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Editorial

# Introduction to the Special Issue entitled "Benthic Foraminiferal Ultrastructure Studies"

Foraminifera are unicellular eukaryotes. These protists have membrane-bound nuclei, a microtubule-based cytoskeleton, intracellular membranous organelles, and anastamosing granuloreticulose pseudopodia. The name derives from the combination of two neo-Latin terms ("foramen", meaning an opening, hole or passage; "fer", meaning bearing), equating to "hole-bearing", referring to the presence of a hole in each chamber wall of the mineralized shells observed by Alcide d'Orbigny, who coined the moniker in 1826 (Lipps et al., 2011). The shell, formally called a "test", is typically considered as protection to the foraminiferal cell and commonly preserved in the geological record. Easily fossilized tests can be composed of either calcium carbonate secreted by the foraminifer, or mineral grains that are adhered with either organic or carbonate cement. Foraminifera with carbonate tests are informally referred to as "calcareous"; those forms that bind inorganic grains are "agglutinated", most of which are multichambered. Some foraminifera such as the "thecate" allogromiids and "naked" forms, have organic tests that do not fossilize well because of the lack of mineralized test components. Most of these organic-walled forms are single chambered, or "monothalamous". These "soft-walled" forms have largely been overlooked until recent decades. There are instances where monothalamid forms also have rigid agglutinated tests (Bowser et al., 1995). For a recent classification of foraminifera, the reader is directed to Pawlowski et al. (2013).

Foraminifera are nearly ubiquitous in the marine environment. To our knowledge, more limited representation exists in freshwater and terrestrial habitats. Thecate forms are generally marine while naked forms are generally freshwater or terrestrial. Three informal groups may be differentiated among common foraminiferal forms, based according to their habitats and life strategies: 1) planktonics, which live in the upper water column of the open ocean, 2) large benthic foraminifera (LBF) living on the seafloor in warm shallow waters and bearing photosynthetic symbionts, 3) "small" benthic foraminifera (SBF), which typically lack photosynthetic symbionts and live in or on substrate such as unconsolidated "soft" sediment or megafauna and megaflora (seaweed). We focus this special issue on the ultrastructure of "small" marine benthic foraminifera; we do not extensively consider small benthic freshwater or terrestrial foraminifera.

During the mid-1800s foraminifera became widely appreciated, with studies by researchers such as Christian Gottfried Ehrenberg, William Benjamin Carpenter, and Henry Bowman Brady. Due to their long and diverse fossil record, much early research on foraminifera focused on biostratigraphy and paleoecology, eventually as a means to identify source rocks for hydrocarbon reserves. The global H. M. S. *Challenger* Expedition (1872–1876) collected hundreds of samples that received devoted study over decades by experts on every marine life form, including foraminifera (e.g., Brady, 1884, see also updated report by Jones (1994)). Naturalists such as Ernst Haeckel popularized foraminifera at the turn of the century by including them in their artistic scientific illustrations. Because foraminifera can be abundant in shallow to deep waters from low to high latitude, their distribution and ecology also received concentrated research efforts by investigators such as Joseph A. Cushman, in the early to mid-1900s. Beginning in the middle of the last century, the chemical records of foraminiferal carbonate tests were used extensively to decipher past oceanographic and paleoclimate conditions (reviewed in Katz et al., 2010). For more thorough insights into the history of foraminiferal research, the reader is directed to recent compilations and historical perspectives (e.g., Bowden et al., 2014).

Due to the nineteenth century and twentieth century focus on fossil, relict and modern foraminiferal remains, relatively little research was done on the living organism. Research of the past few decades has revealed diverse foraminiferal adaptations, in the context of cell biology and physiology, especially to chemocline habitats and additional "stressful" environmental conditions. Such adaptations implicate their importance to biogeochemical cycling.

## 1. A short history of transmission electron microscopy

A transmission electron microscope (TEM) uses an electron beam to pass (or transmit) through a specimen, to produce an image that is dependent on the interaction of the beam with the specimen; the image is subsequently magnified for viewing. Materials investigated by TEM are typically very thin, either being a thin slice ($< 0.1$ μm) of a larger specimen or very fine particles accumulated on an appropriate surface (i.e., grid).

The first functional TEM was designed, built, and operated in the early 1930s by Ernst Ruska (Flegler et al., 1993). The development of the TEM was so influential that, in 1986, Ruska was co-recipient of the Nobel Prize in Physics (Bozzola and Russell, 1999). With the TEM, the use of an electron beam to image an item of interest allowed investigations at a much higher magnification than light microscopy. The resolution of the electron microscope was approximately three orders of magnitude higher than the light microscopes of the 1940s (Flegler et al., 1993). This ability to view materials at a much higher resolution resulted in unprecedented observations, opening entirely novel avenues of investigation. By the 1950s,

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the TEM was being used in many scientific disciplines, including biology, medicine, geology, and material sciences.

Over the years, the instrumentation has seen vast improvements and modifications that are beyond the scope of this short introduction. In sum, transmission electron microscopy has evolved to include a wide range of instrumentation, including High Voltage EM (HVEM), Low Voltage EM, Cryo-TEM, and Scanning TEM (STEM). These instruments can be paired with additional equipment such as an energy dispersive X-ray spectroscopy detector for elemental mapping and chemical characterization. Information on these methodologies is also beyond the scope of this contribution.

Use of the TEM relies not only on the instrumentation, but also on specimen preparation. Because most electron microscopes require a vacuum, and biological specimens are composed mostly of water, preparing specimens properly is crucial to successful TEM investigations. Different biological materials demand different preparation procedures. For example, even within the foraminifera, different protocols are required to adequately remove the test, if necessary. While this special issue includes information on foraminiferal preparations for TEM, the reader is directed to the primary literature for further details.

## 2. Brief history of foraminiferal TEM studies

To our knowledge, the first TEM investigations of foraminiferal cells were in the 1960s (e.g., Wohlfarth-Bottermann, 1961). In 1965, Lee et al. (1965) described for the first time in the literature the general ultrastructure of two planktonic (calcareous) foraminifera. In 1967, Robert Angell published two works on the calcareous benthic *Rosalina floridana*, being the first to document foraminiferal calcification and chamber addition using the TEM (Angell, 1967a,b). Beginning in the late 1960s, several authors used the TEM to study the ultrastructure of soft-walled foraminifera (Table 1). The 1970s, 1980s and early 1990s saw numerous investigations into benthic foraminiferal reticulopods, extensively reviewed by Travis and Bowser (1991) and Bowser and Travis (2000).

Between 1974 and 1990, the number of publications about the ultrastructure of LBF and planktonics, and their photosynthetic symbionts, increased markedly (e.g., Lee et al., 1974, 1979, 1980a,b; Anderson and Bé, 1976a,b; Lee and Bock, 1976; Müller-Merz and Lee, 1976; Schmaljohann and Röttger, 1978; McEnery and Lee, 1981; Bé et al., 1982; Leutenegger, 1977a,b, 1983, 1984; Hemleben et al., 1985; Spero, 1987; Faber et al., 1988, 1989; Lee, 1990; Lee and Anderson, 1991).

Reviews of foraminiferal biology and cell structure exist mainly on planktonic foraminifera and LBFs. In 1977, important reviews were published by Leutenegger (1977a,b) on the ultrastructure of LBFs and planktonic foraminifera and their photosynthetic symbionts. Anderson and Bé (1978) and Anderson and Lee (1991) both describe the main organelles and features observed in foraminiferal cells (e.g., nuclei, ribosomes, endoplasmic reticulum, Golgi, lysosomes and digestive vacuoles, peroxisomes, mitochondria, fibrillar bodies, cytoskeletal structures) but the source of all their TEM images except for one was planktonic foraminifera or LBF. Hemleben et al. (1989) and Schiebel and Hemleben (2017) thoroughly described the cytology of planktonic foraminifera.

A published review presenting the ultrastructure of SBF does not exist, to our knowledge. Here we present a compilation of representative publications that present at least one micrograph of smaller benthic foraminiferal ultrastructure (Table 1). Using information from Table 1, we note that before 1990, most of the major published studies with the TEM micrographs of SBF ultrastructure were based on soft-walled species (Fig. 1) mainly studying attributes to the cell membrane and/or test wall, especially reticulopods (Fig. 2). Starting in the 1990s, increasingly more studies on calcareous SBF ultrastructure were published (Fig. 1) and, while these often focused on specific organelle type(s), some also focused on functions such as reproduction (Fig. 2). Also, in the 1990s, an interest to study cellular adaptations to specific environmental conditions increased. Much of this interest was and is presently focused on the response of foraminiferal ultrastructure to oxygen-depletion in either natural or laboratory-controlled experimental settings. In the 2010s, many correlative investigations are combining innovative methodologies with the TEM.

In general, multilocular (multichambered) agglutinated foraminifera are not well studied by TEM methods (Fig. 1) generally due to the added complexities of removing their test. Even large agglutinated foraminifera, such as the Xenophyophores and komokiaceans, have rarely been the subject of a TEM study (e.g., Lecroq et al., 2009), presumably due to the issues with recovery of deep-water materials.

## 3. Why this special issue?

Of course all foraminifera have similar overall cellular ultrastructure, including organelles typical to most eukaryotic cells. Additionally, the cellular ultrastructure of symbiont-bearing planktonic foraminifera and LBF are highly similar because both predominantly host photosynthetic symbionts. As noted, published reviews of foraminiferal ultrastructure are based on TEM micrographs of LBF (e.g., Leutenegger, 1977b) and planktonic foraminifera (Hemleben et al., 1989); a compilation of studies presenting TEM micrographs of SBF does not exist. This special issue is intended to fill the gap in existing reviews of SBF ultrastructure in a general context.

To improve our knowledge of the role of foraminifera in biogeochemical cycling and ecosystem functioning, it is imperative that we understand their physiology, metabolism and ecology. Such information is also critical to understanding foraminiferal biomineralization and geochemical signatures. One powerful means to better understand foraminiferal physiology, metabolism and ecology is to return to cell-scale studies, using transmission electron microscopy in combination with additional state-of-the-art imaging approaches.

This special issue includes the following contributions. An overview of typical smaller benthic foraminiferal ultrastructure and organelles is presented in LeKieffre et al., which also includes observations on some fine-scale structures of unknown function. An overview and synthesis of the observed associations between smaller benthic foraminifera and prokaryotes, as symbionts or parasites, is presented by Bernhard et al. A synthesis regarding a type of specialized “symbiosis” involving chloroplast sequestration is presented for shallow-water smaller benthic foraminiferal species (Jauffrais et al.). High-pressure freezing and freeze substitution (HPF-FS) were used to document the ultrastructure of the chloroplast-sequestering *Haynesina germanica* (Goldstein and Richardson); this contribution also includes some comparisons between conventional chemical fixation and the HPF-FS approach. The ultrastructural response of *Ammonia* spp. to anoxia is presented by Koho et al. Similarly, the ultrastructural response of selected smaller benthic foraminifera to heavy metals is presented by Frontalini et al. Finally, an overview of methods that merge the TEM with additional powerful analytical tools is presented in Nomaki et al.

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