

# A new technique for observing the internal morphology of foraminiferal tests in transmitted light

Christopher M. McCauley, Galina P. Nestell and Merlynd K. Nestell

Department of Earth and Environmental Sciences, University of Texas at Arlington, Arlington, TX, 76019, USA  
email: christopher.mccauley@mavs.uta.edu; gnestell@uta.edu; nestell@uta.edu

**ABSTRACT:** The technique proposed herein reveals the internal morphology of calcareous and agglutinated foraminifers in transmitted light, aiding in taxonomic identifications. It involves eliminating air bubbles inside tests through the application of a vacuum or high heat during oil immersion, and the subsequent mounting of the oil-clarified tests in compatible QSMM thermoplastics. The technique is rapid, nondestructive, allows internal viewing of large tests and is especially useful for small tests that are difficult to thin section. The technique has been used for mounting other microfossils, such as polycystine radiolarians.

**Keywords:** Foraminifera, Radiolaria, pteropods, interior morphology, transmitted light microscopy

## INTRODUCTION

Detailed observations of the internal morphologies of recent and fossil foraminiferal tests are essential for making correct taxonomic identifications. Viewing free tests in reflected light or under SEM scans is sufficient to identify many genera and species, but important internal morphological details may remain obscured. Thin sectioning of free tests is the most useful method of revealing internal morphological characteristics, including wall structure and wall thickness, septal structure, chamber arrangements, chamber shapes, and the size of the proloculus, which allows the microspheric and megalospheric generations in foraminifers to be distinguished. However, thin sectioning comes with its own challenges. Small or delicate free tests are especially difficult to section, and often the process may destroy the test or otherwise fail to produce a useable section. As a result, it is generally necessary to sacrifice numerous tests to distinguish between species, but this is not an option when few tests are available.

Another well-known technique for observing the internal morphology of free foraminiferal and radiolarian tests is to place the tests in Canada balsam, which has a refractive index ( $n$ ) of 1.520–1.545. For example, Eisenack (1954) mounted delicate specimens of Ordovician and Silurian agglutinated foraminifers in Canada balsam. He noted for some of his agglutinated tests it was the only method capable of revealing the location of apertures, such as in the genera *Ordovicina* and *Amphitremoida* (Eisenack 1954, pls. 1, 3–4). Tests of some of Eisenack's foraminifers such as *Sorosphaera tricella* (Moreman) were opaque in reflected light, but became transparent in Canada balsam (Eisenack 1954, pl. 3, fig. 3, pl. 4, fig. 18). However, the use of Canada balsam has some limitations. Often, foraminiferal tests embedded in Canada balsam retain bubbles in their chambers that obscure internal details. Even so, Canada balsam remains a highly effective means of making thin sections of free

foraminiferal tests (e.g., Boltovskoy and Wright 1976; Fursenko 1978).

In the last several decades, radiolarian researchers began to substitute modern thermoplastic materials for Canada balsam. One such material is Quick-Stick Meltmount (QSMM) produced by Cargille Laboratories. The QSMM ( $n = 1.539$ ) is optically similar to Canada balsam but has the advantage of readily melting on a hotplate and rapidly resolidifying when removed from it. It produces no fumes and is much easier to work with than Canada balsam. Nestell et al. (2012) used this QSMM during the study of Middle Pennsylvanian Desmoinesian radiolarians from south-central Iowa. Agglutinated foraminifers of the genera *Ammobaculites* and *Reophax* were also found in that assemblage. It was impossible to section tests of these foraminifers because they disintegrated during the process. So, tests of these genera were placed in QSMM to enable transmitted light observations of their internal morphology. Unfortunately, the test of the genus *Reophax* remained opaque in QSMM, possibly due to its thickness, but tests of the genus *Ammobaculites* revealed the tiny initial coiled part of the test, which permitted the tests to be assigned to the correct genus and revealed the need to establish a new species, *Ammobaculites marmatonensis* (Nestell et al. 2012, pl. 8, fig. 1).

Test interiors may also be observed by means of oil immersion. Empson-Morin (1981) proposed new techniques for the preparation and transmitted light photography of polycystine radiolarian tests submerged in petrographic immersion oils ( $n = 1.470$ – $1.600$ ). Later, Boltovskoy et al. (1983) proposed immersing radiolarian tests in various vegetable oils such as soy oil, cotton oil, sunflower oil, grape oil, tung oil, and olive oil ( $n = 1.47$ – $1.51$ ).

In this paper, we modify and combine the techniques of oil immersion and QSMM mounting of tests to allow more detailed

transmitted light observations of the internal structures of foraminiferal (Pls. 1-3; Pl. 4, figs. 1, 3-4, 6), and radiolarian (Pl. 4, figs. 5, 7-8) tests. These structures include their chamber arrangement, type of coiling, wall thickness and its structure, and in radiolarians their internal framework. The technique proposed here follows three simple steps. The first involves the immersion of foraminiferal tests in clear oils as has been done previously with radiolarians (Empson-Morin 1981; Boltovskoy et al. 1983). This immersion is followed by the application of heat and/or a vacuum to eject all air bubbles from the tests. Second, it has been discovered that these clarified oil-saturated tests can be transferred into the QSMM thermoplastic to preserve their bubbleless state. Third, a series of photographs are taken of each test in transmitted light under high magnification.

## TECHNIQUE DEVELOPMENT

The need for an efficient means of revealing the internal structures of foraminiferal tests became apparent during the study of Silurian agglutinated foraminifers from Oklahoma, USA. The foraminiferal fauna there is very diverse, consists of many free specimens of different sizes, and because some of the tests are small and delicate, it is impossible to section them. Experiments began with a focus on using various liquids and oils to clarify tests for transmitted light photography: water, glycerin, Hoyer's solution, propylene glycol, soybean oil, food-grade mineral oil, cassia oil, silicone oil, and refractive index oils. Many of these materials were unsuitable or had limited applicability. For instance, the refractive index of water was raised by creating salt and sucrose solutions, but the tests were often coated by crystals when the solution began to evaporate. Heated anhydrous glycerin was found to be unacceptably viscous. Agglutinated foraminifers and radiolarians mounted in Hoyer's medium immediately cracked and fragmented under a cover slip. Propylene glycol ( $n = 1.43$ ) is a convenient test clarifier but unfortunately reacts with the QSMM thermoplastic to form an opaque condensate when saturated tests are mounted, and mounting was only occasionally successful using epoxy (Pl. 1, fig. 4). Whatever liquid was in use, there was a general tendency of many foraminiferal or radiolarian tests to retain large air bubbles, including in QSMM (Pl. 1, fig. 1).

However, when tests immersed in soybean oil were heated above 100°C many bubbles were driven out. The clarification of tests in soybean oil has excellent results, and tests saturated with soybean oil ( $n = 1.47$ ) and subsequently placed in QSMM ( $n = 1.539$ ) can be seen in Plate 1, figs. 2b, 3b, and 5. Most other oils cannot be safely raised to such temperatures, but it is also possible to eject bubbles from immersed tests by using a vacuum chamber at room temperature. The results using a vacuum and food grade mineral oil ( $n = 1.48$ ) (Pl. 1, figs. 6-12; Pl. 2, figs. 1, 3-7, 9, 11; Pl. 3, figs. 1-2; Pl. 4, fig. 7), or Cargille refractive index oils ( $n = 1.540$ - $1.638$ ) (Pl. 2, figs. 2, 8, 10; Pl. 3, figs. 3-7; Pl. 4, figs. 1-4, 6, 8-9) were often excellent.

## Selection of media in specific cases

A number of experiments were performed to determine useful combinations of oils and mounting media for microfossils of differing and specific compositions. For the experiments, a number of tests of agglutinated and calcareous foraminifers, radiolarians, and some pteropod shells were selected from different stratigraphic levels and areas, such as Silurian agglutinated foraminifers from Oklahoma, Pennsylvanian agglutinated foraminifers from North-Central Texas, Middle Permian calcareous foraminifers from the Volga region, Russia, Middle Perm-

ian radiolarians from West Texas, Jurassic radiolarians from Oregon, Late Cretaceous calcareous foraminifers from Central Texas, Holocene calcareous foraminifers from Florida Bay, and calcareous foraminifers and pteropods from the Atlantic Ocean (Vema Cruise # 19).

Oils such as soybean oil and food-grade mineral oil greatly improve internal test visibility, however, the refractive index of vegetable oils and petroleum distillate oils are suboptimal for viewing the internal characteristics of foraminifers. For instance, the difference in how light is transmitted through the round chambers of the genus *Sorospaera*? in mineral oil ( $n = 1.48$ ) and after the oil-saturated test is implanted in the QSMM thermoplastic ( $n = 1.539$ ), is shown in text-figure 1. The combination of mineral oil and QSMM ( $n = 1.539$ ) works well for radiolarians (Pl. 4, fig. 7b), quartz-agglutinated foraminifers (Pl. 1, figs. 6-9), and many calcareous foraminifers, especially when tests have thin walls or have a hyaline wall ultrastructure (Pl. 1, figs. 10-12; Pl. 2, figs. 1, 3-7, 9, 11; Pl. 3, figs. 1-2). In cases where light transmission through a test is restricted, it is often helpful to photograph mounted specimens in reflected light (Pl. 2, fig. 1b; Pl. 4, fig. 4b).

Among calcareous tests, Holocene hyaline tests from Florida Bay are often clearest in refractive index oils ( $n = 1.540$ ) (Pl. 3, fig. 3; Pl. 4 fig. 1), and milky-white porcelaneous tests in refractive index oils ( $n = 1.574$ ) (*Triloculina*, Pl. 2, fig. 8) or ( $n = 1.602$ ) (*Archaias*, Pl. 3, fig. 4). Tests saturated in refractive index oils were often preserved in QSMM ( $n = 1.539$ ) (Pl. 3, fig. 3-4; Pl. 4, fig. 1), but sometimes light transmission decreases as the refractive index of the oil used increases beyond that of the thermoplastic, especially when ( $n = 1.6$ ). This problem is resolved by switching to a higher refractive index QSMM. The QSMM ( $n = 1.662$ ) works well for calcareous foraminifers with thick multilayered radial walls (Pl. 2, fig. 2c), thin porcelaneous walls (Pl. 3, fig. 7b, Pl. 4, figs. 4b-4c), flat hyaline tests (Pl. 3, figs. 5b-5c, 6b-6c; Pl. 4, fig. 6b), viewing radiolarians in high contrast (Pl. 4, fig. 8b), and pteropod shells (Pl. 4, figs. 2b, 9b).

As an example of the impact of the refractive index of media, if a comparison is made between two tests of the planktonic genus *Trilobatus*, one saturated in refractive index oil ( $n = 1.636$ ) and mounted in QSMM ( $n = 1.662$ ) (Pl. 4, figs. 3b-3c) and another saturated with mineral oil ( $n = 1.48$ ) and mounted in QSMM ( $n = 1.539$ ) (Pl. 2, fig. 9b), the former requires a brighter light source, but better reveals the coiling of the early chambers, whereas the latter much more clearly illustrates the pore structure and wall thicknesses of the test's larger chambers.

In summary, the use of various mounting media aid in the discernment of many internal details of foraminiferal tests. In agglutinated genera, apertures and necks can be viewed in profile, coiling arrangements can be seen, and wall structure enhanced (Pl. 1, figs. 1-9). The results for calcareous forms are arguably even better, such as the multilayered wall in a Cretaceous hyaline *Dentalina* (Pl. 2, figs. 2b-2c), or the internal chambers of a recent planktonic *Orbulina* (Pl. 3, fig. 2b). These media are also useful for observing internal details of both smaller (Pl. 2, figs. 3-7) and larger tests (Pl. 2, figs. 1-2, 11; Pl. 3, figs. 1, 5, 6; Pl. 4, fig. 4). Pores, forams, and chamberlets are visible, and even weathered tests may reveal extraordinary interior details when viewed in transmitted light (Pl. 3, fig. 1). The mounting technique works with other microfossils such as pteropods (Pl. 4, figs. 2, 9) and radiolarians (Pl. 4, figs. 5, 7, 8).





TEXT-FIGURE 1

Illustration comparing light transmission through the agglutinated chambers of the foraminiferal genus *Sorosphaera*?, immersed in mineral oil ( $n = 1.48$ ) (A) and following implantation of the mineral oil saturated test in QSMM ( $n = 1.539$ ) (B). Silurian, Pridoli; Lawrence Uplift, south-central Oklahoma, Henryhouse Formation, 9-10 section, sample 11, *Zieglerodina? eosteinhornensis* s. l. conodont Zone.

### DESCRIPTION OF THE NEW TECHNIQUE

The first step in the process is immersing the tests in soybean oil, food-grade mineral oil, or refractive index oils because these oils are compatible with the vacuum enclosure and QSMM thermoplastics. If one does not have a vacuum enclosure, it is possible to drive out bubbles using soybean oil and a hotplate (see step 1-B below). However, the vacuum technique is more efficient and is compatible with a larger number of oils. It requires only the desired oil, a dish, an inexpensive  $\frac{1}{4}$  horsepower single stage rotary-vane vacuum pump, and a two-quart vacuum enclosure, all of which are easily purchasable online. A flowchart for the technique is provided in text-figure 2. Transmitted light photography may take place either before or after implantation in the QSMM.

#### 1-A) Liquid Clarification of Tests and Bubble Ejection Using a Vacuum Chamber

1. Place clean dry tests in a small dish, ensuring they are covered by a shallow pool of oil such as food grade mineral oil, soybean oil, or a refractive index oil.

a. A 3mL clear plastic cosmetic jar works well as a dish, especially for mineral oil and soybean oil. The jars come with a convenient screw-on lid. The lid should be left very loosely covering the dish, which allows air to escape while the vacuum

is pulled and also shelters the tests during repressurization, preventing the tests and the oil from being blown out of the dish.

b. If one uses refractive index oils, we recommend using a glass microscope slide with a concave depression or a watch glass because refractive index oils etch plastic containers and solidify after three days of contact with plastic. A smaller watch glass placed convex side up above the oil and tests may shelter them from rapid airflow and act as an impromptu lid.

2. Transfer the dish into a vacuum chamber. Seal the chamber and turn on the vacuum pump to draw a vacuum, following the instructions of the manufacturer. Once a minimum pressure is achieved, seal the chamber, turn off the pump, and leave the dish and specimens to sit in the vacuum for at least 15 minutes.

a. Lower the air pressure gradually over a period of 20 to 30 seconds. A pressure below approximately 0.03 atmospheres (or 23 mmHg) is recommended to evict all gasses from the tests.

b. Bubbles rising out of the tests indicate air ejection is ongoing. After 5-10 minutes, the vacuum pump can be turned on again and the pump valve reopened to remove any air that has come out of the tests during that time and lower the atmosphere of the chamber once again. Once the pressure is minimized, again close the valve connected to the pump and shut off the pump.

c. With mineral oil or refractive index oils, evacuation may be complete within 15-30 minutes. In general, the greater the viscosity of the oil used, the more time is required.

3. Once a few minutes have passed without any sign of bubbles flowing out of the tests, the vacuum chamber may be opened. Over a period of 30 seconds, slowly bring the vacuum enclosure back to atmospheric pressure. Observe the tests under transmitted light.

a. Air may rush into the vacuum chamber as an unopposed wind. Total evacuation of air bubbles in the tests is generally achieved on the first or second attempt. Reorient any tests that retained air and draw a vacuum again.

#### **1-B) Liquid Clarification of Tests and Bubble Ejection by Heating in Soybean Oil**

1. Place tests in a small metal cup and cover them with a shallow pool of soybean oil.

2. Gradually heat the oil dish on a small hot plate to 100°C or higher. Heat lowers the viscosity of the oil, which flows around the expanding and escaping bubbles in the test interior.

3. Remove the dish from the hot plate using a thin metal spatula or tweezers and transfer it onto a secure surface to cool. Determine if all gas has been ejected from the tests. If not, the tests can be reoriented, and the previous step can be repeated until a desirable result is achieved. Once the oil has cooled, the tests can be transferred to a glass slide or container using a small brush and may be viewed in transmitted light.

#### **2-A) QSMM Mounting for Clarified Tests**

1. Prepare a glass microscope slide with the QSMM

a. Clean a glass microscope slide with alcohol.

b. Turn on any requisite ventilation. The QSMM ( $n = 1.539$ ) contains no hazardous ingredients, but the QSMM ( $n = 1.662$ ) contains pentabromodiphenyl ether and should be heated in a fume hood.

c. Arrange small cut fragments of the QSMM ( $n = 1.539$ ) on the glass slide. If using the softer QSMM ( $n = 1.662$ ) instead, follow Cargille's instructions to transfer the material to the glass slide by pressing the QSMM stick directly against a heated slide on a hot plate.

d. Heat the slide on a hotplate to 60-80°C until the thermoplastic is watery, and any bubbles have popped.

e. Take the slide off the hot plate using metal tweezers and allow the slide to cool to room temperature until the thermoplastic becomes solid.

2. Place and orient oil-filled tests (obtained from step 1-A or 1-B) on the surface of the cool thermoplastic QSMM

a. Remove each test from the oil using a small paint brush such as size 4/0.

b. To limit the amount of oil transferred along with the tests to the thermoplastic, the tests can first be relocated to a secondary surface such as another slide. The brush initially picks up excess oil when the test is grasped, but after the test is placed on a glass slide, the bristles of the brush can be twisted to wring out

the excess oil or the oil can be absorbed by a paper towel. The test can then be properly oriented with the brush and positioned on top of the thermoplastic. The thermoplastic will absorb excess oil once it melts.

c. Oil may begin to drain from tests left exposed to air, allowing bubbles to return. This problem can be overcome by mounting a smaller number of tests at a time or by using a higher viscosity oil, e.g., soybean oil has a higher viscosity than mineral oil.

d. Return the slide to the hotplate at 60-80°C, allowing time for the tests to sink into the Meltmount. For foraminifers it is recommended to wait until the tests fully sink to the surface of the glass slide. Two minutes is often sufficient time for the tests to sink and any excess oil to be absorbed into the thermoplastic.

e. Remove the slide from the hot plate using tweezers, let the slide cool and view the specimens under a microscope. The surface of the thermoplastic is flat enough that a coverslip is not required for imaging under the microscope. Indeed, a coverslip may crush delicate specimens if the thermoplastic is in a liquid state.

3. Cargille recommends QSMM slides be stored horizontally in an environment that is dark, sealed away from dust, and temperature controlled.

a. Slides containing mounted oil-saturated specimens have remained clear more than a year after their creation. Previous slides using dry tests and the thermoplastic have remained in good condition after years in storage. Slides featuring important specimens have been made more durable by encapsulating the solid QSMM bead in epoxy or liquid Canada balsam and a cover slip.

b. Extraction from the mount is possible using a solvent such as acetone, but is not recommended as delicate specimens may be destroyed during the procedure.

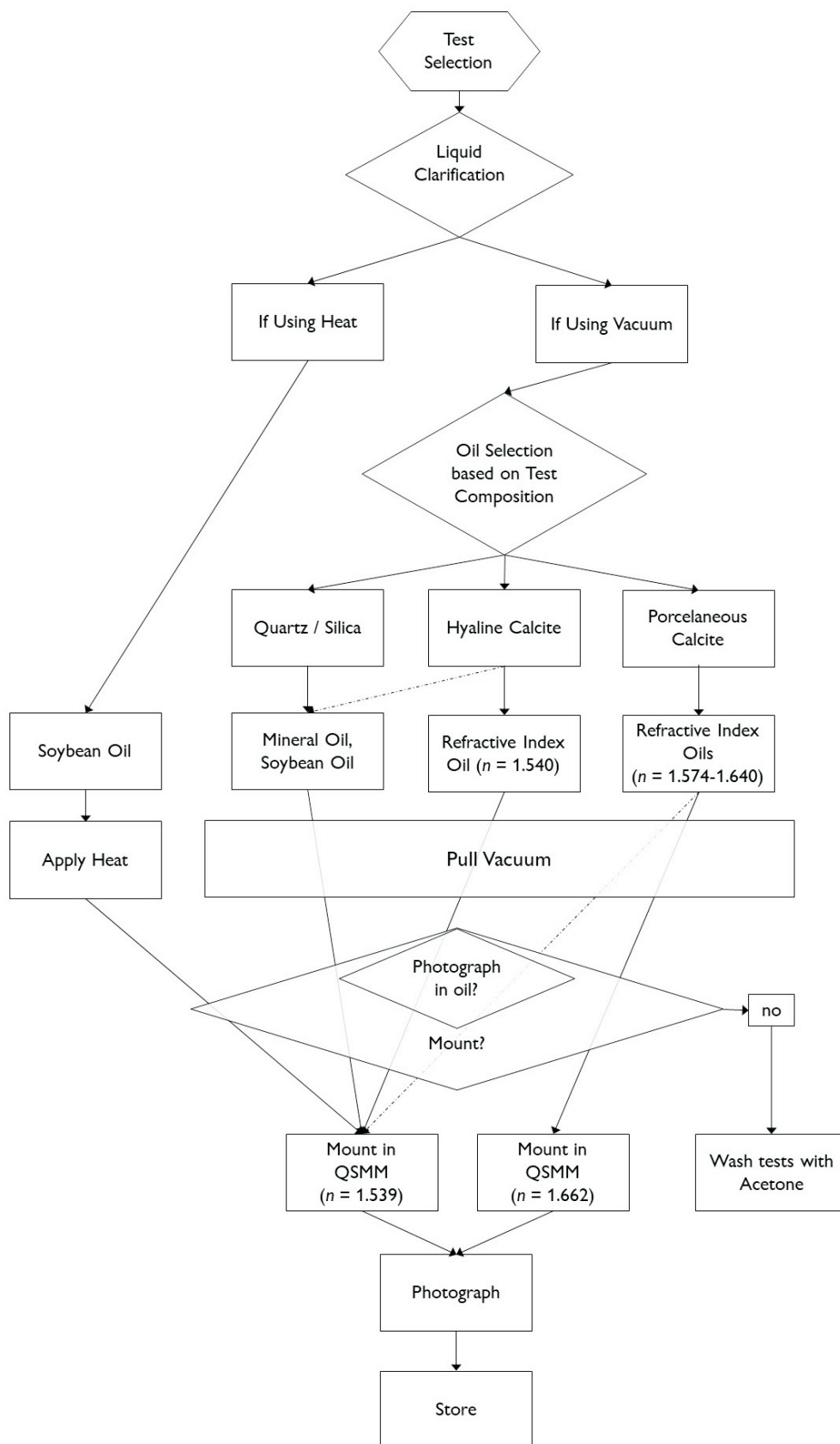
#### **2-B) A Mounting Technique for Larger Specimens**

As the QSMM thermoplastics melt they flatten under gravity, making it challenging to mount specimens with a thickness greater than roughly 0.3 millimeters. A slide with a pre-ground basin or depression, or a ring formed on a slide can confine the mounting material in a pool of greater depth. For this article, one side of a metal washer was coated in clear silicone sealant, and then pressed and rotated onto a glass slide while the hole in the washer was temporarily occupied by a dowel. After the dowel was removed and the sealant dried, the washer remained bonded to the slide by heat resistant silicone. QSMM ( $n = 1.539$ ) was added to the new basin and melted until sufficient depth was achieved to encapsulate larger tests (Pl. 2, figs. 9b, 11b; Pl. 3, fig. 2b). The technique is otherwise equivalent to that of step 2-A.

#### **3) Transmitted Light Photography of Tests**

Clarified tests immersed in refractive index oils are easily photographed by transferring them into beads of oil on a glass slide, and no coverslip is necessary (Pl. 2, figs. 2b, 8, 10). The orientation of each test is easily manipulated in oil, and different details of test structure may be highlighted in oil as compared to the QSMM. For example, details of the multilayered wall in *Dentalina* (Pl. 2, fig. 2b-2c) were arguably better displayed when the test was immersed in refractive index oil ( $n = 1.636$ ) than when the oil-saturated test was mounted in the QSMM ( $n =$





TEXT-FIGURE 2

Summary flowchart of the technique proposed here. The diamond-shaped boxes indicate key decisions, including whether to mount the specimens, the type of oil to use for best transmitted light viewing, and whether to drive bubbles out of tests using heat or, in most cases, a vacuum.

# PLATE 1

Agglutinated (figs. 1-9) and calcareous (figs. 10-12) foraminifers viewed in reflected and transmitted light. Scale bar – 100  $\mu$ m.

- 1 *Webbinelloidea?* sp., mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light. Without vacuum treatment the test retains large air bubbles in the chambers. Silurian, Pridoli; Lawrence Uplift, south-central Oklahoma, Henryhouse Formation, 9-10 section, sample 11A, *Zieglerodina? eosteinhornensis s. l.* conodont Zone.
- 2 *Hyperammina* aff. *harrisi* Ireland, a – test in reflected light, b – soybean oil ( $n = 1.47$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing wall thickness and the shape of the proloculus. Silurian, Ludlow, Ludfordian; Lawrence Uplift, south-central Oklahoma, Henryhouse Formation, 9-10 section, sample 6, *Ozarkodina? snajdri* conodont Interval Zone.
- 3 *Rectoammodiscus* sp., a – test in reflected light, b – soybean oil ( $n = 1.47$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, c – enlarged view of rectangular area of 3b showing the proloculus and planispiral coiling. Silurian, Ludlow, Gorstian; Arbuckle Mountains, south-central Oklahoma, Henryhouse Formation, Dougherty West section, sample 610B, *Kockelella crassa* conodont Zone.
- 4 *Rectoammodiscus* sp., test saturated with propylene glycol ( $n = 1.43$ ), embedded in epoxy ( $n$  approximately 1.50-1.56) and viewed in transmitted light, showing wall thickness and planispiral coiling. Silurian, Ludlow, Gorstian; south-central Oklahoma, Arbuckle Mountains, Henryhouse Formation, Hickory Creek section, sample 12, *Kockelella crassa* conodont Zone.
- 5 *Rectoammodiscus* sp., soybean oil ( $n = 1.47$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing the proloculus, wall thickness, and planispiral coiling. Silurian, Ludlow, Gorstian; Arbuckle Mountains, south-central Oklahoma, Henryhouse Formation, Hickory Creek section, sample 12, *Kockelella crassa* conodont Zone.
- 6 *Lagenammina* aff. *cucurbita* Moreman, a – test in reflected light, b – mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing wall thickness and the interior of the neck. Lower Devonian, Lochkovian; Arbuckle Mountains, south-central Oklahoma, Haragan Formation, Dougherty West section, sample 15, *Caudicriodus hesperius* conodont Zone.
- 7 *Webbinelloidea?* sp., a – test in reflected light, b – mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing the interior shape of eight similarly-sized and mutually appressed hemispherical chambers. Silurian, Pridoli; Arbuckle Mountains, south-central Oklahoma, Henryhouse Formation, Ca2 section, sample 530, *Zieglerodina? eosteinhornensis s. l.* conodont Interval Zone.
- 8-9 *Ammobaculites* sp., 8a, 9a – tests in reflected light, 8b, 9b – mineral oil ( $n = 1.48$ ) saturated tests mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing the planispiral coiling of the initial chambers, wall thicknesses and the openings between chambers, especially in the uncoiled uniserial portion of the test. Upper Pennsylvanian, Kasimovian; North-Central Texas, Bridgeport, Runaway Bay section, sample HP A at 11m.
- 10 *Ichthyolaria* sp., a – test in reflected light, b – mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing wall thickness, and the chevron septa. Middle Permian, lower Kazanian; Volga region, Sok River, Baytugan Village, Baytugan beds, sample BT-2-1/03.
- 11 *Coryphostoma* sp., a – test in reflected light, b – mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing biserial chamber arrangement. Due to a short vacuum period, a single bubble remained in the test (black circle). Upper Cretaceous, Maastrichtian; North-Central Texas, near Coolidge, Navarro Group, Corsicana Formation, Kemp Clay, locality DW-13, sample 41J3-3.
- 12 *Globotruncanella minuta* Caron and Gonzalez Donoso in Robaszynski et al. 1984, a – test in reflected light, b – mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing proloculus and coiling. Upper Cretaceous, Maastrichtian; North-Central Texas, near Coolidge, Navarro Group, Corsicana Formation, Kemp Clay, locality DW-13, sample 41J3-1.







1.662). In addition, if permanent mounting is not desired, tests can be bathed in acetone for a short time to dissolve out the oil and the tests can once again be stored dry or be prepared for imaging in an SEM, as Empson-Morin (1981) did with radiolarians. However, mounting in the correct refractive index QSMM will generally produce a sharper transmitted light image than oil alone and has the additional benefit of preserving the clarified specimen in perpetuity. All tests imaged in transmitted light in this article are embedded in a QSMM thermoplastic excepting (Pl. 1, fig. 4; Pl. 2, figs. 2b, 8, 10).

Transmitted light photographs taken at high magnification may be combined using an (EDF) enhanced depth of field tool to bring all portions of the test into focus in one image. Many images for this article were taken using a Nikon Optiphot-POL microscope fitted with an old Polaroid Model PDMC 1 digital camera communicating with Polaroid's DMC Direct 2.0 soft-

ware operating on MAC OS 9.2.2 (Pl. 1, figs. 1, 2b, 5, 6b, 7b, 10b, 12b; Pl. 2, figs. 2b-2c, 9b, 11b; Pl. 3, figs. 1b-1c, 2b, 7b; Pl. 4, figs. 2b, 4c, 6b, 7b, 8b, 9b). All other transmitted light images were taken on another Optiphot-POL equipped with a PixelINK digital camera. The TIFF images were imported into contemporary AmScope 4.11.18421 software and stitched together using the EDF tool. In some cases, it was best to provide the EDF tool only with images focused on the middle layers of the test to get the clearest composite image of the interior (Pl. 1, fig. 3c; Pl. 2, fig. 3b; Pl. 3, figs. 2b, 6b-6e).

## CONCLUSION

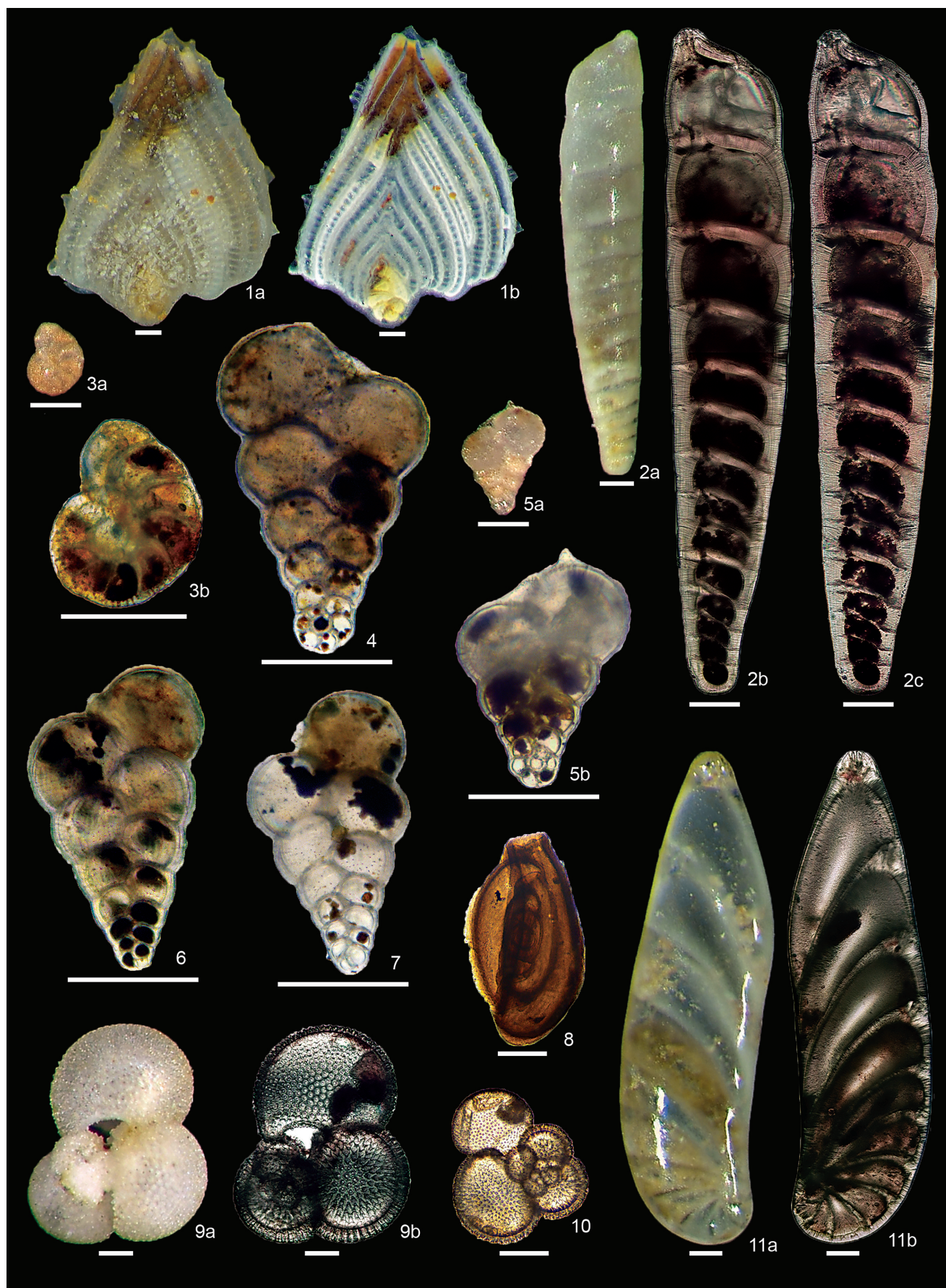
The new proposed technique for observing the internal structures of various microfossils in transmitted light involves eliminating air bubbles inside tests through the application of a vacuum or high heat during oil immersion and subsequent mounting of the oil-clarified tests in compatible QSMM ther-

## PLATE 2

Calcareous foraminifers viewed in reflected and transmitted light.

Tests in figs. 3–7 illustrate the effectiveness of the vacuum technique at clarifying very small tests. Scale bar – 100  $\mu$ m.

- 1 *Palmula reticulata* (Reuss), a – test in reflected light, b – mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in reflected light, showing uncoiling of early chambers, septa, forams, and strongly enveloping chevron-shaped chambers subdivided into chamberlets. Upper Cretaceous, Maastrichtian; North-Central Texas, near Coolidge, Navarro Group, Corsicana Formation, Kemp Clay, locality DW-13, sample 41J3-1.
- 2 *Dentalina* sp., a – test in reflected light, b – test immersed in refractive index oil ( $n = 1.636$ ) and viewed in transmitted light showing multilayered radial wall and forams between chambers, c – test saturated with refractive index oil ( $n = 1.636$ ), mounted in QSMM ( $n = 1.662$ ) and viewed in transmitted light. Upper Cretaceous, Maastrichtian; North-Central Texas, near Coolidge, Navarro Group, Corsicana Formation, Kemp Clay, locality DW-13, sample 41J3-1.
- 3 *Gavelinella* sp., a – test in reflected light, b – mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing the proloculus, septa, and coiling. Upper Cretaceous, Maastrichtian; North-Central Texas, near Coolidge, Navarro Group, Corsicana Formation, Kemp Clay, locality DW-13, sample 41J3-1.
- 4-7 *Heterohelix navarroensis* Loeblich. 5a – test in reflected light, 4, 5b-7 – mineral oil ( $n = 1.48$ ) saturated tests mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light. Tests in figs. 4 and 5 display a developed initial planispiral whorl of early chambers, whereas tests in figs. 6 and 7 have an undeveloped planispiral whorl. Wall thicknesses, forams, and outer costae are visible in some cases. Upper Cretaceous, Maastrichtian; North-Central Texas, near Coolidge, Navarro Group, Corsicana Formation, Kemp Clay, locality DW-13, sample 41J3-1.
- 8 *Triloculina* sp., test immersed in refractive index oil ( $n = 1.574$ ) and viewed in transmitted light, showing internal coiling and wall thickness. Holocene; Florida Bay, shallow water bottom sample TAF-236.
- 9 *Trilobatus* sp., a – test in reflected light, b – mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light. Wall thickness and pore structures are visible. Holocene; Vema Cruz # 19, Latitude 4°S Longitude 5°E, sample 45.
- 10 *Globigerinoides* sp., test immersed in refractive index oil ( $n = 1.540$ ) and viewed in transmitted light, showing coiling of globular chambers, wall thickness, and pore structure. Holocene; Vema Cruz # 19, Latitude 4°S Longitude 5°E, sample 45.
- 11 *Astacolus* sp., a – test in reflected light, b – mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing radial aperture, wall thickness, and the shape of internal chambers. Holocene; Vema Cruz # 19, Latitude 4°S Longitude 5°E, sample 45.





moplastics. The technique is rapid, nondestructive, and allows internal viewing of tests that are small and difficult to thin section. The technique could be used for mounting other microfossils composed of calcite, aragonite, and silica.

## ACKNOWLEDGMENTS

We would like to thank reviewers Profs. M. A. Kaminski and Anna Waskowska for reviewing the manuscript. We would like to thank the Dallas Paleontological Society and the Fort Worth Geological Society for their scholarship support of C. McCauley's research.

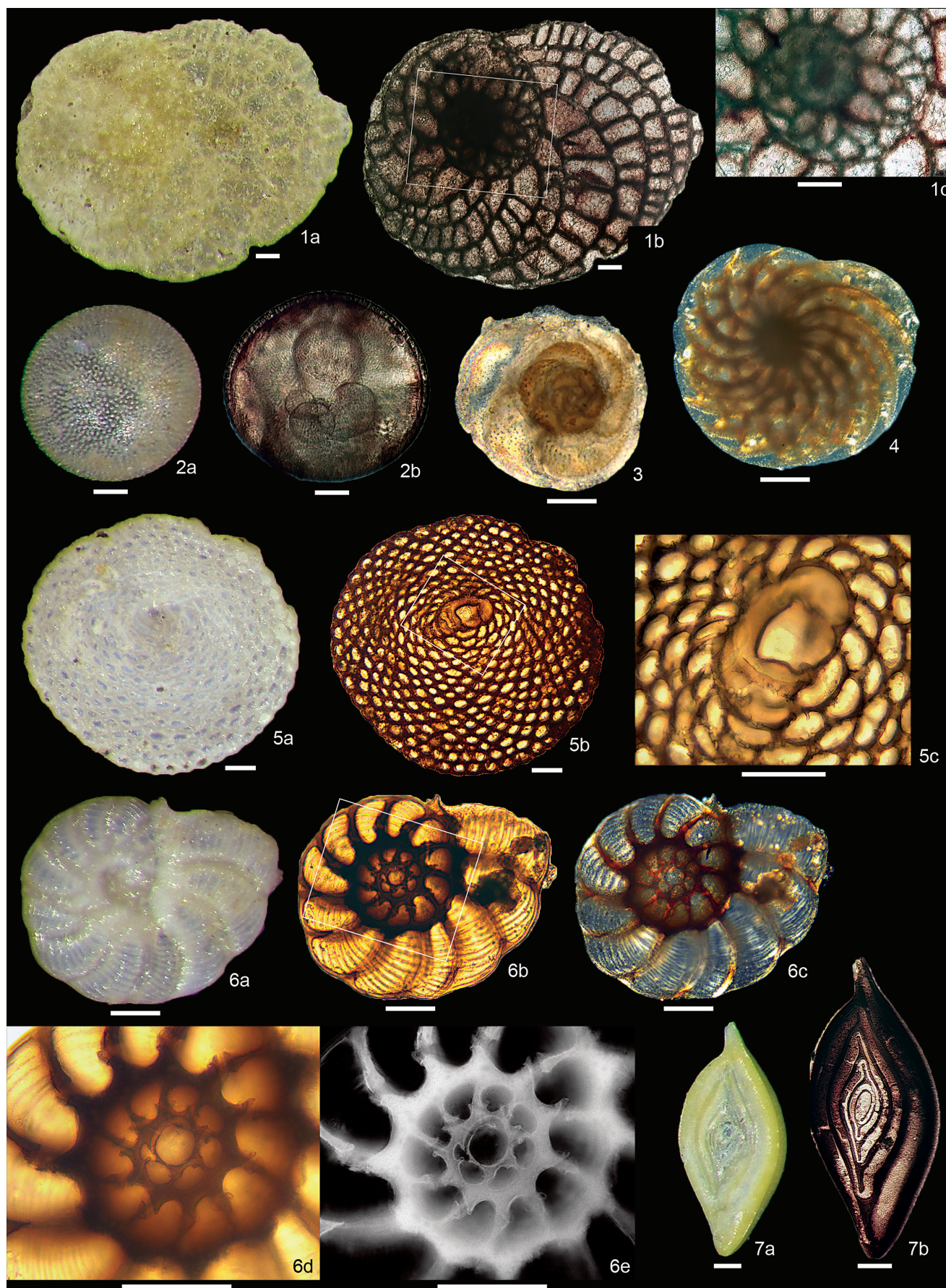
## REFERENCES

- BOLTOVSKOY, E. and WRIGHT, R. C., 1976. Recent foraminifera. The Hague: Dr. W. Junk b.v. Publishers, 515 pp.
- BOLTOVSKOY, D., KOTZIAN, S. B. and PEDROZO, F. L., 1983. Some new techniques for the preparation and illustration of Polycystina (Radiolaria). *Micropaleontology*, 29 (4): 382–390.
- EISENACK, A., 1954. Foraminiferen aus dem baltischen Silur. *Senckenbergiana lethaea*, 35 (1/2): 51–72.
- EMPSON-MORIN, K. M., 1981. Campanian Radiolaria from DSDP Site 313, Mid-Pacific Mountains. *Micropaleontology*, 27 (3): 249–292.
- FURSENKO, A. V., 1978. Vvedenie v izucheniye foraminifer [Introduction to the study of foraminifers]. *Trudy Instituta Geologii i Geofiziki*, 391: 1–242. (In Russian)
- HOTTINGER, L., 2006. Illustrated glossary of terms used in foraminiferal research. Carnets de Géologie/Notebooks on Geology, Brest, Memoir 2006/02 (CG2006\_M02), 126 pp.
- 1 *Heterostegina* sp., a – test in reflected light, b – mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing chamberlets, c – enlarged view of initial coiling. Holocene; Vema Cruz # 19, Latitude 4°S Longitude 5°E, sample 45.
- 2 *Orbulina inversa* d'Orbigny, a – test in reflected light, b – mineral oil ( $n = 1.48$ ) saturated test mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing internal coiled globular chambers and the thickness of the outer wall. Holocene; Vema Cruz # 19, Latitude 4°S Longitude 5°E, sample 45.
- 3 *Eponides* sp., test saturated with refractive index oil ( $n = 1.540$ ), mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light under crossed nicols, showing internal coiling. Holocene; Florida Bay, shallow water bottom sample TAF-236.
- 4 *Archaias* sp., test saturated with refractive index oil ( $n = 1.602$ ), mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light under crossed nicols, showing vortex, spiral chambers (terminology in the sense of Hottinger 2006), and their subdivision into chamberlets. Holocene; Florida Bay, shallow water bottom sample TAF-236.
- 5 *Sorites marginalis* (Lamarck), a – test in reflected light, b – test saturated with refractive index oil ( $n = 1.636$ ), mounted in QSMM ( $n = 1.662$ ) and viewed in transmitted light, c – enlarged view of initial part of the test showing early chambers, septa, foramens, annular passages, and crosswise-oblique stolons (terminology in the sense of Hottinger 2006). Holocene; Florida Bay, shallow water bottom sample TAF-236.
- 6 *Peneroplis pertusus* (Forskal), a – test in reflected light, b – test saturated with refractive index oil ( $n = 1.636$ ), mounted in QSMM ( $n = 1.662$ ) and viewed in transmitted light, c – view under crossed nicols, d – enlarged view of the initial part of the test in transmitted light showing the proloculus, flexostyle, septa, and foramens in early chambers, e – a negative image of fig. 6d using a grayscale gradient map to highlight the walls of the test. Holocene; Vema Cruz # 19, Latitude 4°S Longitude 5°E, sample 45.
- 7 *Spiroloculina* sp., a – test in reflected light, b – test saturated with refractive index oil ( $n = 1.636$ ), mounted in QSMM ( $n = 1.662$ ) and viewed in transmitted light, showing the internal structure including the oval proloculus and flexostyle. Holocene; Vema Cruz # 19, Latitude 4°S Longitude 5°E, sample 45.

## PLATE 3

Calcareous foraminifers viewed in reflected and transmitted light. Scale bar – 100  $\mu$ m.







NESTELL, G. P., POPE, J. P. and NESTELL, M. K., 2012. Middle Pennsylvanian (Desmoinesian) Radiolaria from the Midcontinent of North America. *Micropaleontology*, 58 (3): 217–257.

ROBASZYNSKI, F., CARON, M., GONZALES DONOSO, J. M., WONDERS, A. A. H. and The European Working Group On Plank-

tonic Foraminifera, 1984. Atlas of Late Cretaceous globotruncanids. *Revue de Micropaléontologie*, 26 (3-4): 145–305.

#### PLATE 4

Foraminifers (figs. 1, 3-4, 6), pteropods (figs. 2, 9), and radiolarians (figs. 5, 7-8) viewed in reflected and transmitted light.  
Scale bar – 100  $\mu\text{m}$ .

- 1 *Eponides* sp., a – test in reflected light, b – test saturated with refractive index oil ( $n = 1.540$ ), mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing the proloculus, initial coiling, wall thickness and pores, c – the same test viewed under crossed nicols. Holocene; Florida Bay, shallow water bottom sample TAF-236.
- 2 *Styliola* sp., a – shell in reflected light, b – shell saturated with refractive index oil ( $n = 1.636$ ), mounted in QSMM ( $n = 1.662$ ) and viewed in transmitted light, showing the thin aragonitic wall. Holocene; Vema Cruz # 19, Latitude 4°S Longitude 5°E, sample 45.
- 3 *Trilobatus* sp., a – test in reflected light, b – test saturated with refractive index oil ( $n = 1.636$ ), mounted in QSMM ( $n = 1.662$ ) and viewed in transmitted light, c – enlarged view of initial part of the test, showing the proloculus and coiling of the initial chambers. Holocene; Vema Cruz # 19, Latitude 4°S Longitude 5°E, sample 45.
- 4 *Archaias angulatus* (Fichtel and Moll), a – test in reflected light, b – test saturated with refractive index oil ( $n = 1.636$ ), mounted in QSMM ( $n = 1.662$ ) and viewed in reflected light, showing vortex, spiral chambers and their subdivision into chamberlets (terminology in the sense of Hottinger 2006), c – a portion of the test viewed in transmitted light showing chamberlets and pores. Holocene; Florida Bay, shallow water bottom sample TAF-236.
- 5 *Higumastra* sp., a – test in reflected light, b – test mounted dry in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing the internal structure. Despite no vacuum treatment, this specimen retained no bubbles. Middle Jurassic, Bajocian; Oregon, Seneca, Snowshoe Formation, locality OR-549.
- 6 *Lenticulina* sp., a – test in reflected light, b – test saturated with refractive index oil ( $n = 1.636$ ), mounted in QSMM ( $n = 1.662$ ) and viewed in transmitted light, showing proloculus, internal coiling, and thick keel (or marginal furrow). Holocene; Vema Cruz # 19, Latitude 4°S Longitude 5°E, sample 45.
- 7 *Latentifistula* sp., a – test in reflected light, b – test saturated in mineral oil ( $n = 1.48$ ), mounted in QSMM ( $n = 1.539$ ) and viewed in transmitted light, showing the layers of the patagial wedge. Middle Permian, Roadian; West Texas, Guadalupe Mountains, Cutoff Formation, Williams Ranch Member, Quarry section, sample LC.
- 8 *Rectotortum delicatum* (Nazarov and Ormiston), a – test in reflected light, b – test saturated with refractive index oil ( $n = 1.636$ ), mounted in QSMM ( $n = 1.662$ ) and viewed in transmitted light, showing internal layers. Middle Permian, Roadian; West Texas, Guadalupe Mountains, Cutoff Formation, Williams Ranch Member, Quarry section, sample LC.
- 9 *Creseis* sp., a – shell in reflected light, b – shell saturated with refractive index oil ( $n = 1.636$ ), mounted in QSMM ( $n = 1.662$ ) and viewed in transmitted light, showing thin wall and interior with some planktonic foraminifers. Holocene; Vema Cruz # 19, Latitude 4°S Longitude 5°E, sample 45.

