standard 25 x 75 mm glass slides with cover slips. For more detailed specimen analyses requiring greater magnification, a scanning electron or transmission electron microscope is required. These microscopes show ultrastructure details visible only at greatly increased magnifications.



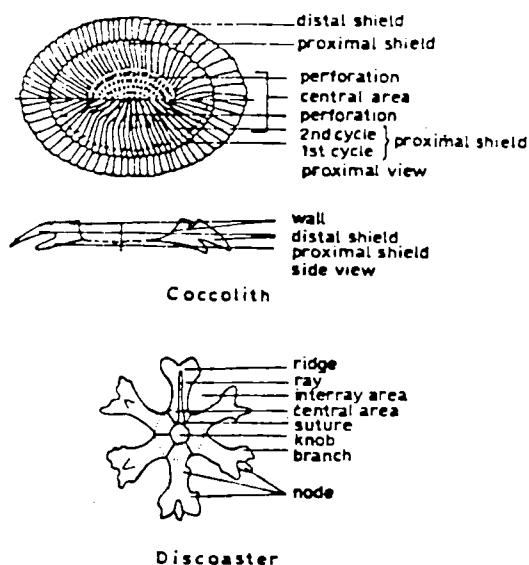
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Figure 2. Coccoliths (figures a under phase-contrast and b under cross nichols)
 1. *Triquetrorhabdulus carinatus* Martini;
 2. *Sphenolithus belemnos* Bramlette and Wilcoxon;
 3. *Helicophaera ampliaperta* Bramlette and Wilcoxon. (photos
 from Haq, 1978).

DISCOASTERS

A group of organisms related to the coccoliths are the now extinct discoasters, which belong to the Family Discoasteraceae. This group, which evolved during the Tertiary (the last 65 million years), may have more importance as a biostratigraphic tool due in part to their diversity, rapid evolution, distinctive morphology and abundance in low-latitude sediments. Discoasters are relatively large when compared to coccoliths and easy to identify. Age range charts of key species have been developed which provide excellent biostratigraphic resolution of the Tertiary species. Bolli et al.(1985) is an excellent reference source and aid which provides a very detailed and comprehensive discussion of coccoliths

and discoasters. Figure 3 is an illustration of coccolith and discoaster terminology.



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Figure 3. Coccolith and discoaster terminology.
(after Farinacci, 1971.)

Sample Preparation

The following procedure is a typical sample preparation of Florida sediments for coccoliths/discoaster separation generally following the procedures of Dr. Sherwood Wise, Florida State University Geology Department.

1. Clean a 100 ml beaker and treat with a 10% solution of HCl acid to remove any carbonate residue; then rinse thoroughly with distilled water to remove the acid.
2. Pour 50 to 75 ml of distilled water into beaker and to this add approximately 1 to 2 cc of sample material.
- 3a. Stir and mechanically disaggregate the sample with a spatula or glass rod. If aggregation occurs, the sample can be boiled to disaggregate clays.

- 3b. An alternate method to disaggregate a hard sample is to place the beaker with sample in an ultrasonic bath for 20 to 30 seconds. A note of caution is in order for this method as coccolith specimens can experience mechanical breakage at high ultrasound settings or prolonged baths.
4. After step 3a or 3b, let sample settle for 30 minutes; pour off liquid and suspended clays. Save residue.
5. Dilute residue with distilled water and store in properly labeled glass vial for subsequent slide preparation.

Glass Slide Preparation

1. Heat hot plate to approximately 45°C.
2. Shake capped glass vial containing previously prepared sample, see sample preparation.
3. Let contents settle for approximately 20 seconds; then, using a small disposable straw or pipette, carefully extract a sample from the middle of the vial.
4. Place several drops on a glass coverslip and dry on hot plate at a temperature of 45°C. Make 2 slides: 1) a thick slide for species counts and 2) a thin slide for specimen photographs.
5. After drying, place 2-3 drops of "Norland Optical Adhesive 61" (available from: Norland Products, Inc., New Brunswick, NJ, 08902), a mounting medium for calcareous microfossils, on the cover slip; place a glass slide over the cover glass, invert and place under a long-wave (350-380 nm) ultraviolet light for at least 1 minute or long enough for the adhesive to harden.

6. Properly label slide and examine under microscope.

Smear Slide Preparation

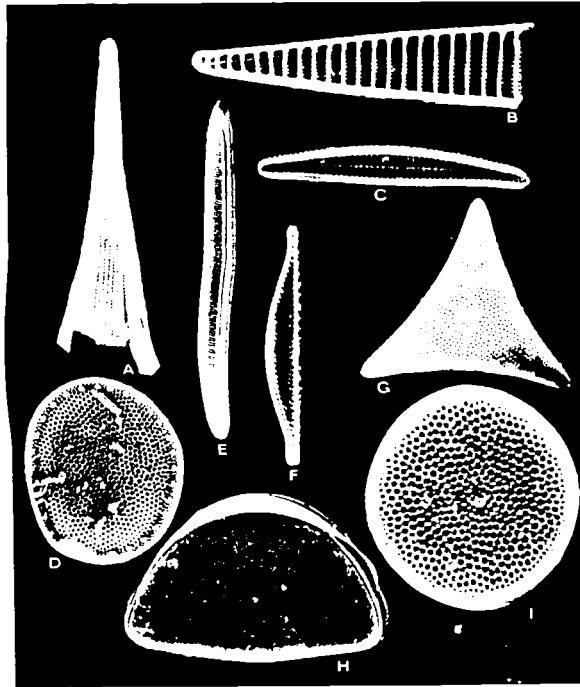
This procedure offers "a quick and dirty" method for determining the presence of coccoliths and/or presence of specific species for age dating (modified from Dr. Sherwood W. Wise, Florida State University Geology Department).

1. Using a wet (distilled water) toothpick to prevent sample from sticking, scrape a small quantity from sample.
2. Smear this material on a slide cover slip.
3. Add 2 drops of water and using toothpick mix water and sample evenly over cover slip.
4. Place cover slip on a hotplate and dry at a temperature of 45°C.
5. After drying, place 2-3 drops of the mounting medium for calcareous microfossils, on the cover slip; place a glass slide over the cover slip, invert and place under a long-wave ultraviolet light for at least 1 minute, or long enough for adhesive to harden.
6. Remove slide, properly label and examine under microscope.

DIATOMS

Diatoms are single-celled algae constructed of distinctive, two-part siliceous tests. Belonging to the Class Bacillariophyceae of the Division Chrysophycophyta, they are present in many aquatic and subaquatic environments. Diverse in make-up, they may be planktonic (free floating) or sessile (attached) and include freshwater, brackish, near shore, and open ocean species.

Diatoms can generally be categorized on the basis of symmetry as to being either centric or pennate types (Figure 4). Centric forms are commonly abundant in open marine waters in contrast to pennates which are more common in fresh and brackish water environments.



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Figure 4. Representative genera demonstrating basic patterns in centric and pennate diatoms. A. *Rhizosolenia* sp., pennate form. B. *Nitzschia* sp., pennate form. C. *Pseudoeunotia* sp., pennate form. D. *Roperia* sp., centric form. E. *Nitzschia* sp., pennate form. F. *Nitzschia* sp., pennate form. G. *Triceratium* sp., centric form. H. *Hemidiscus* sp., centric form. I. *Coscinodiscus* sp., centric form. (From Burckle, 1978).

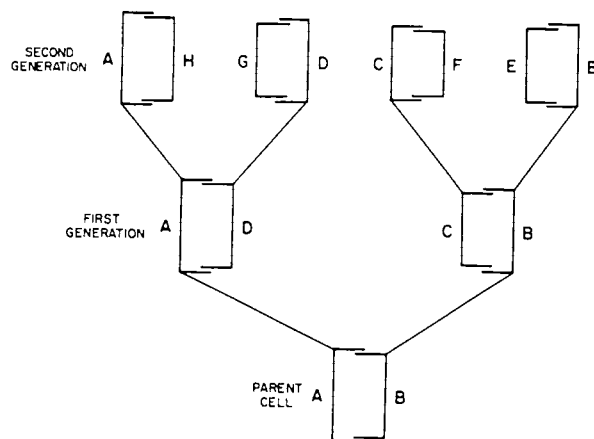
Diatoms have been found to be excellent biostratigraphic and paleoecologic indicators. They are of primary importance in high latitudes where calcareous microfossils are sparse or entirely absent. Much of the modern biostratigraphic information is tied to siliceous diatom species whose stratigraphic value has been recognized and developed only in recent decades. Additionally, information on water chemistry, paleosalinity, paleodepth, paleotempe-

perature, and paleonutrient concentrations can be deduced from the diatom species present in the sediments (Patrick and Reimer, 1966; Burckle, 1978).

Diatoms are a highly definitive tool that can be used in the correlation and environmental analysis of Florida's siliciclastic sediments. Advances in worldwide diatom biostratigraphic correlation, especially the sequence of events in the sediments of the Atlantic Coast from the Miocene (Abbott, 1978; Andrews, 1979) have made possible comparative biostratigraphic studies of siliciclastic sediments of the Hawthorn Group. These studies include a biostratigraphic investigation of the Hawthorn Group sediments along Florida's east coast (Hoenstine, 1984). Such studies, which have found close relationships between oceanic diatom assemblages and similar age sediments from coastal regions, have enhanced the value of coastal zonations in biostratigraphic investigations.

Diatoms secrete an external test (frustule) composed of opaline silica (Figure 5). The frustule consists of two halves (valves) that overlap and are connected by a circular band called a girdle. Frustules are variable in size, ranging from approximately 15 μm for smaller diatoms for such genera as Rouxia to as much as 120 μm for larger diatoms of the genus Coscinodiscus. Fossil diatom taxonomy is primarily based upon valve shape and features. For this reason, only diatom specimens showing valve side up (valve view) can be used for identification. Like coccoliths, diatoms are viewed with a transmitted light microscope in plain light. Mounts are made on standard 25 x 75 mm glass slides with cover slips.

Scanning electron and transmission electron microscopes are used for more detailed specimen analyses requiring greater magnifications. The following sample preparation technique generally follows the procedures of Schrader (1974) and Abbott (1978).



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Figure 5. Girdle views of diatom valves (frustules) through several reproductive phases (from Burkle, 1978).

Sample Preparation

1. Place approximately 2 cc of sample material in a cleaned 400 ml beaker.
2. Add approximately 50 to 75 ml of distilled water and mechanically disaggregate the sample with a spatula or glass rod.
3. Add an equal quantity of 30% hydrogen peroxide to remove organic components. (Caution - hydrogen peroxide is a strong oxidizing agent which readily attacks exposed skin. Rubber gloves are recommended).

4. Add 25 to 35 ml of a 10% solution of HCl acid if sample contains carbonate material to further concentrate the silica.
5. Heat sample for approximately 40 minutes at 100°C.
6. Remove beaker from heat, shake and pour supernatant liquid into 50 ml test tubes.
7. Centrifuge for 2 minutes at a speed of 1200 revolutions per minute. Pour off suspended clay minerals and resuspend residue with distilled water. Repeat this procedure seven times (**Caution** - this step is optional and serves to concentrate diatoms, however, breakage can occur.)
8. If clay fraction is flocculated, add 0.5 percent sodium hexametaphosphate to disaggregate, then centrifuge 4 more times following the procedures of step 7.
9. Dilute the resulting residue with distilled water and store in glass vial for later slide preparation.

Slide Preparation

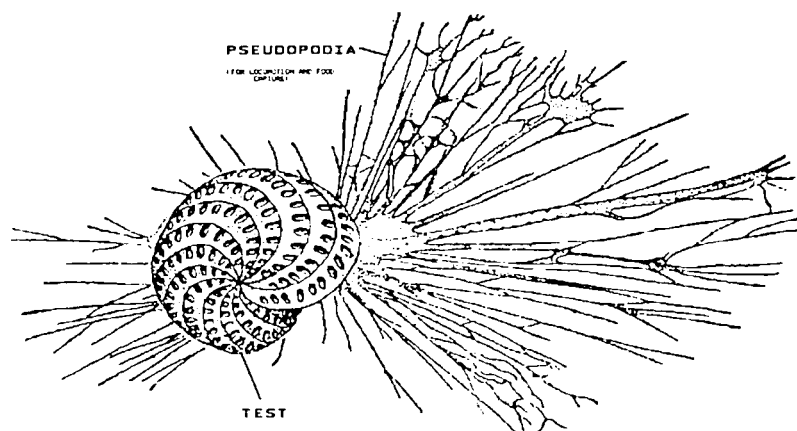
1. Heat hot plate to approximately 45°C.
2. Shake capped glass vial containing diatom suspension in order to mix sample.
3. Let contents settle for approximately 20 seconds, then, using a small disposable straw or pipette, carefully extract a sample out of the middle of the bottle.
4. Place one or two drops of this sample on a cover glass and dry on hot plate. (**Caution** - if suspended material is too dense, dilute with distilled water; a toothpick can be used to evenly distribute sample).

5. After drying, place one drop of Hyrax (available from Custom Research and Development, 8500 Mt. Vernon Road, Auburn, CA 95603), a commercial mounting medium for siliceous microfossils, on the coverglass; place a glass slide over the coverglass, invert and heat the slide for approximately 5 minutes at 150° to 175°C to set the Hyrax.
6. Frequently air bubbles form under the cover glass during the heating process. Applying light pressure with a toothpick can remove the majority of these air bubbles by causing them to migrate to the edge of the cover glass where they dissipate.
7. After bubbling stops, remove the slide and place it on a cool (room temperature) surface. Remaining air bubbles will contract.
8. Properly label slide and examine under microscope.

FORAMINIFERA

Foraminifera are single celled organisms belonging to the Phylum Protozoa, Class Rhizopodea and Order Foraminiferida. Inhabiting the ocean bottom as well as floating in the water column, foraminifera are one of the most studied calcareous microfossils (Figure 6). Ranging in size from approximately 0.1 to 25 mm, this group, generally viewed with a reflected light stereomicroscope with a magnification range of 10X to at least 40X, has proven to be one of the primary means for determining depositional dates and paleoenvironments of ancient carbonate sediments. Biostratigraphy using foraminifera was initially developed based on the stratigraphic ranges of benthic

foraminifera. This created problems because benthic foraminifera are associated with sediments that accumulate in an environment that shifts geographically with time resulting in an age variation at different localities. As a consequence, these time-transgressive problems associated with benthics resulted in the development of low-latitude zonation schemes based on planktic foraminifera.

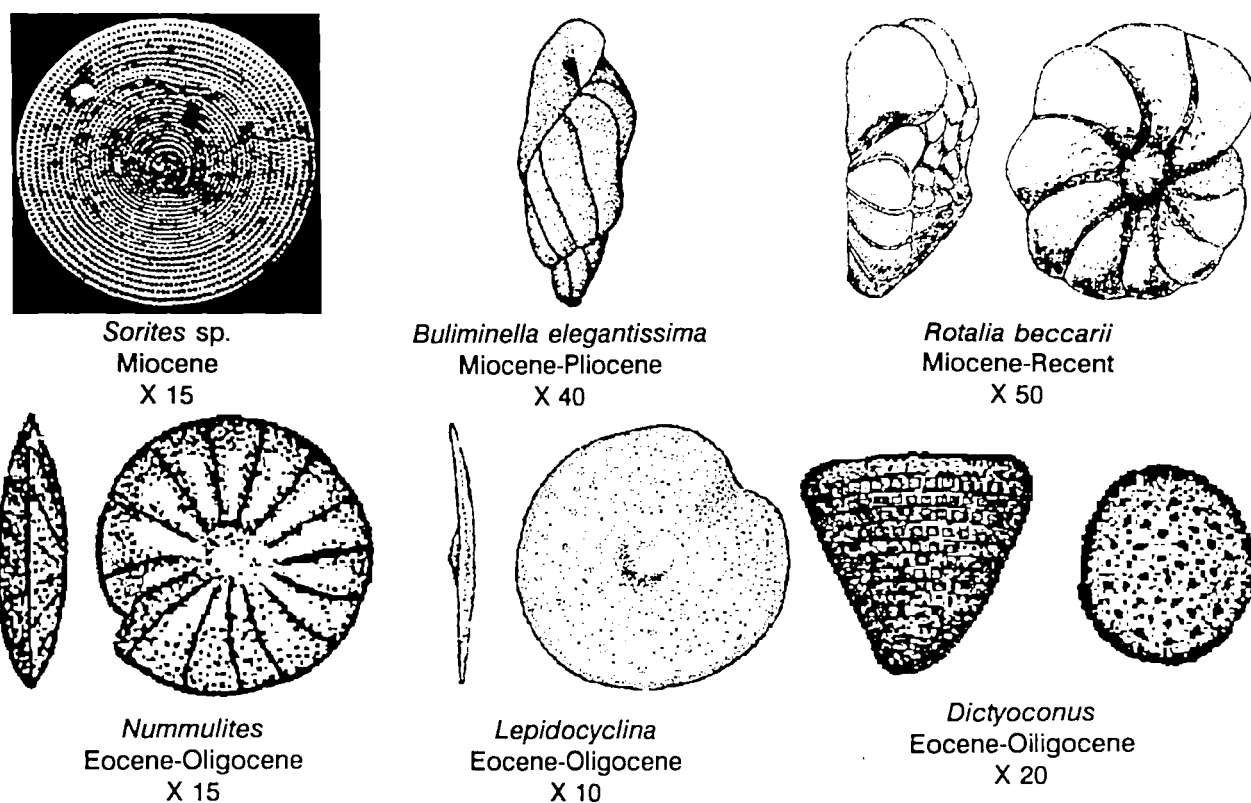


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Figure 6. A typical living benthic foraminifera. (from Rupert, 1992).

To date, a number of foraminifera species have been identified in Florida's sediments which have proven to be useful in characterizing Florida's paleoenvironments and correlating biologic assemblage zones. These include identified species which are

correlated with specific paleosalinity, paleoalkalinity and trace and nutrient elements limits, as well as such physical parameters as water depth and temperature (Bolli et al., 1985). Rupert (1989) depicts several of these stratigraphically significant foraminifera species in a Florida Geological Survey poster. Figure 7 represents a sampling of Florida benthic foraminifera. Figure 8 illustrates several planktonic foraminifera.



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Figure 7. A sampling of Florida benthic foraminifera (from Rupert, 1992).

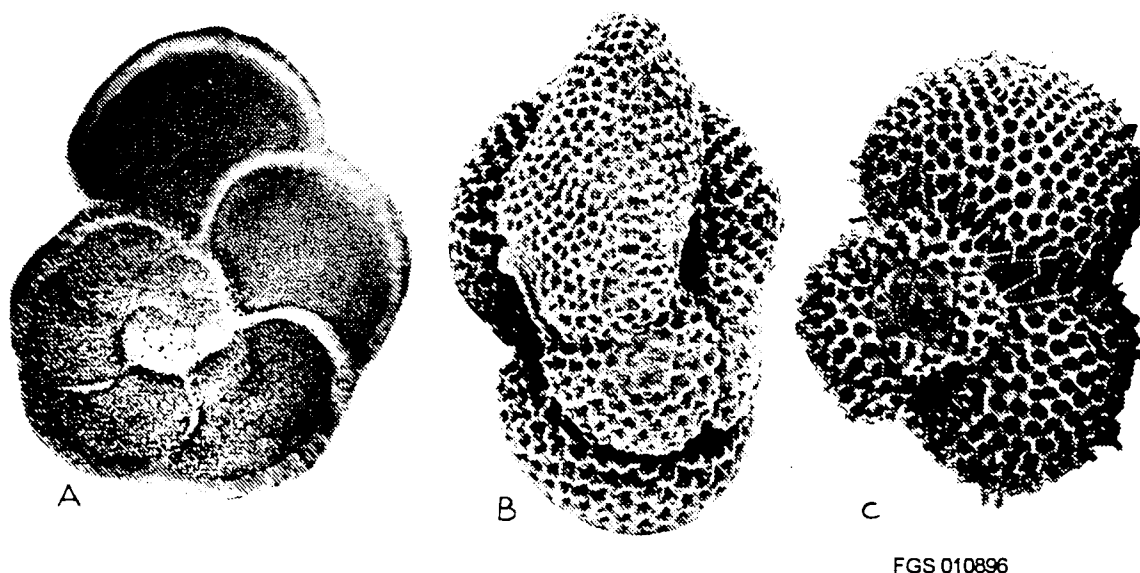
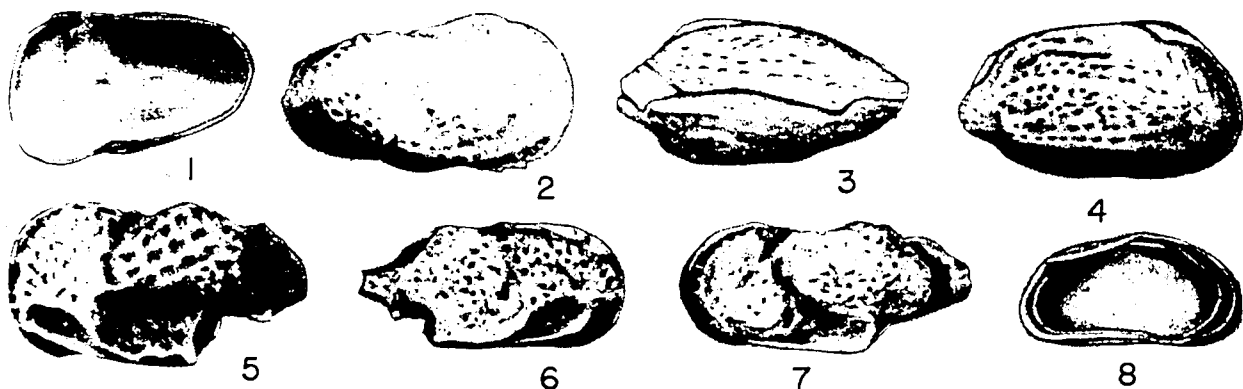


Figure 8. Typical planktonic foraminifera genera:
a. *Globorotalia*
b. *Globigerinoides*
c. *Globigerina*
(modified from Boersma, 1978)

Ostracods

Commonly present with foraminifera are ostracods, another important microfossil group present in Florida's sediments and rocks (Figure 9). Living in fresh, brackish and saline waters, these organisms belong to the Phylum Arthropoda and Class Crustacea. Present in the fossil record since the Cambrian Period (570-500 million years before present), ostracod fossils are primarily benthic forms, many of which are excellent paleo-environmental indicators. Of primary importance is their use as paleobathymetric and paleosalinity indicators.

The taxonomy of fossil ostracods is primarily based on shell morphology and hingement characteristics. Although some recent swimming forms reach lengths of 80 mm, fossil shells generally range in length from 0.15 to 2 mm.



FGS 010996

Figure 9. Ostracods present in Florida's Eocene age Ocala Limestone. 1-5 *Bairdia ocalana* (Puri); 6-8 *Bairdia nagappai* (Puri); 10-13 *Bythocypris gibsonensis* (Howe and Chambers) (from Puri, 1957).

Sample Preparation

The following sample preparation is a common generic technique for the separation of foraminifera and ostracods. Procedures for quartz separation and a list of helpful aids for examining specimens is included.

1. Place sample material* in a 65 mesh (62 micron, no. 230) sieve.
2. Wash sample using a small hose attached to a faucet. At the same time, using fingers, gently desegregate and push unwanted fine material** through the sieve.
3. When sample is washed of all clay, remove the sieve material by carefully turning sieve over on filter paper or a paper towel. If necessary, any material remaining in the mesh may be washed from sieve with a fine stream of water.

4. Dry sieve sample in oven on a low heat setting (40-50°C).
5. Place sample into properly labeled sample bags for future examination.

*Clayey samples can be soaked overnight in a calgon (sodium hexametaphosphate) solution to facilitate disaggregation before following above steps.

**The majority of foraminifera are larger than 40 microns. Those specimens present in the 40 to 60 micron size sieves are tiny and difficult to identify and the majority are juvenile forms. For this reason, those specimens smaller than 60 microns are usually ignored.

Quartz Separation

Flotation is a commonly used method to separate foraminifera tests from quartz grains in thoroughly dried samples. Quartz and calcite have specific gravities (sg) of 2.65 and 2.72, respectively. Most foraminifera have chambers that may be filled with clay or may be hollow, which float in heavy liquids having a specific gravity between 2.2 and 2.4, while quartz grains sink to the bottom. A combination of tetrabromoethane (sg 2.89) and acetone gives a mixture with an sg between 2.2 and 2.4 (**caution** - fumes are hazardous and work with this organic liquid must be done under a ventilated hood).

NOTE: The flotation method is not suitable for quantitative analyses because certain foraminifera species either sink or float in greater proportion than others.

Examination Procedures

(for additional information on techniques, see Hannibel, 1989)

- . Carefully spread the dried sample out in a thin layer in a flat viewing tray. Placing the sample on a tray with a black background provides for maximum contrast.
- . Specimens can be moved within the tray and oriented for various diagnostic views with a thin brush (size #000 or finer).
- . Specimens can be removed for identification or mounting with a wet brush. Some foram workers dampen the brush tip with their tongue. Other more hygiene-conscious individuals keep a small vial of water nearby.
- . The light source should be placed on the upper left or right hand side of the microscope so as to simulate natural light conditions. Light from a single direction provides a shadow effect, allowing the observation of fine foram-test details not clearly visible in diffuse light.
- . Resolution is improved using low power oculars and high power objectives.
- . 25 X 75 mm cardboard mounting slides for foraminifera are available from scientific supply houses such as Curtin Matheson Scientific, Inc. (P.O. Box 1546, Houston, TX (713) 820-9898). These slides are available in a single-well style, with an acetate cover glass, or with a large rectangular well containing a numbered grid. Foraminifera may be mounted on these slides using a thin, water-soluble adhesive. Profes-

sional labs mix an adhesive solution of powdered gum tragacanth (Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA 15219, (412) 562-8300) and water. Several drops of oil of clove may be added to this to retard mildew growth. This solution is painted on the slide mounting area and allowed to dry. When foraminifera species are placed on the slide with a damp brush, the moisture activates the gum and allows the foram to stick in place. They may be moved at any time using a wet brush.

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