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Research paper

An overview of cellular ultrastructure in benthic foraminifera: New observations of rotalid species in the context of existing literature

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ABSTRACT

We report systematic transmission electron microscope (TEM) observations of the cellular ultrastructure of selected, small rotalid benthic foraminifera. Nine species from different environments (intertidal mudflat, fjord, and basin) were investigated: *Ammonia* sp., *Elphidium oceanense*, *Haynesina germanica*, *Bulimina marginata*, *Globobulimina* sp., *Nonionellina labradorica*, *Nonionella* sp., *Stainforthia fusiformis* and *Buliminella tenuata*. All the observed specimens were fixed just after collection from their natural habitats allowing description of intact and healthy cells. Foraminiferal organelles can be divided into two broad categories: (1) organelles that are present in all eukaryotes, such as the nuclei, mitochondria, endoplasmic reticulum, Golgi apparatus, and peroxisomes; and (2) organelles observed in all foraminifera but not common in all eukaryotic cells, generally with unknown function, such as fibrillar vesicles or electron-opaque bodies. Although the organelles of the first category were observed in all the observed species, their appearance varies. For example, subcellular compartments linked to feeding and metabolism exhibited different sizes and shapes between species, likely due to differences in their diet and/or trophic mechanisms. The organelles of the second category are common in all foraminiferal species investigated and, according to the literature, are frequently present in the cytoplasm of many different species, both benthic and planktonic. This study, thus, provides a detailed overview of the major ultrastructural components in benthic foraminiferal cells from a variety of marine environments, and also highlights the need for further research to better understand the function and role of the various organelles in these fascinating organisms.

1. Introduction

Despite a number of studies regarding the ultrastructure of benthic foraminifera revealed by transmission electron microscope (TEM) observations, only a small fraction of the organelles in these single-celled organisms have been identified and their function understood (see compiled review of prior publications in Bernhard and Geslin, this issue. Recent studies have attempted to correlate ultrastructural imaging of the cytoplasmic structures to physiological processes using correlative imaging approaches (Bernhard and Bowser, 2008; LeKieffre et al., 2017; Nomaki et al., 2016). The inability to confidently link form and structure with function warrants an improved understanding of the foraminiferal cell at the ultrastructural level.

Based on the literature, we present here an inventory of the common organelles found in benthic foraminifera, to which we add new TEM observations on the cytoplasm of nine benthic rotalid foraminiferal species. These foraminifera were sampled in three different locations and environments (Fig. 1 and Table 1): *Ammonia* sp. (phylotype T6, Hayward et al., 2004; Holzmann, 2000), *Elphidium oceanense* (*excavatum* species complex, Darling et al., 2016), and *Haynesina germanica* (Ehrenberg, 1980) from an intertidal mudflat in Bourgneuf Bay on the Atlantic coast of France; *Bulimina marginata* (d'Orbigny, 1826), *Globobulimina* sp., *Nonionellina labradorica* (Dawson, 1860), *Nonionella* sp., and *Stainforthia fusiformis* (Williamson, 1848) from the Gullmar Fjord in

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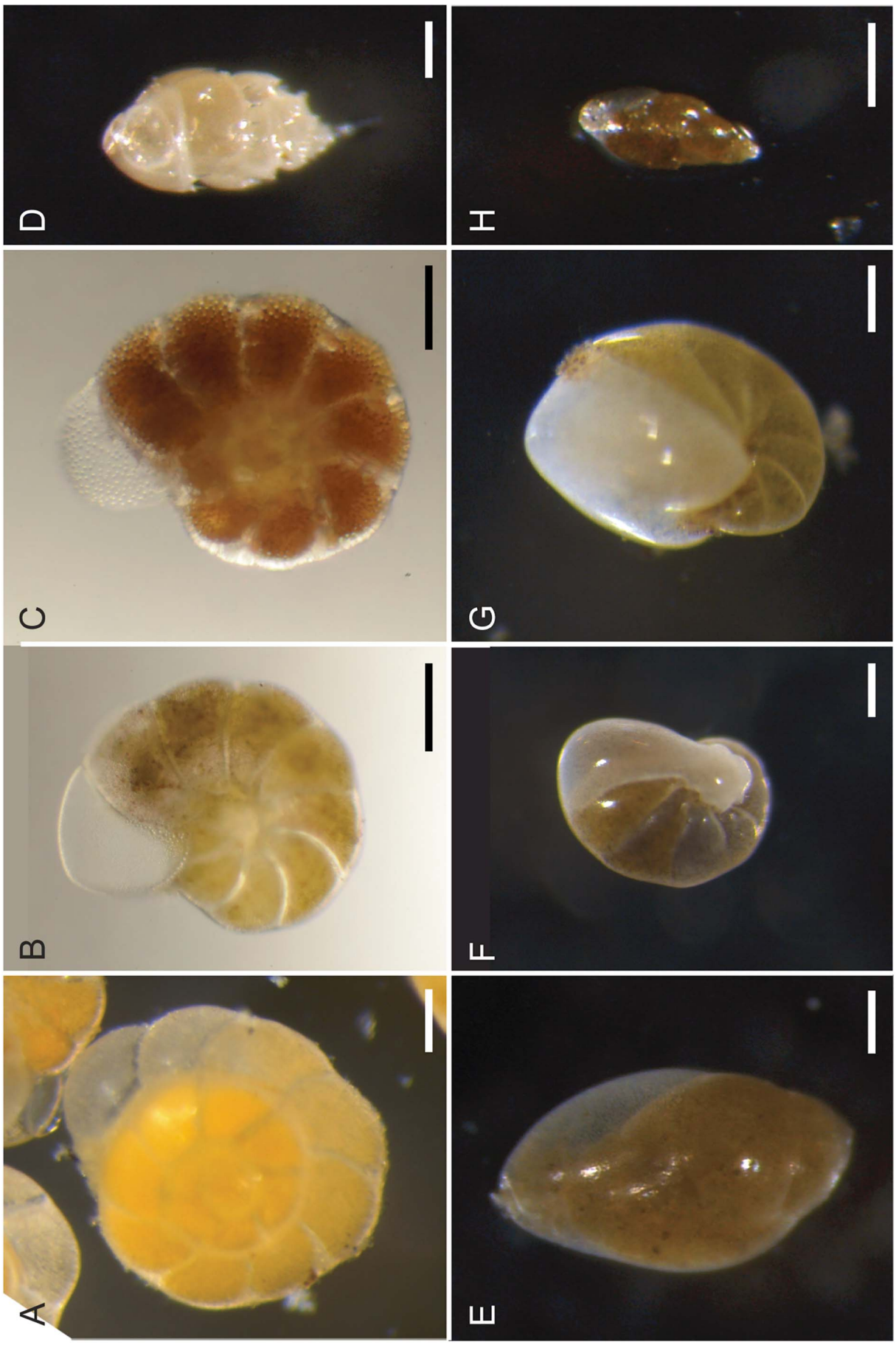

Fig. 1. Light micrographs of the foraminifera from the Atlantic French coast intertidal mudflat and the Gullmar fjord.
A: *Ammonia* sp. (phylotype T6); B: *Haynesina germanica*; C: *Elphidium oceanense* (*excavatum* species complex), D: *Bulimina marginata*; E: *Globobulimina* sp.; F: *Nonionella* sp.; G: *Nonionellina labradorica*; H: *Stainforthia fusiformis*. Scales: A–D, F = 100 μm; E, G, H = 200 μm.

Table 1
Species, sampling location, and number of specimens observed using TEM or reported in this study.

Species	Site	Numbers of specimens analyzed
Ammonia sp. (phylotype T6)	Bourgneuf Bay, Atlantic coast, intertidal mudflat, France	3
Elphidium oceanense (excavatum species complex)		3
Haynesina germanica		4
Bulimina marginata	Gullmar Fjord, Sweden (70 m/117 m depth)	1
Globobulimina sp.		3
Nonionellina labradorica		6
Nonionella sp.		4
Stainforthia fusiformis		3
Buliminella tenuata	Santa Barbara Basin, California, USA (approximately 580–598 m) (Bernhard and Bowser, 2008)	3

Sweden, which is a silled basin with restricted circulation, and *Buliminella tenuata* from the silled, typically stagnant, Santa Barbara Basin (USA, samples from Bernhard and Bowser, 2008). These species are all small (< 500 μm) rotalid benthic foraminifera for which a description of each typical organelle is presented along with discussion of their known or inferred function(s).

2. Material and methods

2.1. Collection sites

Foraminifera from intertidal environments (*Ammonia* sp., *E. oceanense* and *H. germanica*) were sampled on March 7, 2016, on the intertidal mudflat in the bay of Bourgneuf at la Couplasse station (Loire-Atlantique, France; 47°0′47″N, 2°1′17″W). The top 2 cm of sediment was collected at low tide, transferred to plastic jars, and immediately chemically fixed (1:1 volume sediment: fixative solution; see below). Living foraminifera from the Gullmar fjord, Sweden (*B. marginata*, *Globobulimina* sp., *N. labradorica*, *Nonionella* sp. and *S. fusiformis*) were collected on October 22, 2014, at two locations using the R/V *Skagerak* of the Sven Lovén Centre for Marine Science (University of Gothenburg): site DF3 (117-m depth; 58°19.096′N, 11°32.398′E) and site DF70-2 (70-m depth, 58°17.071′N, 11°30.636′E). At the time of collection, both locations were characterized by a temperature of approximately 8 °C and salinity of 34.6. Sediment samples were collected with a box corer. Immediately after the cores were brought on board, the top 2 cm (approximately) of the sediment was collected in plastic jars and chemically fixed (1:1 volume sediment: fixative solution; see below). *Buliminella tenuata* specimens were collected as described in Bernhard and Bowser (2008) from sediments in the Santa Barbara Basin (centered on 34°16′N, 120°02′W).

2.2. Chemical fixation and TEM observations

The fixative solution contained 4% glutaraldehyde and 2% paraformaldehyde diluted in cacodylate buffer solution (NaCaco 0.1 M, Sucrose 0.4 M, NaCl 0.1 M). Following fixation and rinsing with the cacodylate buffer, cytoplasm-bearing foraminifera were, based on the color of their cytoplasm, picked using a binocular microscope (Leica, M165C) and transferred individually into microtubes for decalcification in 0.1 M Ethylenediaminetetraacetic Acid (EDTA) diluted in 0.1 M cacodylate buffer solution. They were then post-fixed with 2% osmium tetroxide (OsO_4) for 1 h at room temperature, dehydrated in successive ethanol baths (50, 70, 95 and 100%) and finally embedded in LR White acrylic resin. Embedded individuals were cut into 70-nm sections with an ultramicrotome (Reichert ultracut S) equipped with a diamond knife (Diatome, Ultra 45°) and placed on formvar-carbon coated copper TEM slot grids. The sections were post-stained for 10 mins with 2% uranyl acetate and observed with a transmission electron microscope (TEM), either a Philips 301 CM100 at the Electron Microscopy Facility of the University of Lausanne (Switzerland) or a JEOL JEM 1400 at the SCIAM (Service Commun d'Imagerie et d'Analyses Microscopiques) platform at the University of Angers (France). Semi-thin sections (500 nm) for light microscopy observation were also cut and stained with toluidine blue and basic fuchsin. Both thin and semi-thin sections were taken in the middle of the foraminiferal cell in order to obtain sections bisecting the maximum number of chambers. *Buliminella tenuata* specimens were processed, and imaged as described in Bernhard and Bowser (2008).

3. TEM observations of benthic foraminiferal cells

The TEM micrographs presented in this study depict foraminifera that were alive when collected and preserved. The vitality of each specimen was checked by observing the appearance of mitochondria and membranes as described by Nomaki et al. (2016). Only specimens with well-preserved mitochondria and membranes are reported here.

Two regions of cytoplasm in foraminifera are usually distinguished in thin sections: the cell body (located inside the test (shell); also called “endoplasm”) and the reticulopodial net (reticulated pseudopods) typically, but not always, located outside the test (the reticulopodial net can also be gathered within the younger chambers of the foraminifer) (Alexander and Banner, 1984; Anderson and Lee, 1991). The cell body is usually denser (i.e., contains more electron-opaque organelles and thus appears darker in TEM images) than reticulopodial net, which has a granular appearance (Alexander and Banner, 1984). The cell body is the focus of the present study.

3.1. Cell body and “empty” vacuoles

The appearance of the cell body was highly variable among foraminiferal species, both between conspecifics and between chambers within a single individual. A main difference between intertidal and fjord/basin species was the numerous, large (between 10 and 200 μm in diameter) empty vacuoles, which were typical in the cell body of most of the fjord species, except *B. marginata* (Fig. 2). These vacuoles certainly had lost their soluble compounds during sample preparation. Similar, albeit smaller (i.e., 5–20 μm diameter) vacuoles were sometimes observed in the youngest chambers of species from intertidal mudflats (for instance in *H. germanica* and *E. oceanense*, Fig. 2B and C). Portions of the cell body in the two or three youngest chambers of a foraminifera had a particular appearance: they contained very few lipid droplets but more “empty” and degradation vacuoles (and thus appeared less dense) than the cell body portions of older chambers. Besides these cell body portions of the youngest chambers often had a patchy aspect: that is, more electron-opaque organelles were grouped in certain areas within the cell body, thus appearing darker in TEM images than the surrounding cytosol (e.g. Fig. 2C).

Because *Globobulimina* sp. from Gullmar Fjord and several other species inhabiting the same type of environment, such as *Stainforthia fusiformis* or *Nonionella* sp., are known to store nitrate in their cell (Piña-Ochoa et al., 2010), it is possible that the large “empty” vacuoles play a role as internal reservoirs of nitrate, as suggested by Bernhard et al. (2012). The intertidal species *Ammonia tepida* (same type T6 as our specimens) and *Haynesina germanica* are not known to contain any intracellular nitrate (Piña-Ochoa et al., 2010). Thus the vacuoles observed in the cell body of intertidal species may have a different origin and/or function than the common, large “empty” vacuoles observed in fjord specimens. As hypothesized in some studies (e.g., Erez, 2003; Bentov et al., 2009), the vacuoles in intertidal species could serve as storage for ions intended for test formation inside the foraminiferal cell body (pool of HCO_3^- ions in *Amphistegina lobifera*). The brighter appearance of the

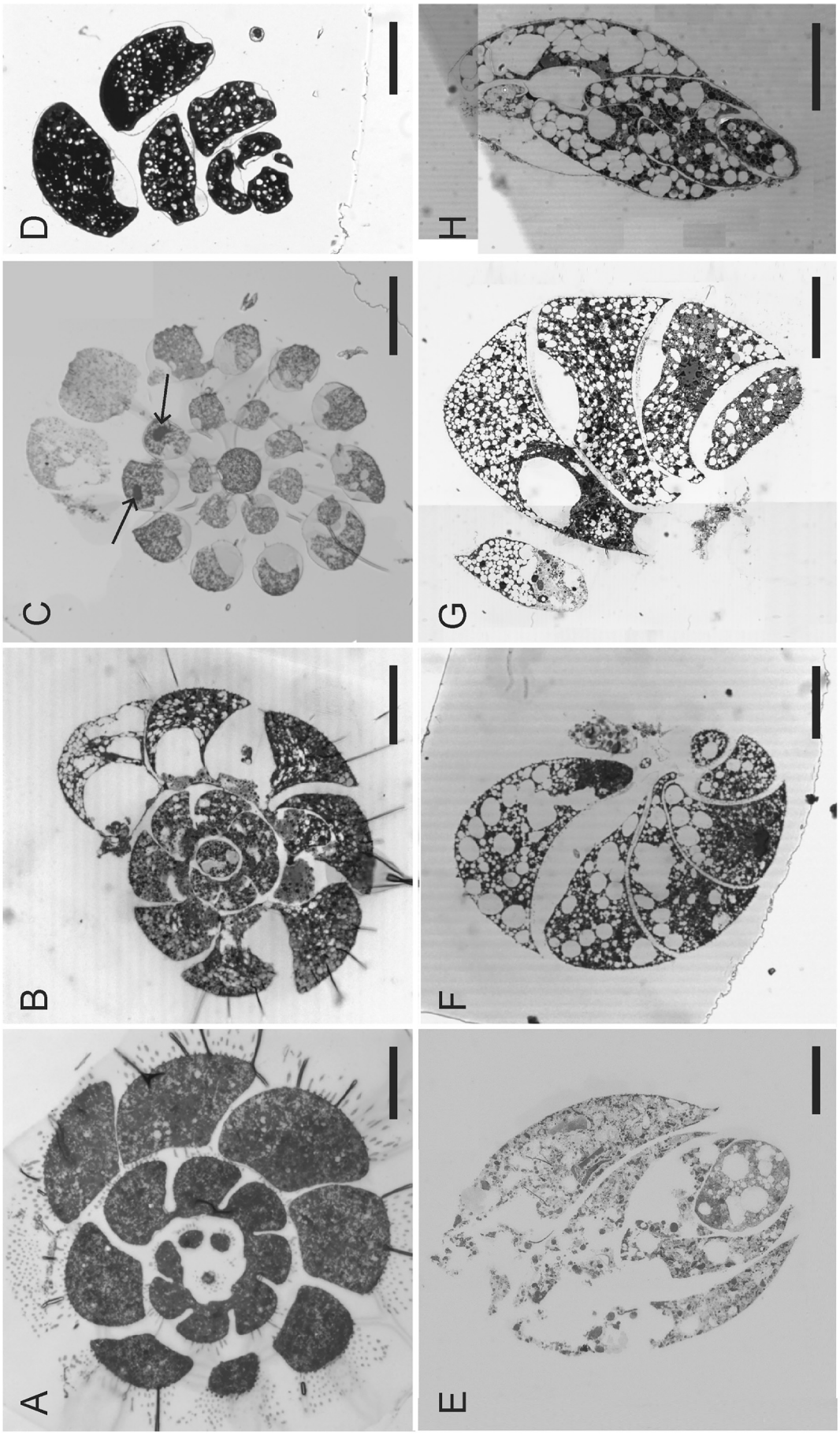

Fig. 2. Light micrographs of semi-thin sections of the foraminifera from the Atlantic French coast intertidal mudflat and the Gullmar fjord.
A: *Ammonia* sp. (phylotype T6); B: *Haynesina germanica*; C: *Elphidium oceanense* (*excavatum* species complex), D: *Bulimina marginata*; E: *Globobulimina* sp.; F: *Nonionella* sp.; G: *Nonionellina labradorica*; F: *Stainforthia fusiformis*. Arrows: nuclei. Scales: A, B, C, E, F, H = 100 μm; D, G = 200 μm.

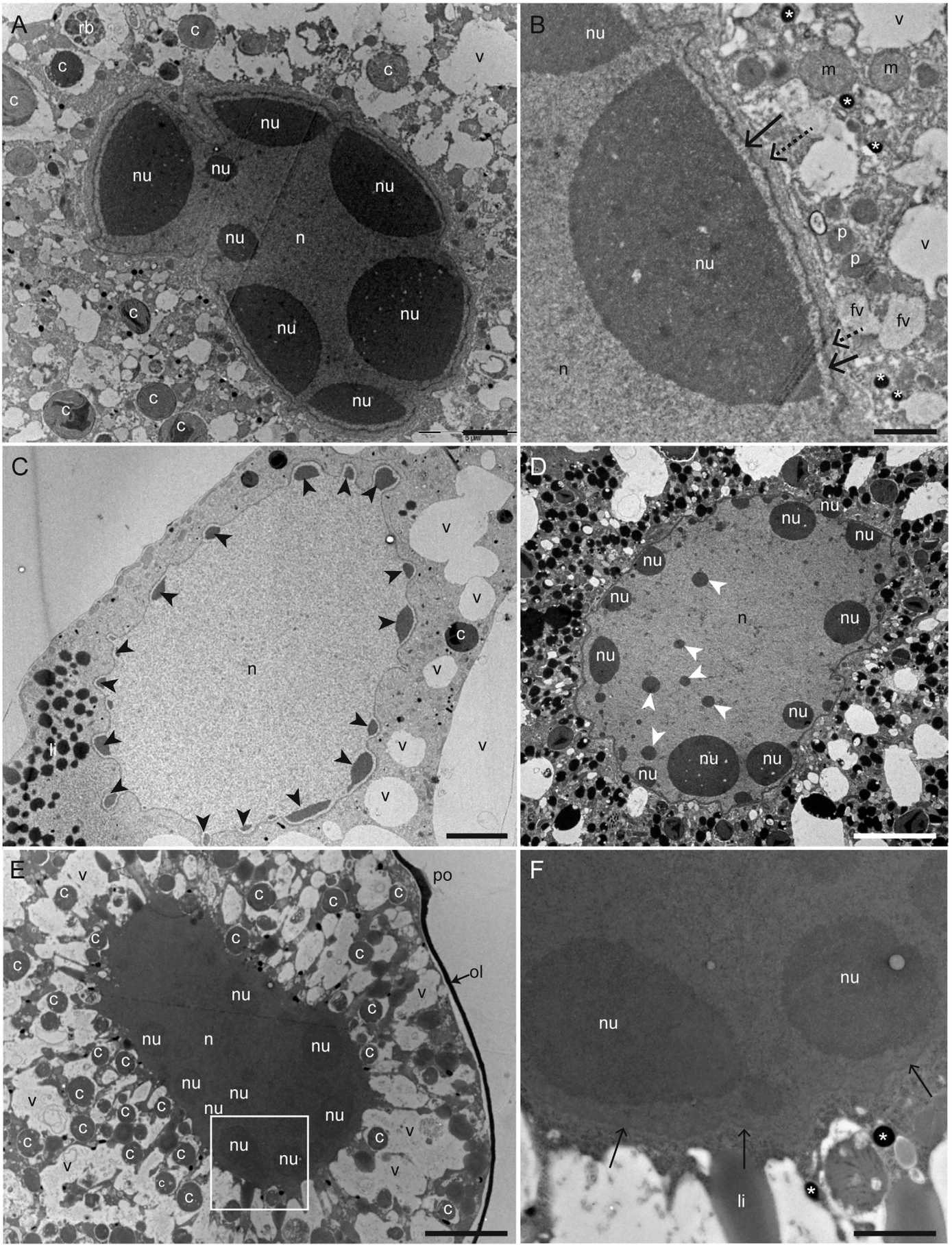

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Fig. 3. Transmission electron micrographs of benthic foraminiferal nuclei.
A: *Nonionella* sp. nucleus with numerous nucleoli. B: Higher magnification of *Nonionella* sp. nucleus showing a nucleolus at the nucleus periphery and the nuclear envelope (arrows) with a thin cytoplasmic layer (dotted arrows). C: *S. fusiformis* nucleus with numerous small peripheral nucleoli (black arrowheads). D: *Nonionellina labradorica* nucleus with a few small central nucleoli (white arrowheads). E: *Elphidium oceanense* nucleus with "cytoplasmic outgrowths". F: Higher magnification of the nucleus of *Elphidium oceanense* (arrows: nuclear envelope). Arrows: nuclear envelope, dotted arrows: thin cytoplasmic layer, white asterisks: electron opaque bodies, c: chloroplasts, fv: fibrillar vesicles, li: lipid droplets, m: mitochondria, n: nucleus, nu: nucleolus, ol: organic lining, p: peroxisomes, po: pore, rb: residual bodies, v: vacuoles. Scales: A = 2 μm; B, F = 1 μm; C, E = 5 μm; D = 10 μm.

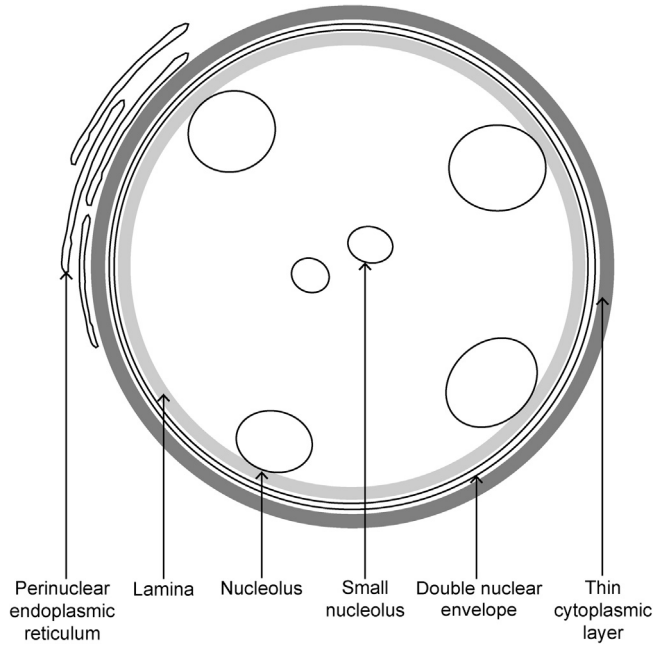

Fig. 4. Schematic ultrastructure of a foraminiferal nucleus.
The double nuclear envelope is surrounded by a thin cytoplasmic layer against which perinuclear endoplasmic reticulum stand in a continuous or intermittent layer. On the inner part of the nuclear envelope a lamina can be seen, separating the peripheral nucleoli from the envelope. Smaller nucleoli can also occupy a central position. The nucleus is represented in a circular shape for convenience as it can also be in a lobate form (see details in the text).

cell body portions of the youngest chambers is likely due to its location near the aperture from where the reticulopods extend, as this requires the presence of numerous microtubules which are not electron-dense organelles (e.g., Alexander and Banner, 1984). The numerous degradation vacuoles sometimes observed can also be explained by the proximity to the aperture, where food is internalized by phagocytosis.

3.2. Organelles with known function

3.2.1. Nucleus

The nucleus can be located at different locations within the foraminiferal cell, from older to younger chambers in multi-chambered species (reviewed in Anderson and Lee, 1991). In our spiral-shaped foraminifera, the nucleus in one *Ammonia* sp. (phylotype T6) and two *E. oceanense* occurred in chambers of the penultimate whorl. In all specimens where a nucleus was observed, it seemed that these individuals were uninucleate, except for one *E. oceanense* where two nuclei were noted (Fig. 2C). The uninucleate feature seemed to be typical for foraminiferal gamonts (e.g. McEnery and Lee, 1976; Goldstein, 1988, 1997; Goldstein et al., 2010; Raikov et al., 1998). The observed nuclei exhibited various shapes, from nearly spherical to partially lobate. Published accounts also document nuclei with various shapes, from spherical to multilobate (reviewed in Raikov et al., 1998). The lobate forms may derive from the spherical form in response to an increased need of membrane before gametogenesis (Raikov et al., 1998).

In the rotalid species investigated in this study, the nucleus had a size typically ranging from about 20 to 50 μm in diameter with numerous nucleoli and a lamina (Figs. 3A and 4), which is in the same range as previously observed nuclei in different monothalamous or rotalid species (e.g., Altin et al., 2009; Altin-Ballero et al., 2013; Anderson and Lee, 1991; Dahlgren, 1967a, b; Goldstein and Richardson, 2002; Goldstein et al., 2010; Raikov et al., 1998; Schwab, 1972; Schwab and Schwab-Stey, 1979). The lamina is a layer of nucleoplasm (i.e., matrix of the nucleus) in contact with the inner membrane of the nuclear envelope (Fig. 4). Although the lamina appears very similar to the nucleoplasm, the former is easily visualized because of the space it creates between the nuclear envelope and the nucleoli (Fig. 3B). The lamina between the nucleoli and the inner membrane has been described in other species (e.g., Dahlgren, 1967a, b. In the allogromiid foraminifer *Myxotheca* sp. a "prominent non-chromatin containing" space was also observed between the nucleoli and the nuclear envelope but was not interpreted as a lamina (Goldstein and Richardson, 2002). However it might not exist in all species because it was not observed in *Heterostegina depressa* and *Globobulimina turgida* (Hottinger and Dreher, 1974). At higher magnification (Fig. 3B), the double-membrane nuclear envelope was observable. The distribution of the nucleoli in the nucleus varied among species: all the nucleoli we observed were distributed at the periphery of the nucleus, flattened against the lamina or nuclear envelope as illustrated for *S. fusiformis* (Fig. 3C). Sometimes, however, additional small nucleoli were seen in the central part of the nucleus as in *N. labradorica* (Fig. 3D). The literature also notes that different distributions of nucleoli occur in different species (see Raikov et al., 1998). For example, Spindler et al. (1978) described a central dispersion of the nucleoli in the planktonic species *Hastigerina pelagica*, without nucleoli adjacent to the nuclear envelope, while Anderson and Lee (1991) reported nucleoli distribution either centrally or at the periphery of the nucleus. A peripheral repartition of nucleoli was also observed in the monothalamous *Hippocrepinella alba*, the rotalid *Globobulimina turgida* (Dahlgren, 1967b) and the allogromiid *Hyperammina* sp. (Goldstein and Richardson, 2002).

Perinuclear endoplasmic reticulum was often noted on the outside of the nuclear envelope (see Section 3.2.3). A thin layer (approximately200 nm) of cytoplasmic material with a particular fibrous aspect surrounded the nuclei in some species, such as *Nonionella* sp. or *Nonionellina labradorica* (Fig. 3A, B and D). This layer was absent from the nucleus of *S. fusiformis* (Fig. 3C). The thin layer of fibrous cytoplasm around the nuclei has been described in larger benthic foraminifera (Soritidae) and planktonic species (Leutenegger, 1977) where it was separated from the cytosol by small vesicles or lacunas (electron-light cytoplasmic layer). Small vesicles surrounding the nucleolar membrane were also observed in different monothalamous species: *Psammophaga sapela*, *Xiphophaga minuta*, *Niveus flexilix*, *Myxotheca* sp., *Cribrothalammina alba* and *Hyperammina* sp. (Altin et al., 2009; Altin-Ballero et al., 2013; Goldstein and Richardson, 2002; Goldstein et al., 2010). However in the present study, the thin layer of cytoplasm with a fibrous aspect surrounding the nuclei in some rotalid species was not clearly separated from the rest of the cytosol and no accumulation of small vesicles was detected. This feature could correspond to the "nuclear villi" observed by Dahlgren (1967a) in the monothalamous species *Ovammina opaca* and described as protrusions projected into the cytoplasm from the nuclear envelope. The double nucleated *E. oceanense* exhibited atypical nuclei with outgrowths (Fig. 3E). At higher magnification (Fig. 3F), the circular nuclear envelope was distinguished between the nucleus and the "outgrowths". These "outgrowths" were formed of an electron-dense matrix and included various organelles like lipid droplets or electron-opaque bodies. This matrix was probably linked to the thin layer of cytoplasm, although its role remains

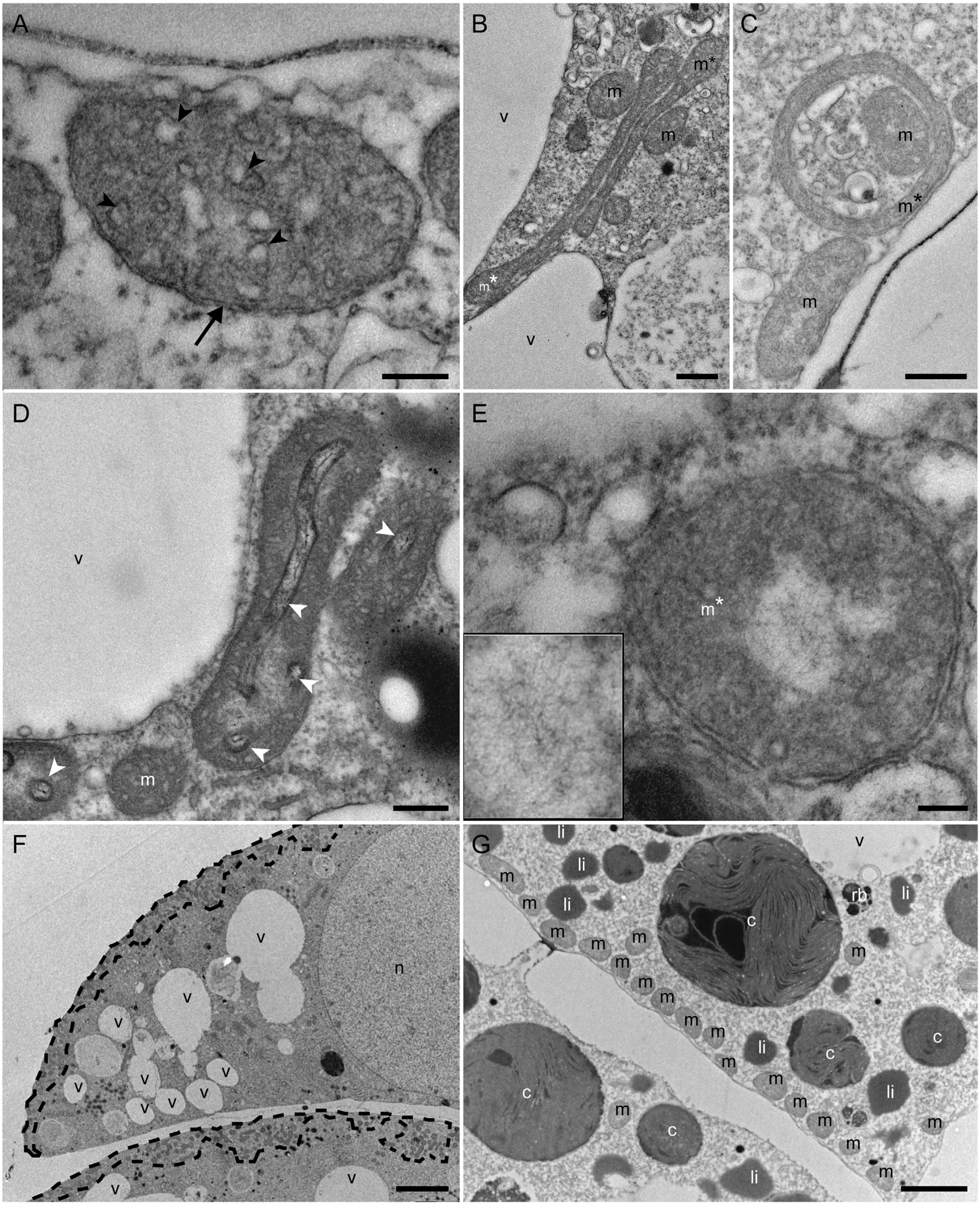

Fig. 5. Transmission electron micrographs of benthic foraminiferal mitochondria.
A: Classic structure of a mitochondrion observed in *Stainforthia fusiformis* (arrowheads: cristae, arrows: double membrane). B: Elongated mitochondria (asterisks) in *Nonionella* sp. C: Circular mitochondrion (asterisk) aside two classic mitochondria in *Nonionella* sp. D: Tubular inclusions (white arrowheads) in two mitochondria of a specimen of *Nonionellina labradorica*. E: Mitochondria with a less electron opaque central part (white asterisks) in *Nonionella* sp. Inset: High-magnification micrograph of the fibrils in this central part. F and G: Numerous mitochondria at the periphery of the chambers (areas surrounded by a dashed black line in F) in *Stainforthia fusiformis*. c: chloroplasts, m: mitochondria, li: lipid droplets, n: nucleus, rb: residual bodies, v: vacuoles. Scales: A, D = 200 nm; B, C = 500 nm; E = 100 nm; F = 5 µm; G = 2 µm.

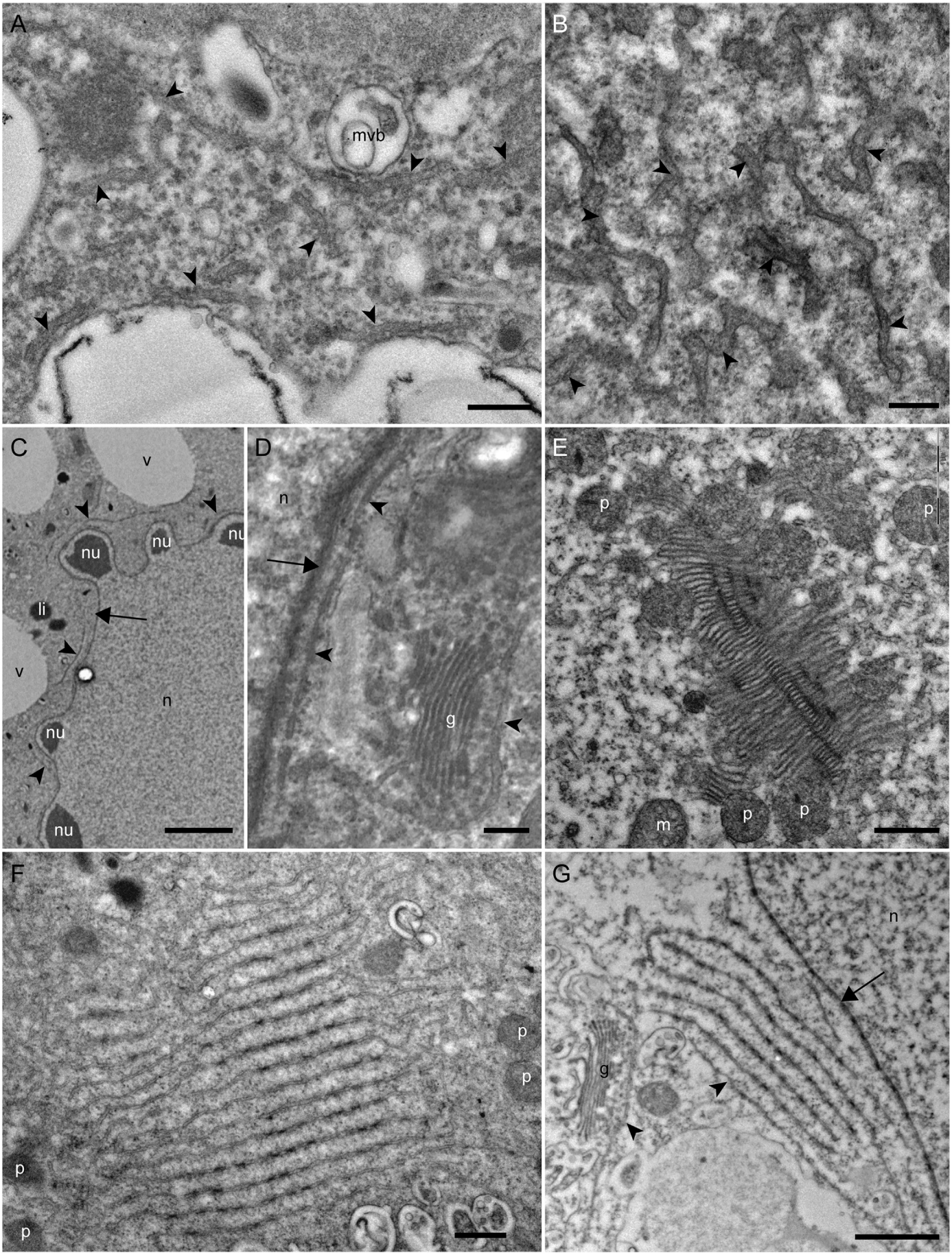

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Fig. 6. Transmission electron micrographs of benthic foraminiferal endoplasmic reticulum.
A: Area of the cytoplasm rich in endoplasmic reticulum in *Nonionella* sp. B: Higher magnification of endoplasmic reticulum in *Nonionellina labradorica*. C, D: Endoplasmic reticulum at the periphery of a nucleus in C: *Nonionellina labradorica* and D: *Stainforthia fusiformis*. E: Particular structure made of endoplasmic reticulum in one specimen of *Stainforthia fusiformis*. F, G: Endoplasmic reticulum stacked in a parallel pattern in another specimen of *Stainforthia fusiformis*. Black arrowheads: endoplasmic reticulum, arrows: nuclear envelope, g: Golgi apparatus, m: mitochondria, mvb: multivesicular bodies, n: nucleus, nu: nucleolus, li: lipid droplets, p: peroxisomes. Scales: A, B, D = 200 nm; C = 2 μm; E, F = 500 nm; G = 1 μm.

unknown. It could also be a fixation artifact.

The TEM micrographs of our study did not allow us to observe the pores of the nuclear envelope, but other studies have noted their presence in both planktonic and benthic species (e.g. Altin et al., 2009; Anderson and Lee, 1991; Dahlgren, 1967b; Goldstein, 1997; Hemleben et al., 1989; Leutenegger, 1977). These pores may allow communication with the cell body, in particular the migration of RNA (Anderson and Lee, 1991).

3.2.2. Mitochondria

Mitochondria were studied in all the observed specimens. As they are the site of Adenosine TriPhosphate (ATP) production (Sherratt, 1991), their presence and integrity are one of the main tools to attest to the vitality of the specimen at the time of fixation (Nomaki et al., 2016). Main features of mitochondria, which are also observed in our study (Fig. 5A), are their double membrane and the presence of cristae in their matrix (Sherratt, 1991). Mitochondria usually appeared oval or kidney shaped in cross section with a length in the range of 0.5 to 1 μm (Fig. 5A–C), although they can be bigger and take various shapes (Fig. 5B and C). In some foraminiferal species mitochondria with atypical morphologies could be observed. In two of the observed *N. labradorica*, the mitochondria exhibited tubular inclusions (Fig. 5D). Also, in other specimens of *N. labradorica,* as well as in *H. germanica* and *Nonionella* sp., a less electron opaque central area was seen in the mitochondria (Fig. 5E). At higher magnification, filaments could be identified in this less electron-dense central part (Fig. 5E inset).

In all analyzed specimens except those of *Stainforthia fusiformis*, the mitochondria seemed to be homogeneously distributed throughout the cell body. However in the three analyzed individuals of *S. fusiformis*, although a few mitochondria were seen dispersed through the entire cell body, most of them were clearly concentrated close to the plasma membrane of the external parts of the chambers, i.e., parts of the chambers that are in contact with the environment (or were in contact before the addition of a new chamber (Fig. 5F and G).

To our knowledge, the tubular inclusions observed in some mitochondria have not been described in any other type of organism. We suggest here that they could be elongated cristae. The less electron-opaque central part of mitochondria observed in some species of this study can also be seen in the mitochondria of the planktonic species *Hastigerina pelagica* and *Globigerinoides ruber* (Hemleben et al., 1989). Seen at higher magnification, the filaments resembled fibrils of mitochondrial DNA (Nass and Nass, 1963). The accumulation of mitochondria under the pores is known in some benthic foraminiferal species such as *Nonionella stella* and *Bolivina pacifica* (Bernhard et al., 2010a; Leutenegger and Hansen, 1979). This specific distribution was interpreted as an adaptation to low-oxygen environments. In the *S. fusiformis* specimens studied here, the mitochondria were not only distributed under the pores but all along the plasma membrane of the external part of the chambers. Thus we cannot conclude that a similar role occurs in this species.

3.2.3. Endoplasmic reticulum

Generally in eukaryotic cells, two types of endoplasmic reticulum occur: rough endoplasmic reticulum (RER) that has ribosomes on its membrane, and smooth endoplasmic reticulum (SER) that lacks ribosomes (Vertel et al., 1992). The former type is a site of protein synthesis, while the latter is involved in lipid synthesis and other synthesis activities (Vertel et al., 1992). Because the visualization of ribosomes at the resolution of the TEM is not assured, in this contribution both types will be grouped under the name of “endoplasmic reticulum”.

Endoplasmic reticulum (ER) was observed in all foraminiferal species studied here (e.g., Fig. 6A and B), and has been documented in many other foraminiferal species (reviewed in Anderson and Bé, 1978). The ER is very often observed associated with Golgi apparatus (Fig. 6D and E) and peroxisomes (Fig. 6E and F - see Sections 3.2.4 and 3.2.5). It was also present around each nucleus observed in this study, as shown in Fig. 6C and D. In Fig. 6E–G, structures apparently made of ER can be observed. These structures, all observed in *S. fusiformis* specimens, were formed of numerous ER cisternae stacked in parallel.

The particular association of ER with the nucleus was established in different foraminiferal species, rotalid or monothalamous (e.g. Altin et al., 2009; Altin-Ballero et al., 2013; Anderson and Bé, 1978; Dahlgren, 1967a, b; Hottinger and Dreher, 1974) and described as “perinuclear reticulum”, however it seems to be absent in some monothalamous species such as *Myxotheca* sp., *Cribrothalammina alba* and *Hyperammina* sp. (Goldstein and Richardson, 2002). In certain specimens of *S. fusiformis,* this perinuclear ER was intermittent (Fig. 6C), while in specimens of *N. labradorica* it formed a continuous layer around the nucleus (Fig. 6D). Finally, the ER organized in parallel stacks (Fig. 6E–G) were similar to the annulate lamellae of the planktonic foraminifer *Hastigerina pelagica* (d'Orbigny) (Anderson and Lee, 1991; Hemleben et al., 1989; Spindler and Hemleben, 1982). These annulate lamellae made of ER are formed before the gametogenesis of a foraminifera and may provide membranous material for the formation of the nuclear envelope of the new nuclei (Anderson and Lee, 1991; Hemleben et al., 1989; Spindler and Hemleben, 1982). Goldstein (1997) also described a pregametic nucleus surrounded by several layers of endoplasmic reticulum in the rotalid *Ammonia* sp. She also hypothesized that those structures are involved in foraminiferal gametogenesis.

3.2.4. Peroxisomes

Peroxisomes, which were observed in all the specimens from the three sites, are spherical vesicles, approximately 500 nm in diameter and containing a crystalline structure (Fig. 7A). At high magnification, the regular organization of the crystalline structure was observed (Fig. 7B inset). Peroxisomes were always observed associated with ER (Fig. 7A, C and D) and, in the cell body of *S. fusiformis* and *Globobulimina* sp., they were organized in a particular structure: numerous peroxisomes were associated with a high density of ER (Fig. 7E).

The existence of peroxisomes in the cytoplasm of foraminifera was first demonstrated in planktonic foraminifera by Anderson and Tuntivate-Choy (1984), who used cytochemical analysis to document the presence of peroxidases, which are enzymes typical of peroxisomes. Peroxidase activity was also demonstrated in benthic foraminiferal peroxisomes (Bernhard and Bowser, 2008). Furthermore, Bernhard and Bowser (2008) measured the spacing of the benthic foraminiferal peroxisomal internal crystals, documenting its identity as catalase, which is present in all peroxisomes (De Duve and Baudhuin, 1966). Catalase is an enzyme that converts hydrogen peroxide (H_2O_2) into water and oxygen, a reaction which produces metabolically useful molecules. Another role that peroxisomes play in eukaryotic cells, including foraminifera, is gluconeogenesis, i.e., the production of carbohydrates (Hemleben et al., 1989).

The association of peroxisomes with ER has been established previously for other benthic foraminiferal species (Bernhard et al., 2001; Bernhard and Alve, 1996; Bernhard and Reimers, 1991; Nyholm and Nyholm, 1975). Also, the specific organization of stacks of peroxisomes associated with copious endoplasmic reticulum (so-called peroxisome-endoplasmic reticulum complexes; P-ER; Fig. 7E) has been observed in

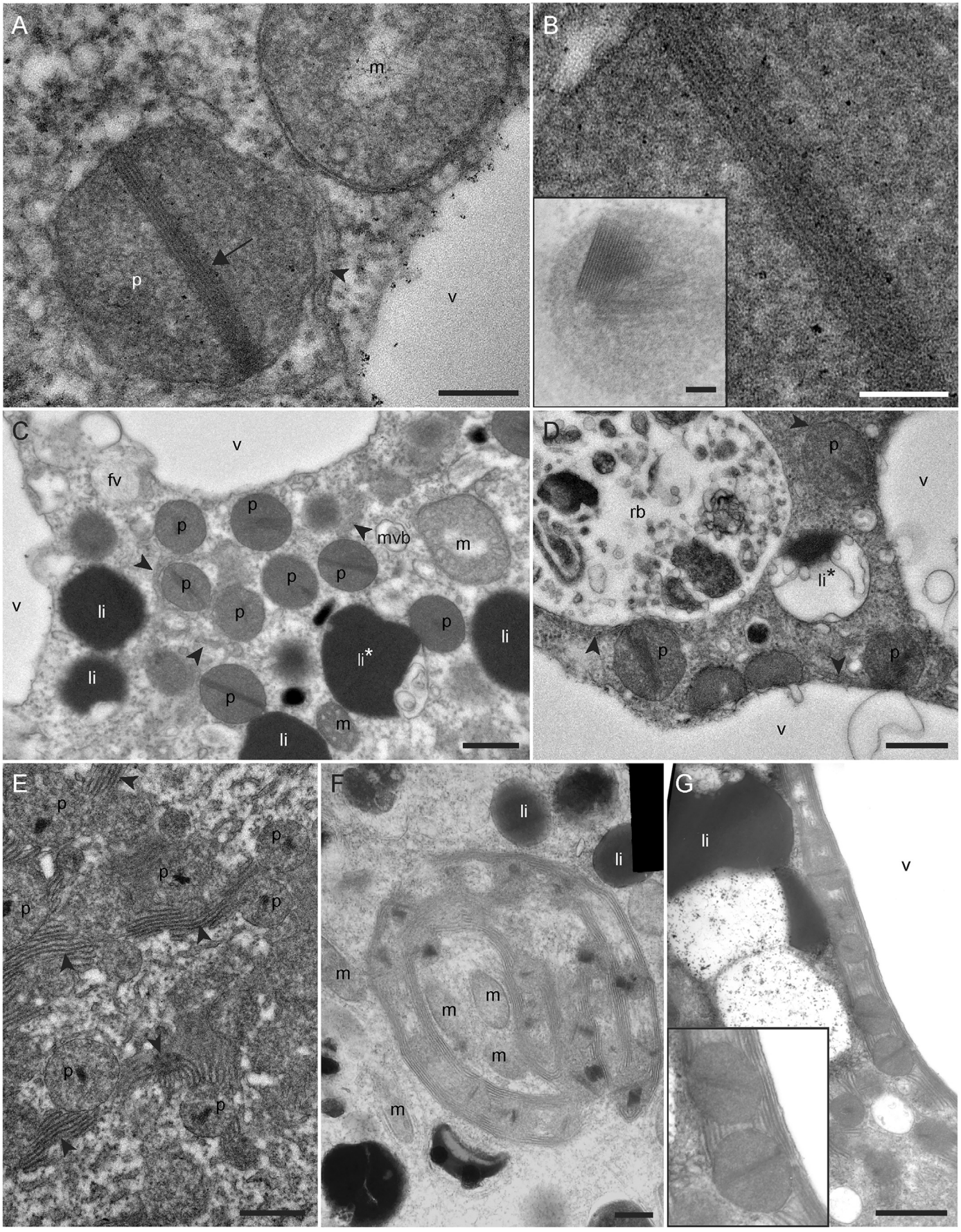

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Fig. 7. Transmission electron micrographs of benthic foraminiferal peroxisomes.
A: Classic structure of a peroxisome surrounded by endoplasmic reticulum (ER) in *Nonionellina labradorica*. B: High-magnification image of the crystalline structure of the peroxisome seen in A; inset: triangular core in *Buliminella tenuata* peroxisome (specimen from Bernhard and Bowser, 2008). C and D: Peroxisomes in the cytoplasm of C: *Nonionella* sp. and D: *Nonionellina labradorica*. E: Peroxisome-endoplasmic reticulum (P-ER) complex in *Stainforthia fusiformis*. F: Circular P-ER ring encircling mitochondria in *Buliminella tenuata* (specimen from Bernhard and Bowser, 2008). G: "Railroad track" of P-ER along the edge of a large vacuole in *Buliminella tenuata* (specimen from Bernhard and Bowser, 2008). Inset: higher magnification showing two of the peroxisomes forming the track and the fibrils of ER linking them. Arrowheads: ER, arrow: crystalline inclusion, fv: fibrillar vesicles, li: lipid droplets, li*: lipid droplets in lysis, m: mitochondria, mvb: multivesicular bodies, p: peroxisomes, rb: residual bodies, v: vacuoles. Scales: A = 200 nm; B = 100 nm; C, D = 500 nm; F, G = 1 µm.

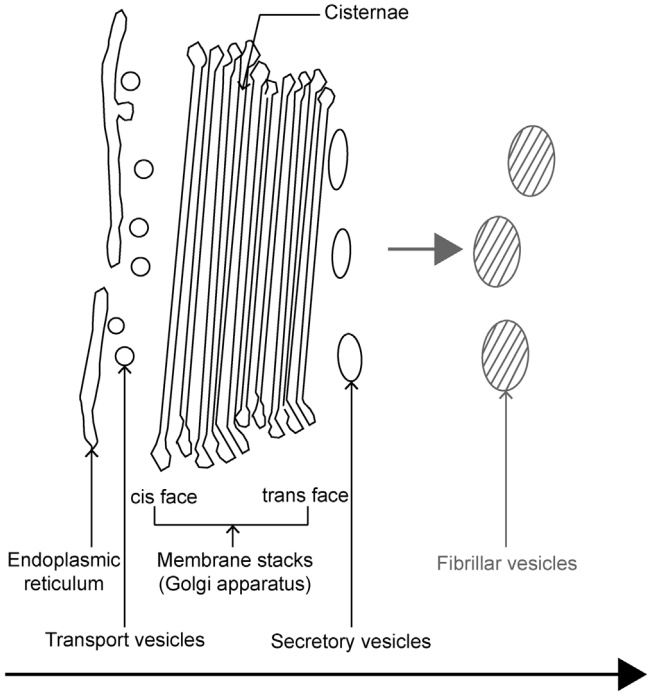

Fig. 8. Schematic ultrastructure and relations between the reticulum endoplasmic, Golgi apparatus and fibrillar vesicles in the foraminiferal cell.
The endoplasmic reticulum (ER) is secreting the transport vesicles containing the ER secretory products (proteins or lipids) which arrive on the cis face of the Golgi apparatus. After maturation through the Golgi saccules the proteins (or lipids) are excreted on the trans face in secretory vesicles. The gray part is speculative: the secretory vesicles would further be transformed into fibrillar vesicles (see details in the text). The arrow represents the direction of the metabolic process, from the secretion by the endoplasmic reticulum to the excretion in secretory vesicles and putative transformation in fibrillar vesicles. Modified from Anderson and Lee (1991).

benthic foraminifera inhabiting chemocline environments, such as low-oxygen areas or seeps (reviewed in Bernhard and Bowser, 2008) (Fig. 7F and G). Our observations are consistent with these prior studies because we noted P-ER complexes in both *S. fusiformis* and *N. labradorica* from the Gullmar Fjord, which often exhibits episodes of hypoxia (Filipsson and Nordberg, 2004; Nordberg et al., 2000).

Peroxisomes can also be closely associated with mitochondria (Bernhard and Bowser, 2008; Fig. 7F) and large vacuoles (Bernhard and Bowser, 2008; Fig. 7G). Bernhard and Bowser (2008) hypothesized the conversion of peroxide to oxygen and water allowed mitochondrial use of oxygen; the association of peroxisomes with vacuoles suggests a potential source of reactive oxygen species in the vacuoles.

3.2.5. Golgi apparatus

Generally, the Golgi apparatus plays a role in the maturation of proteins, as well as in the formation of some lipids (and polysaccharides in plant cells) (Farquhar and Palade, 1998; Staehelin and Moore, 1995). The Golgi processes proteins received from the endoplasmic reticulum through incoming transport vesicles on the *cis* face. These proteins are then sent to their next destination from the *trans* face in secretory vesicles (Farquhar and Palade, 1998; Staehelin and Moore, 1995; Fig. 8). The Golgi apparatus is made of membrane stacks surrounded by different types of vesicles, these membranes form tubular vesicles called cisternae; the space within the cisternae is called the lumen (Farquhar and Palade, 1998; Staehelin and Moore, 1995; see Fig. 8). In our study, the Golgi apparatus observed in the rotalid species' cell bodies also presented this specific organization (Fig. 9).

The Golgi apparatus observed in our foraminiferal cells had a typical structure where the different elements described above can be clearly identified (Fig. 9A). The membrane stacks were made of about ten cisternae and were often surrounded by ER on the *cis* face, with spherical transport vesicles about 70 nm in diameter existing between the ER and the cisternae (Fig. 9A, B and C). The secretory vesicles on the *trans* face were elongated and slightly longer (150–200 nm) (Fig. 9A). Frequently, a single stack of membrane was observed (Fig. 9B) but groups of two and sometimes more were also noted (Fig. 9C).

The Golgi apparatus is a common organelle that has been described in planktonic (e.g., Anderson and Bé, 1976a; Anderson and Lee, 1991), benthic (e.g., Bernhard et al., 2010a, b; Frontalini et al., 2015), and large benthic foraminifera (LBF) (e.g., Hottinger and Dreher, 1974; Leutenegger, 1977). In planktonic foraminiferal cells they may be involved in the formation of fibrillar vesicles (see Section 3.3.1.).

3.2.6. Organelles involved in feeding metabolism

3.2.6.1. Degradation vacuoles. Often in the literature the degradation vacuoles containing food are described and referred to as either food vacuoles or digestive vacuoles. Anderson and Bé (1976b) and Hemleben et al. (1989) differentiated between food vacuoles and digestive vacuoles: the former are vacuoles containing food that has recently been ingested, but not yet degraded; and the latter are vacuoles in the next stage, i.e., after a food vacuole has fused with a primary lysosome carrying digestive enzymes that have triggered the onset of degradation. In our study no food vacuoles with clearly identifiable food particles were observed. Concerning the digestive vacuoles, it is challenging to determine from TEM images if the source of the degraded material is an external source (food) or autophagocytosis of foraminiferal organelles (i.e., self-digestion of damaged or non-functional organelles). Thus, we do not distinguish between digestive vacuoles, and autophagocytosis and consequently lump them together under the label of "degradation vacuoles".

Degradation vacuoles had highly variable dimensions (Fig. 10A, B, C and D), with diameters ranging between 2 and 10 µm. They were mainly localized in the younger chambers of the foraminiferal cell, close to the aperture where food is phagocytosed. They were observed in all specimens examined in this study, in varied abundances. In *Ammonia* sp. (phylotype T6), diatoms at different stages of digestion could be seen in the youngest chambers (*n* to *n* – 5, i.e., the last six chambers from the aperture).

The different sizes and abundances of degradation vacuoles might depend on the foraminiferal metabolism and feeding strategies. Indeed, a starved foraminifer or a foraminifer with an alternative metabolism, such as denitrification, mixotrophy, or symbiosis with algae (or bacteria) might contain fewer endoplasmic degradation vacuoles. Furthermore, the morphological appearance of these vacuoles might depend on the nature of the ingested food and/or feeding strategy (reviewed by Goldstein and Corliss, 1994). In our study, one example is illustrated by the particular appearance of degradation vacuoles in *Ammonia* sp. (phylotype T6). As *Ammonia* sp. feeds on diatoms, including the diatom frustules, the cell body portions of the youngest chambers in this species exhibited diatoms at different stages of

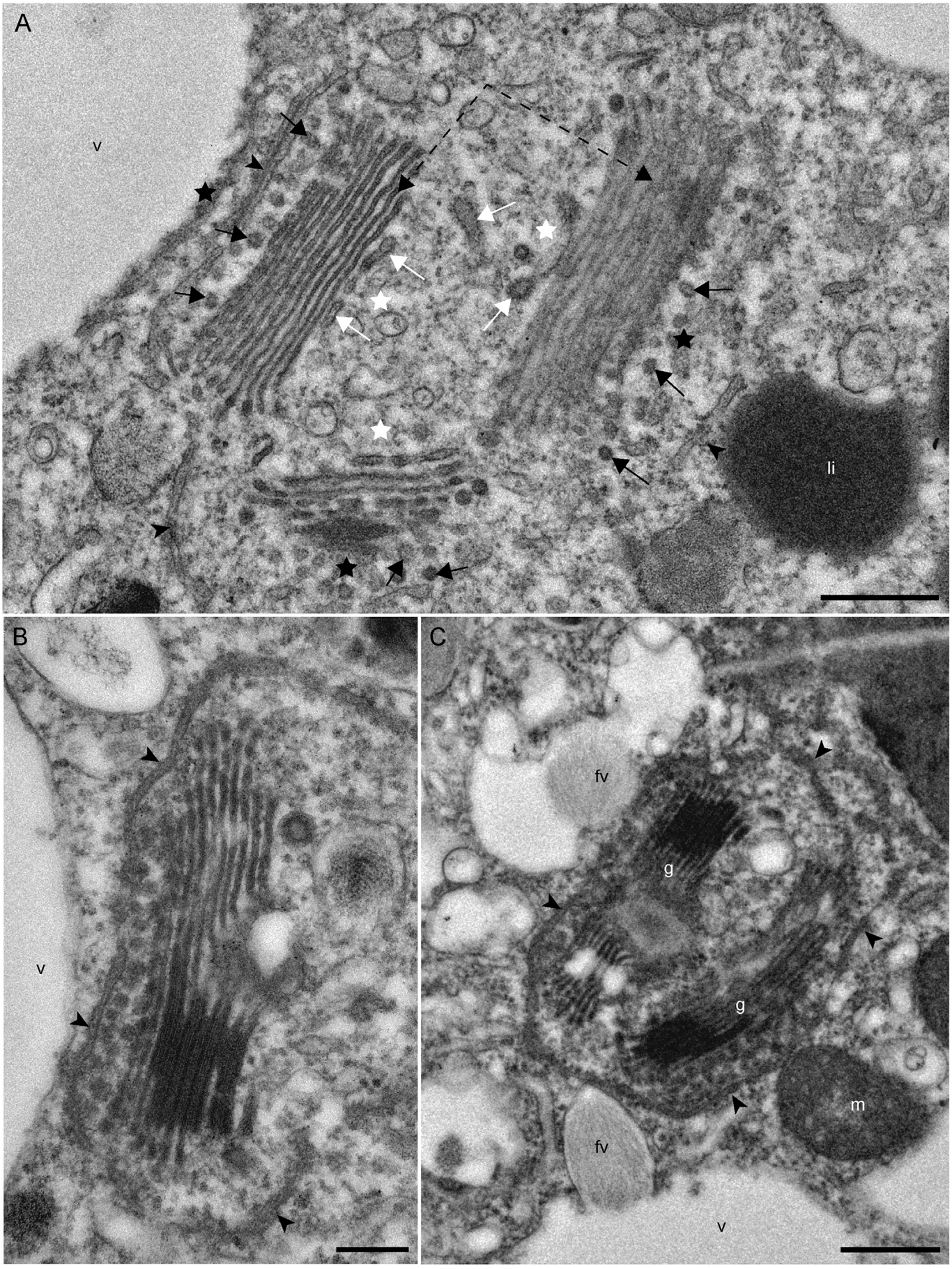

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Fig. 9. Transmission electron micrographs of benthic foraminiferal Golgi apparatus.
A: Golgi apparatus made of three stacks of membranes in *Stainforthia fusiformis*. B and C: Golgi apparatus alone (B) or organized in pair (C) surrounded by endoplasmic reticulum in *Nonionellina labradorica*. Black stars: *cis* face, white stars: *trans* face, arrowheads: endoplasmic reticulum, dotted arrows: cisternae, black arrows: incoming transport vesicles, white arrows: secretory vesicles, fv: fibrillar vesicles, m: mitochondria, li: lipid droplets, v: vacuoles. Scales: A, C = 500 nm; B = 200 nm.

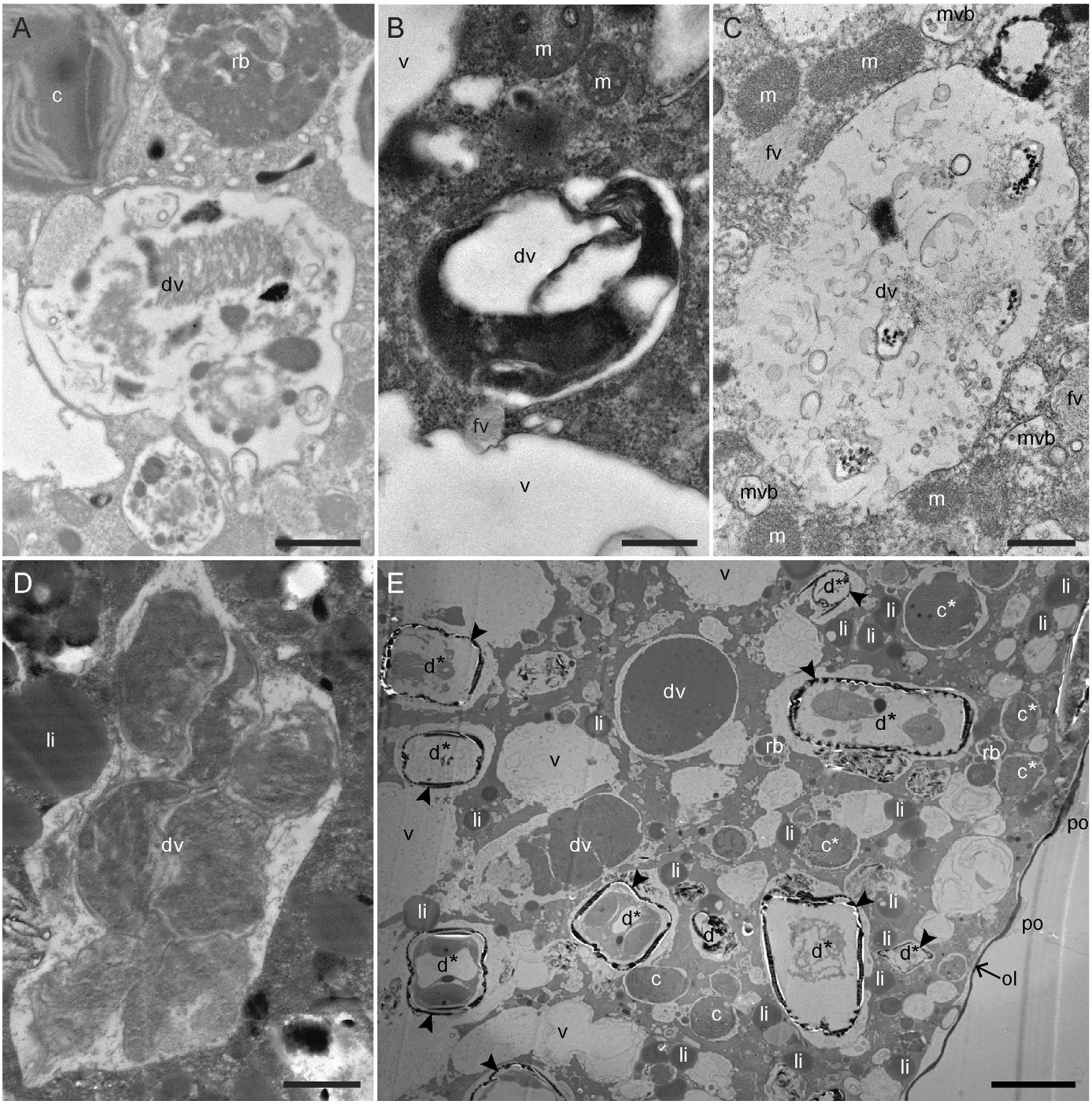

Fig. 10. Transmission electron micrographs of different types of degradation vacuoles.
Degradation vacuoles in the cytoplasm of A: *Nonionella* sp. B: *Nonionellina labradorica*, C and D: *Ammonia* sp. (phylotype T6). E: Cytoplasm of the antepenultimate chamber in *Ammonia* sp. (phylotype T6) exhibiting numerous diatom frustules (arrowheads), in which the diatom cytoplasm is being digested (d*) and diatom chloroplasts are in degradation (c*). c: chloroplasts, c*: chloroplasts in degradation, d*: diatom in degradation, dv: degradation vacuoles, fv: fibrillar vesicles, li: lipid droplets, m: mitochondria, ol: organic lining, po: pore, rb: residual bodies, v: vacuoles. Scales: A, C, D = 1 μm; B = 500 nm; E = 5 μm.

digestion: from the nearly intact diatoms with identified organelles to empty frustules (Fig. 10E). Engulfed frustules have been observed in other studies of *Ammonia* spp. (Goldstein and Corliss, 1994; LeKieffre et al., 2017) as well as in the large benthic foraminifera *Amphisorus hemprichii* and *Amphistegina lessonii* (McEnery and Lee, 1981).

3.2.6.2. Residual bodies. Residual bodies are vacuoles with heterogeneous content, often with electron-dense circular particles

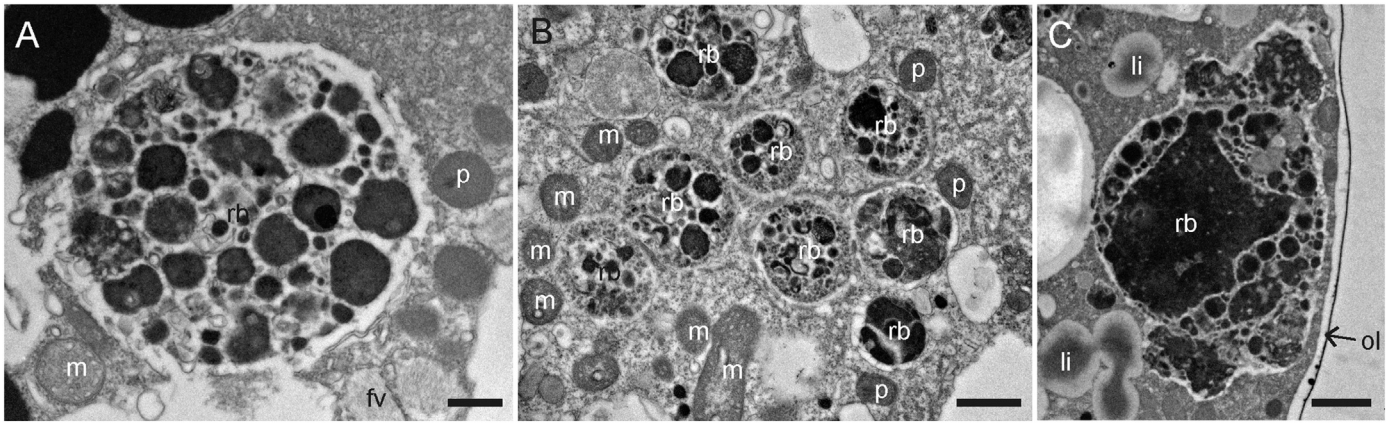

Fig. 11. Transmission electron micrographs of benthic foraminiferal residual bodies.
A: Round shaped residual body in *Nonionella* sp. B: High abundance of residual bodies in the cytoplasm of *Nonionellina labradorica*. C: Irregular shaped residual body in *Bulimina marginata*. fv: fibrillar vesicles, m: mitochondria, ol: organic lining, p: peroxisomes, rb: residual bodies. Scales: A = 500 nm; B, C = 1 μm.

(Hemleben et al., 1989; Leutenegger, 1977). In this study, residual bodies were circular with diameters from 1 to 5 μm (Fig. 11A, B), except in the species *B. marginata* where they were slightly bigger (4 to 8 μm) with irregular shapes (Fig. 11C). These residual bodies were observed in all species studied here, although in different abundances, and appear equivalent to the residual bodies observed in the cell body of LBF Amphisteginidae and Nummulitidae (Leutenegger, 1977), and to inclusions in *Heterostegina depressa* with "strikingly inhomogeneous content" (Hottinger and Dreher, 1974. Leutenegger, 1977 described the residual bodies as autophagocytosis vacuoles, i.e., autolysis vacuoles containing foraminiferal organelles in degradation. Note that there was no clear evidence for degrading organelles in any of the residual bodies observed in this study. In general, it is difficult to establish if the electron-dense particles inside these residual bodies result from the degradation of external material (food) or from foraminiferal organelle autophagocytosis. Hemleben et al. (1989) described residual bodies as vacuoles containing non-digestible debris, which is consistent with the observed accumulation of isotopic labeled compounds derived from diatoms in residual bodies of *Ammonia* sp. (LeKieffre et al., 2017). Le Cadre and Debenay (2006) and Morvan et al. (2004) noted that residual bodies proliferated when *Ammonia tepida* specimens were under stress from different forms of pollution or contamination of their environment.

3.2.6.3. Lipid droplets. Lipid droplets were often spheroidal (Fig. 12A), but can take a variety of shapes in the cytoplasm (Fig. 12B, C). A particular feature of lipid droplets was the absence of apparent enclosing membrane(s). Sizes vary from about 500 nm to 10 μm in diameter, with an average diameter approximately 2 μm. In nearly all specimens studied by us, a few lipid droplets were observed in degradation (i.e., part of the lipid droplet was missing, 'replaced' by apparently empty space in the TEM micrographs; Fig. 12B). Sometimes the opacity of the lipid droplets was not uniform; it could be brighter on the periphery than in the center, as observed, e.g., in *N. labradorica* and *B. marginata* (Fig. 12C and D).

Described as the primary carbon storage in foraminiferal cells (Hottinger, 1982; Hottinger and Dreher, 1974; Leutenegger, 1977; Pawlowski et al., 1995), lipid droplets are osmiophilic vesicles and thus they appear electron dense in TEM micrographs. The level of opacity depends on the osmium tetroxide (OsO4) concentration of incubation media, as well as on the degree of fatty acid saturation, which might explain observed variations among different species, assuming identical sample preparation procedures, including OsO_4 staining (which is the case in our study). Metabolic state and diet can contribute to this variability. LeKieffre et al. (2017) have demonstrated a clear link between lipid droplets and food in digestive vacuoles by tracing ^{13}C–enrichment from ingested food, via degradation vacuoles, to lipid droplets.

Lipid droplet distribution in the cell body in the present study was in agreement with previous studies. Anderson and Lee (1991) also observed such droplets in all chambers, except in the last (youngest) and penultimate chambers, from where benthic foraminifera extend their reticulopods. The proportion of lipid droplets in a state of degradation is highly variable, likely depending on metabolic state of the individual. In a 28-day incubation in which foraminifera were fed only once, at the beginning of the experiment, LeKieffre et al. (2017) observed how lipid droplets were initially formed and then were gradually consumed nearly to the point of disappearance.

A relatively high abundance of lipids has been observed under stressful conditions in different experimental studies testing the response of foraminifera to heavy-metal contamination (Frontalini et al., 2015; Le Cadre and Debenay, 2006) and anoxia (Koho et al., this issue). Finally, lipid droplets with a brighter periphery were also observed in the case of contamination with lead and described as "electron-dense core lipid vacuoles" (Frontalini et al., 2015). In our study we suggest that the lipid brighter at the periphery observed in *N. labradorica* and *B. marginata* could be a fixation artifact, although we are not able to explain it.

3.2.7. Paracrystals of tubulin

Paracrystals of tubulin provide the cell with molecular building blocks for their microtubular network supporting, e.g., the foraminiferal reticulopods (reviewed in Travis and Bowser, 1991), which are the primary mean of foraminiferal food acquisition. They are elongated structures (2 to 50 μm in length) and recognizable due to their regular organization at high magnification (Fig. 13B), sometimes exhibiting regular crystalline pattern akin to a honeycomb structure (Fig. 13C and D) depending on the plane of section.

We observed these structures in the cell body of Gullmar Fjord species *N. labradorica*, *Nonionella* sp. and *Globobulimina* sp. (Fig. 13A), but not in intertidal foraminiferal species. Note that the higher density of the cell body in the latter might have partially obstructed their observation. Our observations are similar to the paracrystals in the cell body and reticulopodial net of other benthic foraminiferal species (reviewed in Travis and Bowser, 1991).

3.3. Organelles with unknown function

3.3.1. Prokaryotes and sequestered chloroplasts

Many benthic foraminiferal species are known to have prokaryotic associates and/or sequester chloroplasts in their cytoplasm (see Bernhard et al., this issue and Jauffrais et al., this issue for reviews). The

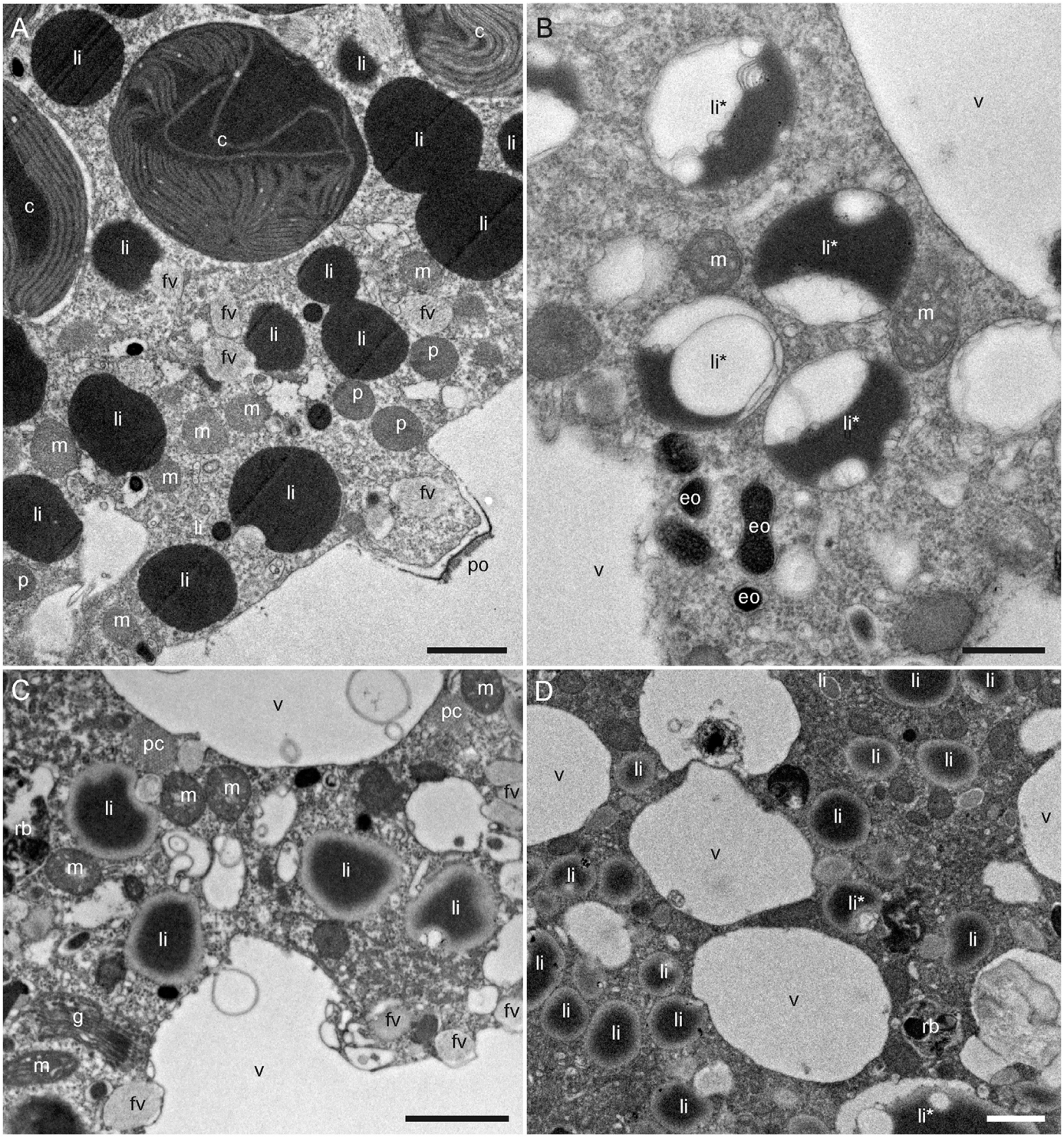

Fig. 12. Transmission electron micrographs of benthic foraminiferal lipid droplets.
A: Lipid droplets in the cytoplasm of *Nonionella* sp. B: Lipid droplets in degradation in *Nonionellina labradorica*. C, D: Less electron-opaque lipid droplets at the periphery of *Nonionellina labradorica* (C) and *Bulimina marginata* (D). c: chloroplasts, eo: electron-opaque bodies, fv: fibrillar vesicles, li: lipid droplets, li*: degraded lipid droplets, m: mitochondria, p: peroxisomes, po: pore, rb: residual bodies, v: vacuoles. Scales: A, C, D = 1 μm; B = 500 nm.

prokaryotes seemingly can be symbionts beneficial to the host foraminifer or can be parasites, which are detrimental to the foraminiferal host. Kleptoplasts are believed to confer considerable advantage to the foraminiferal host. While the role of these subcellular entities clearly impacts the foraminifer, details on the functions of these structures are not well understood. We refrain from further discussion of these entities and direct the reader to the noted publications.

3.3.2. Fibrillar vesicles

Fibrillar vesicles were abundant in all foraminiferal species observed in this study (Fig. 14A). These structures are small oval vesicles approximately 500 nm in length. They contained fibrils that, depending on the cutting plane, appeared as thin threads or as nanometer-scale spots (Fig. 14B). In some TEM micrographs, we observed a space between the fibrils and the membrane enclosing them (Fig. 14C), which

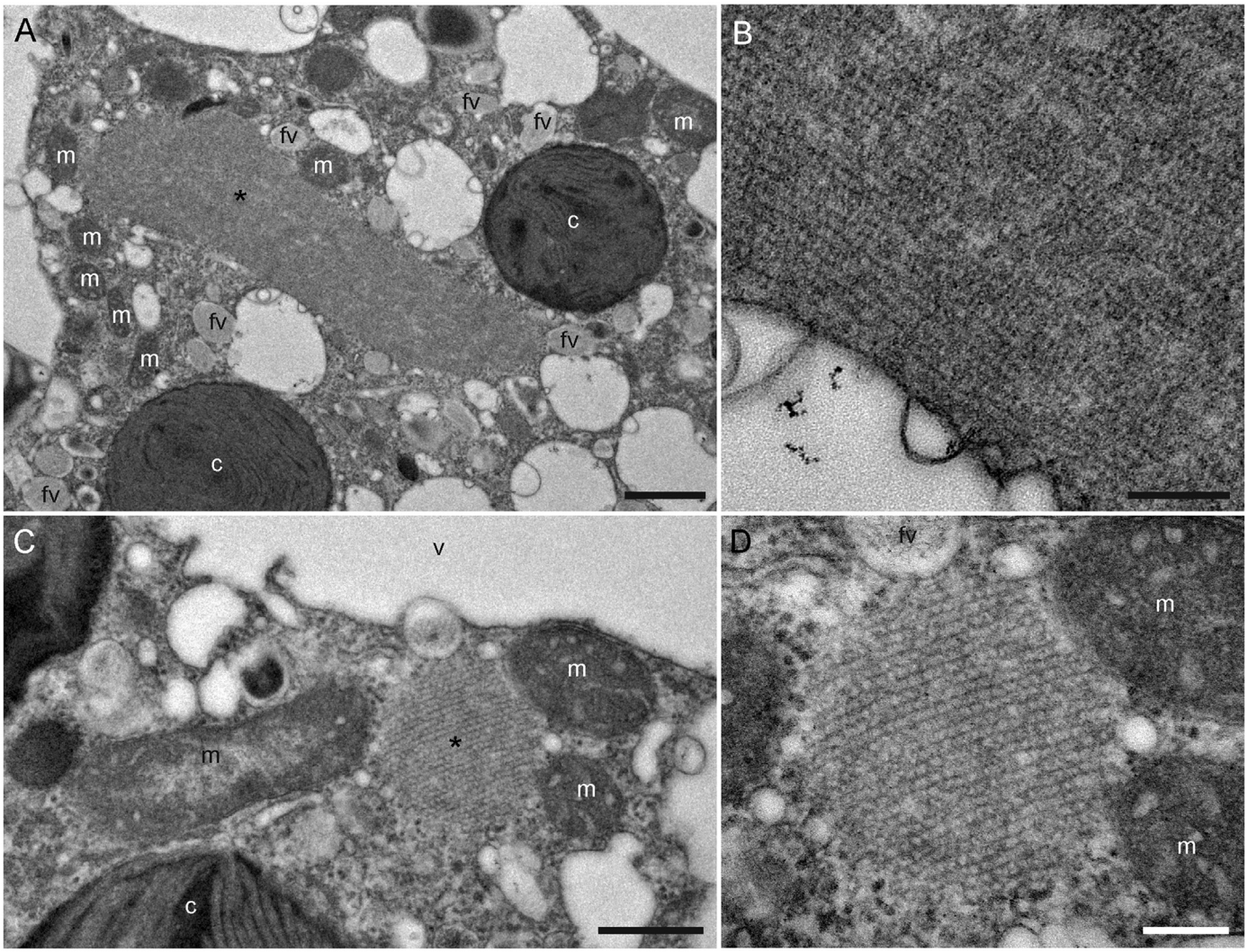

Fig. 13. Transmission electron micrographs of paracrystals of tubulin in the cytoplasm of *Nonionellina labradorica*. A: Longitudinal section of a tubulin paracrystal. B: High-magnification image revealing the regular pattern of the crystalline structure observed in A. C: Cross section of the crystalline structure. D: High-magnification image revealing the regular pattern of the crystalline structure observed in C. Asterisks: paracrystals of tubulin, c: chloroplasts, fv: fibrillar vesicles, li: lipid droplets, m: mitochondria. Scales: A = 1 μm; B, D = 200 nm, C = 500 nm.

may be a fixation artifact. Fibrillar vesicles were sometimes observed to exocytose into white "empty" vacuoles or to be fused with residual bodies (Fig. 14D, E, F and G).

First described by Angell (1967), small fibrillar vesicles have been observed both in planktonic and benthic foraminifera throughout the cell body as well as in the reticulopods (Anderson and Bé, 1976a; Goldstein and Barker, 1988; Hemleben et al., 1989; Leutenegger, 1977). Different authors have hypothesized that the fibrillar vesicles are formed by the stacks of membranes of the Golgi apparatus (Anderson and Bé, 1976a, 1976b; Anderson and Lee, 1991; Leutenegger, 1977) (Fig. 8). Similar fibrillar vesicles were also observed in dinoflagellate cells (Dodge, 1974; Leadbeater and Dodge, 1966) where they were identified as Golgi vesicles. Langer (1992) hypothesized that the fibrillar vesicles are involved in the transport of glycosaminoglycans (GAGs; sulfated polysaccharides) from their production site in the Golgi apparatus, to the place they would be used. Some studies have argued that the fibrillar vesicles could play a role in the secretion of mucilaginous substances for the reticulopods (Anderson and Bé, 1976a, 1976b). Because of the small size of fibrillar vesicles and their abundance in the peripheral cytoplasm of the species studied, Leutenegger (1977) suggested a role in the formation of organic matrix, such as organic linings, which seems consistent with observations of high densities of fibrillar vesicles in the last chamber of foraminifera, prior to the formation of a new chamber in planktonic foraminifera (Angell, 1967; Leutenegger, 1977; Spero, 1988).

Note that the fibrillar vesicles observed here differ from the fibrillar system (also called fibrillar bodies or microvillus system) observed in planktonic foraminifera (Anderson and Bé, 1976a; Anderson and Lee, 1991; Hemleben et al., 1989; Leutenegger, 1977; Spero, 1988). The fibrillar system in planktonic foraminifera is made of larger fibrillar bodies, which are vacuoles containing larger fibrils with a tubular aspect; possibly serving as flotation devices (Anderson and Bé, 1976a; Hemleben et al., 1989; Leutenegger, 1977), or playing a role as Ca storage vacuoles (Spero, 1988).

3.3.3. *Electron-opaque bodies*

Electron-opaque bodies are small (200 to 500-nm in diameter) spherical (Fig. 15A) to oval-shaped (Fig. 15B) dense bodies. Some of these bodies were surrounded by a seemingly empty space and a membrane (Fig. 15A–C, G–H); the space below the membrane could be due to shrinkage during chemical fixation. Others did not seem to possess a space below the membrane (Fig. 15C–H), and they are so electron-opaque that the presence of a membrane is difficult to establish. Such electron-opaque bodies, i.e., with or seemingly without

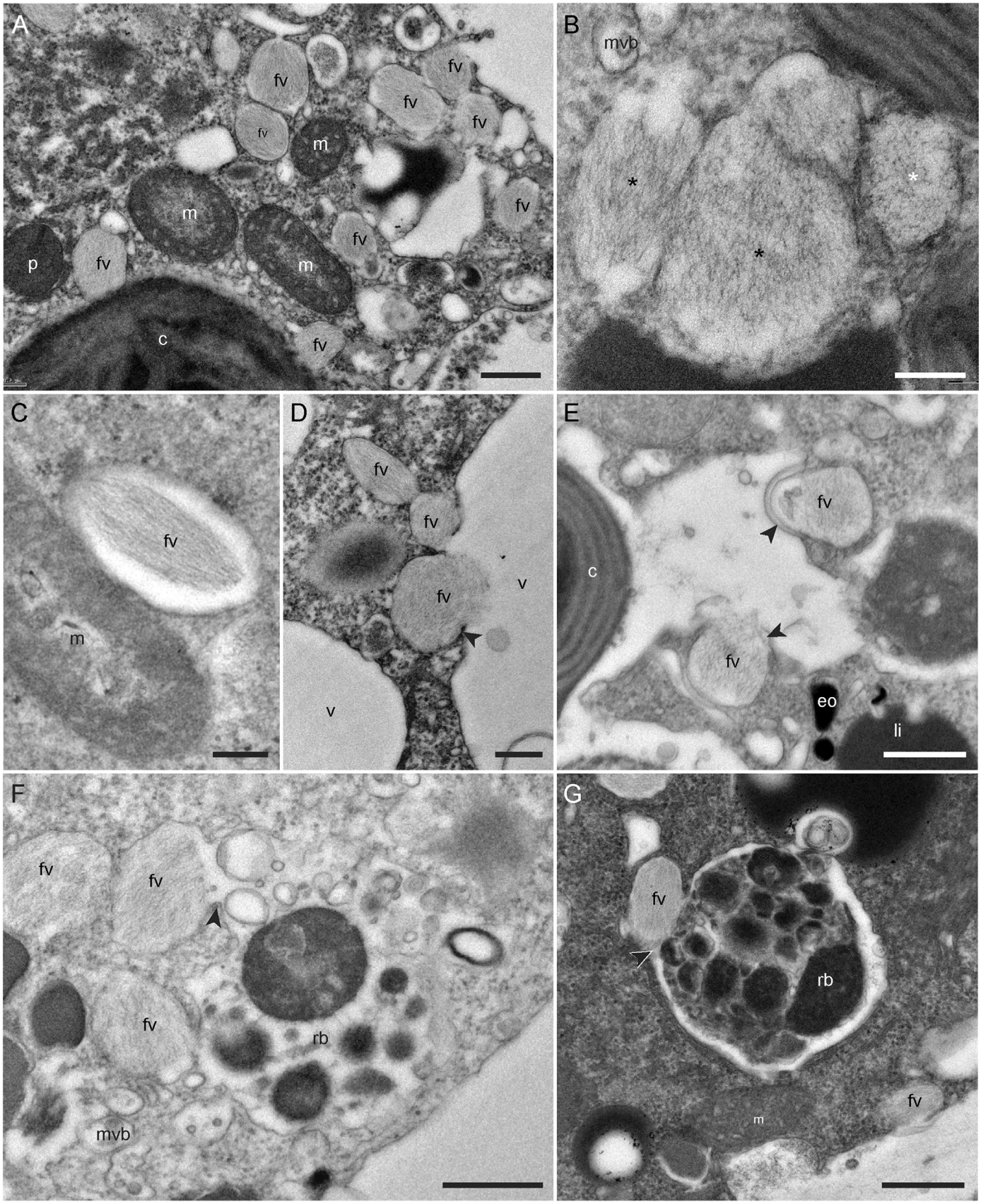

Fig. 14. Transmission electron micrographs of benthic foraminiferal fibrillar vesicles.

A: Fibrillar vesicles in the cytoplasm of *Nonionellina labradorica*. B: High-magnification image of a group of fibrillar vesicles in *Nonionella* sp.; sections parallel (black asterisks) or perpendicular (white asterisk) to the fibrils. C: Fibrillar vesicle surrounded by a space between the fibrils and the vesicle membrane in *Haynesina germanica*. D and E: Fibrillar vesicles merging with vacuoles (arrowheads) in D: *Nonionellina labradorica* and E: *Nonionella* sp. F and G: Fibrillar vesicles merging with residual bodies (arrowheads) in F: *Stainforthia fusiformis* and G: *Nonionellina labradorica*. c: chloroplast, eo: electron-opaque bodies, li: lipid droplet, m: mitochondria, mvb: multivesicular bodies, p: peroxisomes, rb: residual body, fv: fibrillar vesicles. Scales: A, E, F, G = 500 nm; B, C, D = 200 nm.

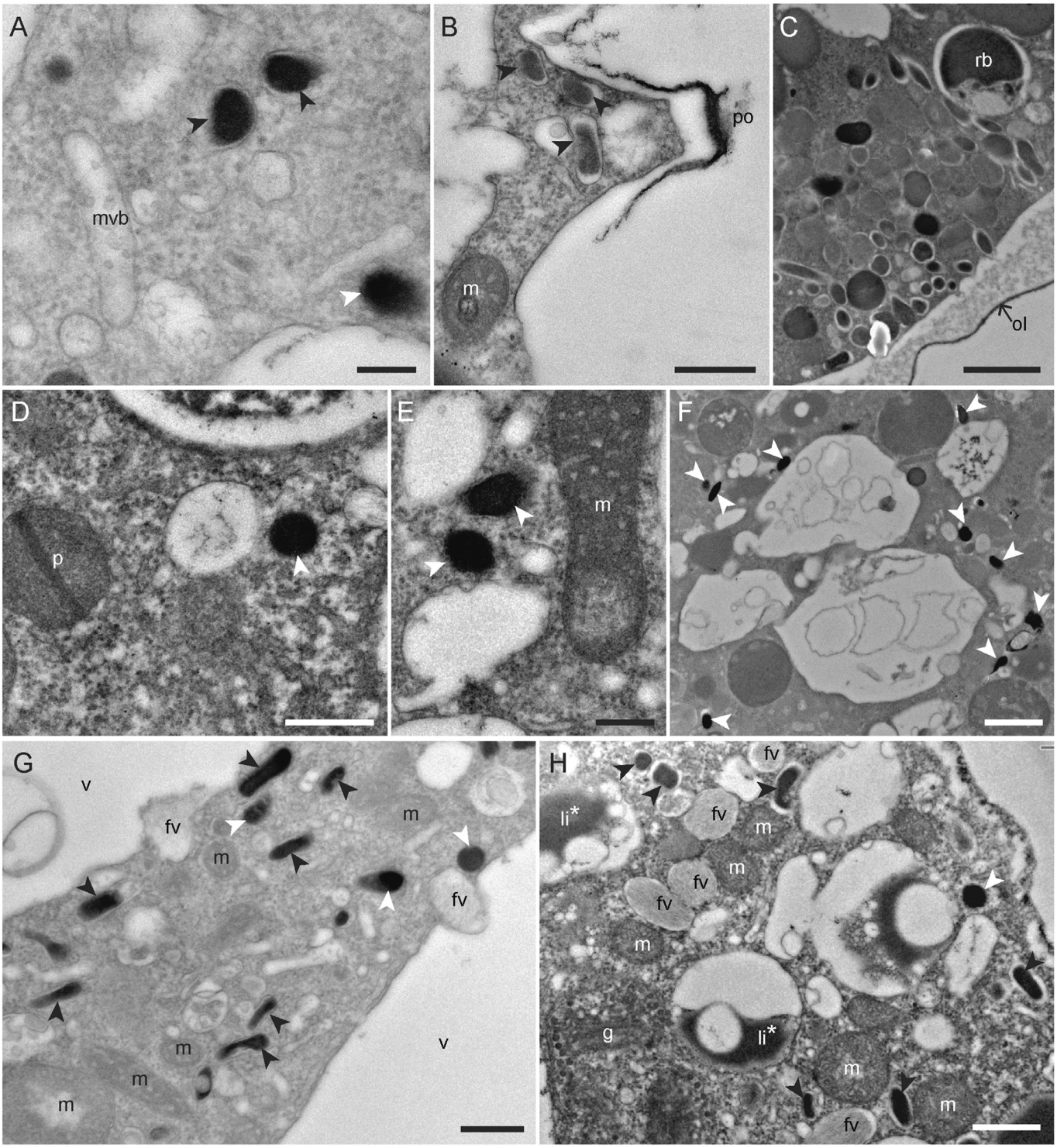

Fig. 15. Transmission electron micrographs of benthic foraminiferal electron-opaque bodies.
A and B: Electron-opaque bodies with membranes (black arrowheads) in the cytoplasm of A: *Nonionella* sp. and B: *Nonionellina labradorica*. C: Clusters of electron-opaque bodies in the cytoplasm of *Ammonia* sp. (phylotype T6). C, D and E: Electron-opaque bodies without distinguishable membranes (white arrowheads) in the cytoplasm of C, D: *Nonionellina labradorica* and E: *Haynesina germanica*. G, H: Both shapes of electron-opaque bodies in the cytoplasm of G: *Nonionella* sp. and H: *Nonionellina labradorica*. Black arrowheads: electron-opaque bodies with membranes, white arrowheads: indistinguishable membrane electron-opaque bodies, fv: fibrillar vesicles, g: Golgi apparatus, li*: lipid droplets in lysis, m: mitochondria, mvb: multivesicular bodies, p: peroxisomes, rb: residual bodies, v: vacuoles. Scales: B, D-F = 500 nm; A, G = 200 nm; C, H: 1 μm.

membrane, were observed in the cell body of all species studied here, sometimes equally distributed but occasionally clustered at the cell periphery, as in the *Ammonia* sp. cell body (Fig. 15C). The visualization of the membrane might also depend on the plane section view and could explain that it was clearly visible in some cases, and sometimes not.

We assume that whether or not they possess a seemingly empty space and a membrane, all the electron-opaque bodies are in fact the same structure, but further studies are required. Leutenegger (1977) did not differentiate the osmiophilic granules observed in the cell body of larger foraminifera, whether a membrane could be distinguished or not (e.g., Plate 17, Fig. c; Plate 29, Fig. d and Plate 43, Figs. b and c in

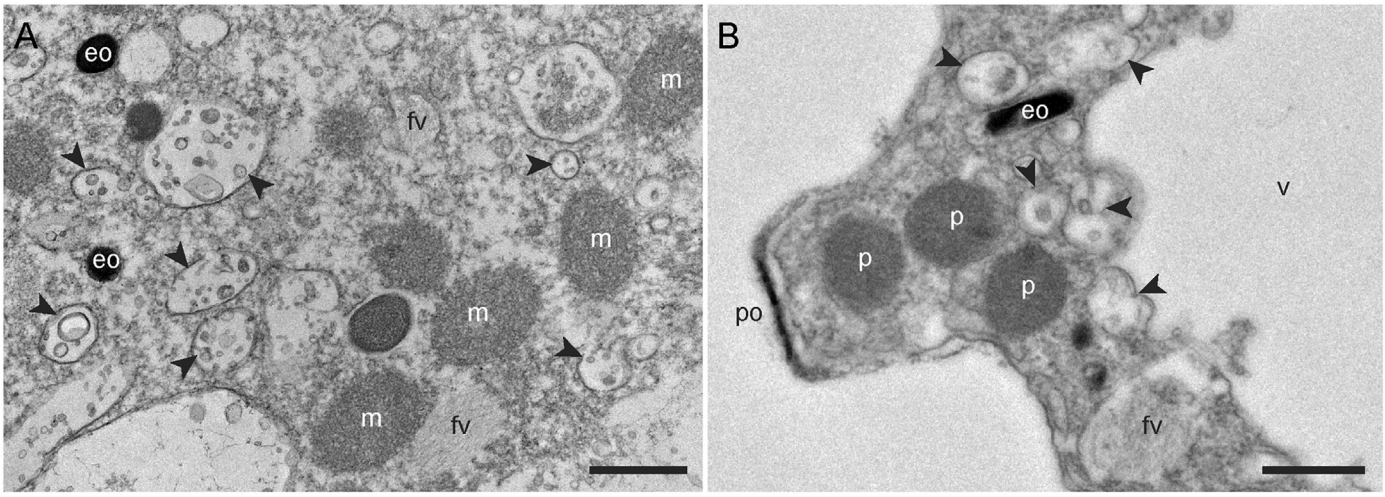

Fig. 16. Transmission electron micrographs of benthic foraminiferal multivesicular bodies.
Multivesicular bodies in the cytoplasm of A: the penultimate chamber of one *Ammonia* sp. (phylotype T6) specimen and B: *Nonionella* sp. Black arrowheads: multivesicular bodies, eo: electron opaque bodies, fv: fibrillar vesicles, m: mitochondria, p: peroxisomes. Scales: A, B = 500 nm.

Leutenegger, 1977). The Leutenegger osmiophilic granules were approximately the same size as the electron-opaque bodies observed in our study. It is noted that these structures occur in the TEM micrographs of several publications, both on planktonic and benthic foraminiferal cells, although they have not always been described (e.g., Anderson and Bé, 1976a; Bernhard et al., 2010a, 2010b; Hemleben et al., 1989; Le Cadre and Debenay, 2006). They may correspond to the "electron-dense bodies" surrounded by membranes observed by Nomaki et al. (2016), who observed these structures clustered close to the cell periphery, but only in specimens incubated in anoxia. Although their role is not understood, it has been shown that these structures had a relatively high sulfur content when compared to other types of organelles (Nomaki et al., 2016).

3.3.4. Multivesicular bodies

Multivesicular bodies are tiny spheroidal vacuoles with a diameter of 200–500 nm (Fig. 16A and B). They contain vesicles of 10 to 50 nm; the number of these vesicles can vary from one or two to more than a dozen per multivesicular body. They were observed in the cell body of all the species studied here and were more abundant in the younger chambers (arrowheads in Fig. 16B).

The role and function of these multivesicular bodies are unknown. These structures could correspond to the multivesicular bodies in the microtubule-transport model of Langer (1992, [Fig. 3]). Vesicles attached to microtubules were also observed by Anderson and Lee (1991, [Fig. 27]), although they were simply referred to as "vesicles". They could also correspond to the "fuzzy coated vesicles" associated with microtubules in the reticulopods (Bowser and Travis, 2000 [Fig. 2b]; Travis and Allen, 1981 [Fig. 3]; Travis and Bowser, 1991 [Fig. 9]): these fuzzy coated vesicles are more elongated than spheroids but their size is similar to the structures observed here. Similar vesicles were also observed in the canal plasma of *Operculina ammonoides*. However, in that case, the vesicles were associated with microtubules (Hottinger and Dreher, 1974 [Fig. 11]). Finally, the same kind of vesicles was also seen in the reticulopodial net of *Peneroplis planatus* and near a pore in *Amphistegina lobifera* (Leutenegger, 1977 [Plate 45, Fig. a; Plate 52, Fig. a]). All these observations are consistent with the higher abundances of multivesicular bodies in younger chambers.

Although, to our knowledge, multivesicular bodies have not been studied in foraminifera, similar structures are observed in additional eukaryotic organisms and a number of studies have shown a role in autophagy and in endocytosis processes (e.g., Piper and Katzmann, 2007; Fader and Colombo, 2008). These functions were also demonstrated in some other protists such as amoeboflagellates (Herman, et al., 2011). From our observations it not possible to conclude to a similar role in foraminiferal cells. Whatever their function, it seems that multivesicular bodies are ubiquitous among benthic foraminiferal species.

4. Conclusion

The ultrastructure of foraminifera is highly variable among species. First, the overall aspect of the cell body is variable, mainly because of the absence/presence and abundance of large vacuoles. Second, although organelles involved in basic functioning of the cell (e.g., nucleus, mitochondria, ER, Golgi apparatus, peroxisomes) are present in all the species, their appearance, size, abundance, or location vary. Third, the degradation vacuoles are found in all the species studied here but because there are many different types of feeding metabolism, there are also many different types of degradation vacuoles. Moreover, the physiological state (environmental stress, starvation state, stage in the reproduction cycle, etc.) of a specimen can have an impact on its cellular ultrastructure, resulting in ultrastructural variations within conspecifics (Koho et al., this issue; Frontalini and Nardelli, this issue).

Finally, this work emphasizes the need for further ultrastructural investigations to determine the role of recurrent but poorly understood organelles, such as fibrillar vesicles, electron-opaque bodies, or multivesicular bodies, as well as the metabolic interactions between all types of organelles.

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