A phylogenetic perspective on the evolution of test morphology in Foraminifera

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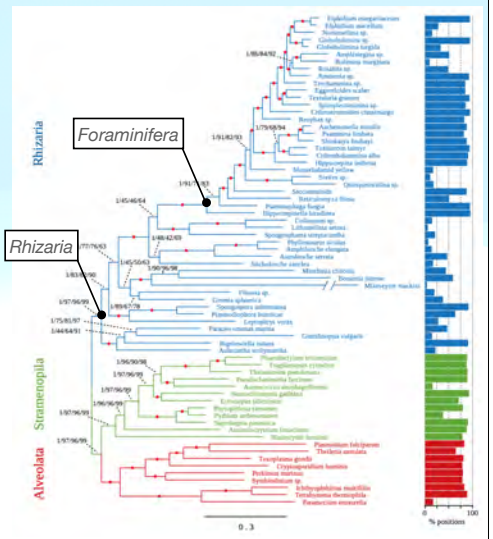
SAR

Rhizaria

Foraminifera

- *Foraminifera* branches as a subclade within the more inclusive clade *Rhizaria* (blue) as sister to *Polycystinea* (polycystine radiolarians)
- Rhizarian traits:
  - Mostly single-celled eukaryotes; one group has aggregative multicellularity (*Guttulinopsis vulgaris*)
  - Free-living & parasitic taxa (both plant & marine invertebrate parasites)
  - Heterotrophic & photosynthetic (*Bigelowiella natans*, a chlorarachniophyte alga)
  - Testate/skeletons & naked forms
  - Pseudopodial & flagellated stages

Molecular phylogeny of the SAR clade



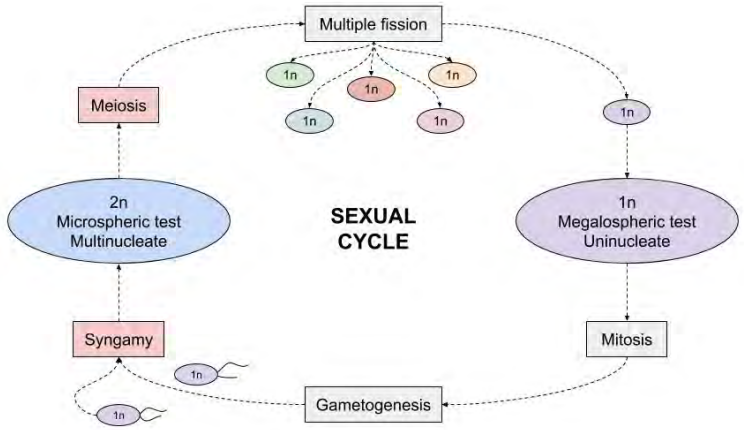
Molecular phylogeny of *SAR* clade showing the major subclades *Stramenopila*, *Alveolata* & *Rhizaria* (Sierra et al. 2022: Fig. S3)

Foraminifera

Diagnostic Traits

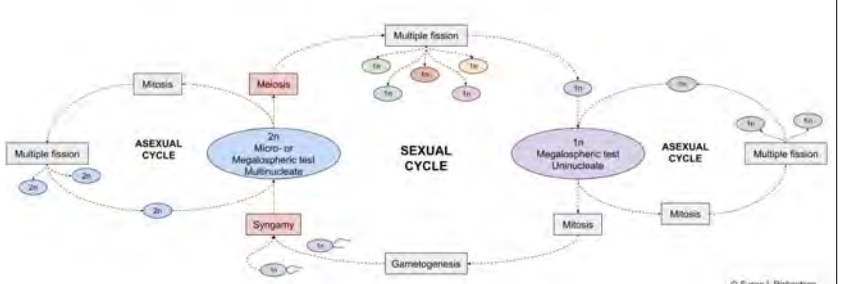
- **Granuloreticulopodia**: anastomosing extensions of cytoplasm underlain by microtubules
  - Functions include: food gathering & digestion; motility; test construction; facilitate mating in gamontogamous taxa
- **External test**: organic-walled test; agglutinated test w/organic or calcareous cement; biomineralized test (calcite/aragonite); some "naked taxa w/o test (e.g. *Reticulomyxa*);
- **Life cycle of alternating haploid & diploid generations**:
  - Sporic meiosis: meiosis occurs in diploid generation; gametes produced by haploid generation
  - Diploid generation: multinucleate; reproduces by meiotic or ameoitic multiple fission
  - Haploid generation: uninucleate; reproduces by gametogenesis or apogamic multiple fission

The Foraminiferal Life Cycle



The basic foraminiferal life cycle is a sexual cycle that consists of alternating diploid & haploid generations.

The Foraminiferal Life Cycle: Variations



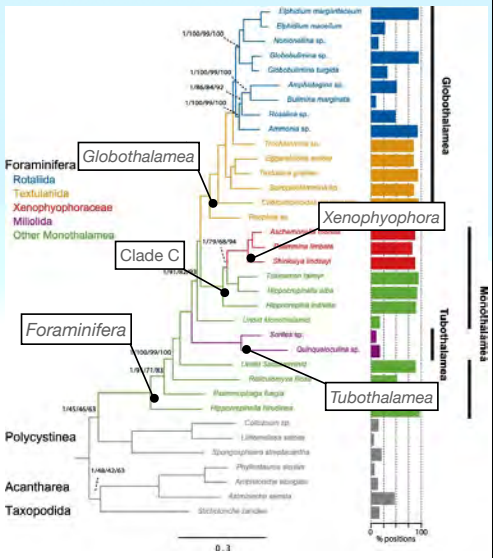
Repeated cycles of asexual reproduction may occur in either the diploid or haploid generations.

Foraminifera

Major Subclades

- "Monothalameans"[green lineages]
  - Paraphyletic
  - Single-chambered taxa with organic wall, agglutinated wall or no wall
- *Tubothalamea* [purple lineages]
  - Two-chambered tests (early lineages)
  - Multichambered tests evolve later
  - Agglutinated & biomineralized tests
- Clade C [red & green lineages]
  - *Xenophyophora* [red]
  - Single-chambered taxa [green]
- *Globothalamea* [blue & orange lineages]
  - Multichambered tests
  - Agglutinated tests [orange] & biomineralized tests [blue]

Molecular phylogeny of the SAR clade



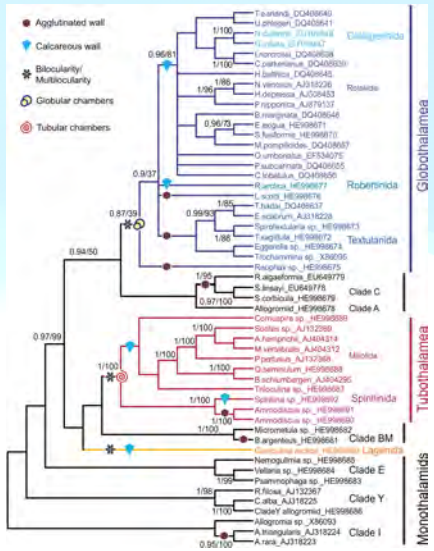
Molecular phylogeny based on transcriptomics of *Foraminifera* & radiolarian outgroups (Sierra et al. 2022: Fig. 1)

Foraminifera

Crown clade

- Phylogeny of *Foraminifera* based on transcriptomics in Sierra et al. 2022 (Fig. 1) is congruent with earlier molecular phylogenies of *Foraminifera* generated using SSU rDNA sequence data; e.g., Pawlowski & Holzmann, 2012 (Fig. 1)
- Important differences in taxonomic composition of analyses:
  - Pawlowski & Holzmann (2013) include *Glandulina arctica*, a multi-chambered species with calcareous tests (Lagenida) & *Bathysiphon argenteus* (Clade BM), a species with a tubular, agglutinated test
  - Sierra et al. (2022) include more taxa with single-chambered, non-mineralized tests

Molecular phylogeny of Foraminifera



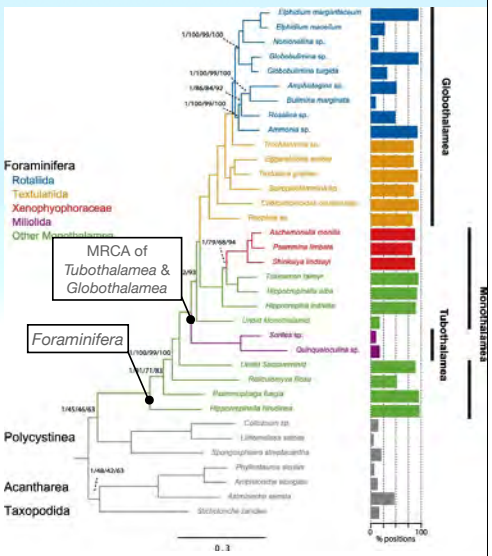
Molecular phylogeny based on SSU rDNA of *Foraminifera* (Pawlowski & Holzmann, 2013: Fig. 1)

Foraminifera

Major growth trends

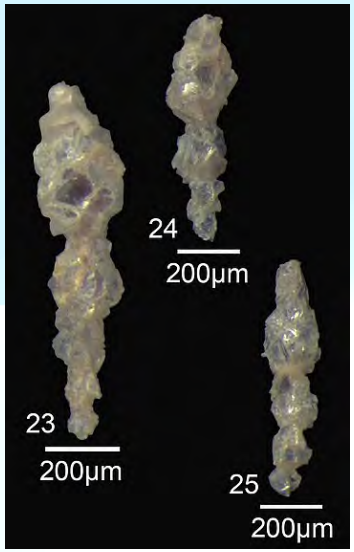
- Basal lineages of single-chambered taxa; e.g. *Allogromia*
  - Growth by expansion
- Later lineages with tubular tests; e.g., *Hyperammina*
  - Growth by terminal addition
  - Episodic growth, but no discrete chambers
- Derived lineages with multi-chambered tests; e.g., *Reophax*
  - Growth by terminal addition of discrete chambers
  - Episodic periods of chamber formation

Molecular phylogeny of the SAR clade



Molecular phylogeny of Foraminifera & radiolarian clades (Sierra et al. 2022: Fig. 1)

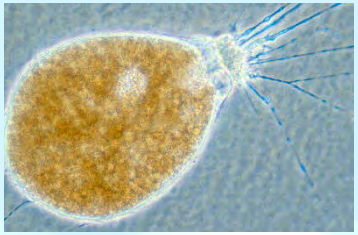
Multichambered foraminiferans



https://foraminifera.eu/

Reophax scorpiurus De Montfort, 1808

Single-chambered foraminiferans



https://eol.org/pages/46591920

Allogromia laticollaris Arnold, 1948

Two-chambered foraminiferans

https://foraminifera.eu/

Hyperammina elongata Brady, 1878

9

Foraminifera

Cell growth & TOR

- **Growth** is an increase in size (biomass, volume) over time; balance between anabolic & catabolic processes (Hohenegger, 2018)
- **TOR** (Target of Rapamycin) = an evolutionarily conserved protein kinase that regulates cell growth
  - **TORC1**: temporal control of cell growth; coordinates cell growth & metabolism with environmental cues (e.g., nutrients); promotes anabolic processes (transcription & ribosome biogenesis, translation, biosynthesis of proteins, lipids & nucleotides); inhibits catabolic processes (degradation of cytosolic components, recycling & autophagy)
  - **TORC2**: spatial control of cell growth; signals cytoplasm; absent in algae & plants
- ***Reticulomyxa filosa* genome** (Tatebe & Shiozake, 2017):
  - **TORC1**: TOR kinase, RAPTOR subunit, LST8
  - **TORC2**: TOR kinase, RICTOR regulatory subunit, LST8, SIN1 regulatory subunit

10

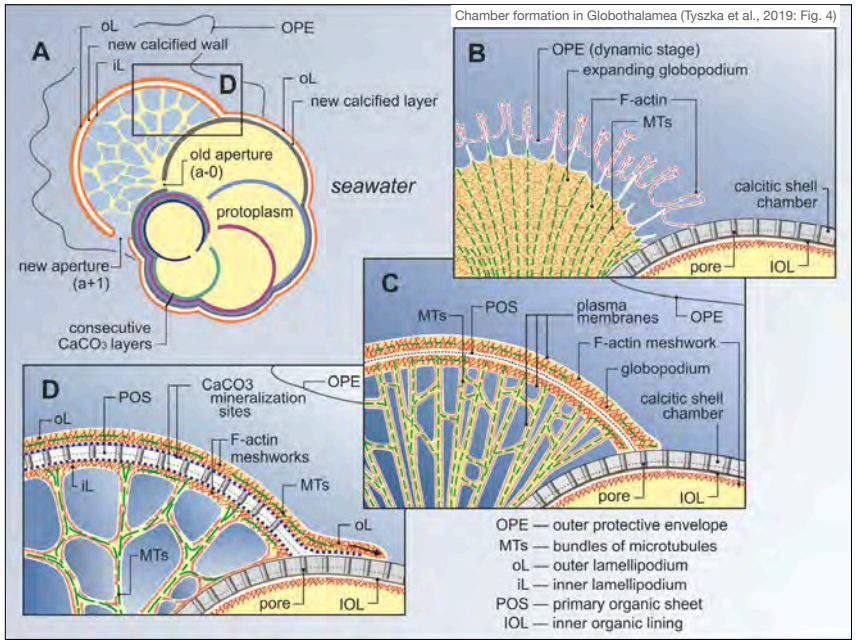
Foraminifera

Polarized cell growth & chamber formation

- **Polarized cell growth** is growth by cell elongation; common feature of polarized cell growth is that a self-similar shape is formed at the apex of the cell (Jaffar & Davidson, 2013)
- **Chamber morphogenesis** in Globothalamea (Tyszka et al., 2017; 2019)
  - Chamber formation in *Amphistegina* investigated using confocal microscopy & fluorescent live staining of cytoskeletal components (actin & tubulin)
  - Chamber shape is predefined by a globular structure (**globopodium**) that acts as dynamic scaffold supported by the F-actin (filamentous actin) meshwork
  - F-actin meshwork interacts with microtubules & associated proteins involved in morphogenesis & biomineralization of chamber

11

Chamber formation in Globothalamea (Tyszka et al., 2019: Fig. 4)



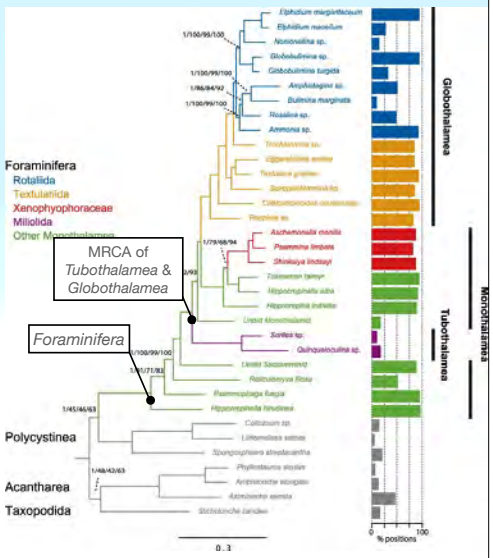
12

Foraminifera

Questions

- How many times did multichambered tests evolve?
  - Once: Multichambered tests evolved in ancestor to the MRCA of *Tubothalamea* & *Globothalamea*
  - More than once: Multichambered tests evolved independently in:
    - *Tubothalamea*
    - *Xenophyophora*
    - *Globothalamea*

Molecular phylogeny of the SAR clade



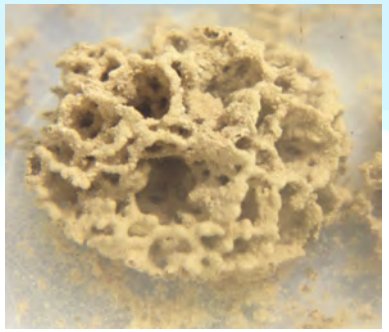
Molecular phylogeny of Foraminifera & radiolarian clades (Sierra et al. 2022: Fig. 1)

Xenophyophora

Classification

- First described as deep-sea sponges
- For many years, xenophyophores were classified in separate phylum from *Foraminifera*
- DNA sequence data from *Syringammina corbicula* showed that xenophyophores are foraminiferans
- Currently 90 named living species classified in 23 genera
- 202 GenBank sequences (mostly SSU rDNA) representing 20 named species & at least 20 unnamed species to date

Live xenophyophore



Syringammina corbicula Richardson, 2001
from the Cape Verde Plateau, Atlantic Ocean

Xenophyophora

Phylogeny

- *Xenophyophora* branches as a monophyletic clade within Clade C
- Clade C (Xenos + single-chambered taxa) is sister to *Globothalamea*
- Tree shows 3 major xenophyophore clades
  - None of the clades have bootstrap values >70%
  - The relationships to each other are unresolved

Molecular phylogeny of Xenophyophora

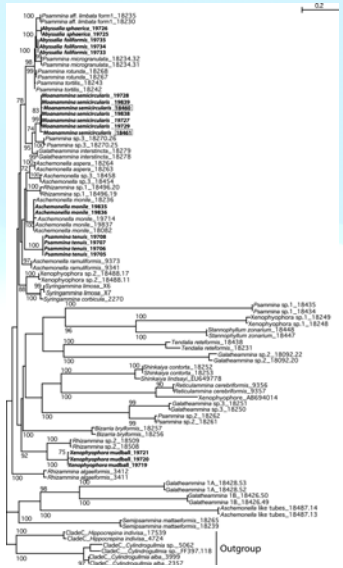


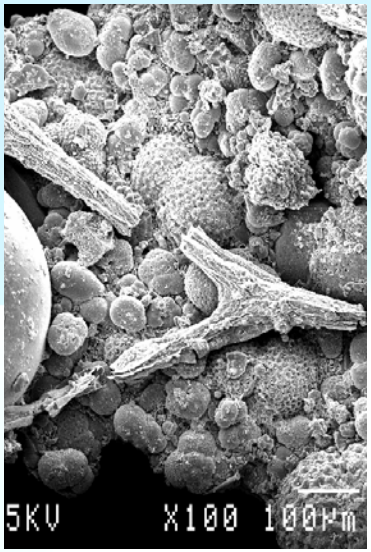
Fig. 4 (Gooday et al., 2020)

Xenophyophora

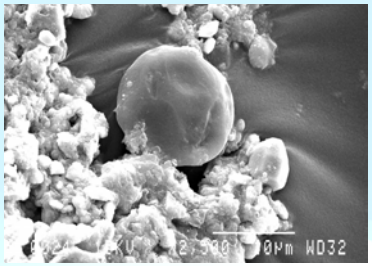
Diagnostic Traits

- **Compartmentalization**: Xenophyophores differ from other forams in the degree of compartmentalization of the test
  - **Xenophyae**: Agglutinated particles that make up test; held together by organic cement; functions as scaffold to support granellare & stercomare
    - Particles include: sponge spicules, foram tests, radiolarian skeletons, coccoliths, quartz & other mineral grains, carbonized plant fragments
  - **Granellare**: Branched, tubular structures that enclose the cell body (cytoplasm & nuclei); bounded by organic envelope; 2-5% of test volume
  - **Stercomare**: Branched, sometimes anastomosing, structures that enclose the stercomes (pellets of indigestible waste); bacteria associated with stercomes may serve as additional food resource; 40-82% of test volume
- **Large size**: *Stannophyllum venosum* Haeckel, 1889 can grow to a test size of 25 cm; Stannophyllum pertusum Haeckel, 1889 reaches sizes >20 cm
- **Habitats**: In modern oceans, xenophyophores occupy epibenthic, infaunal &epifaunal (attach to polymetallic nodules) habitats in the deepsea underlying highly productive surface waters.

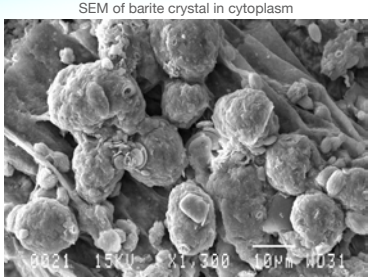
Syringammina corbicula Richardson, 2001



SEM of granellare (branched tube containing cell body)



SEM of barite crystal in cytoplasm



SEM of stercomata (fecal pellets) in stercomare tube

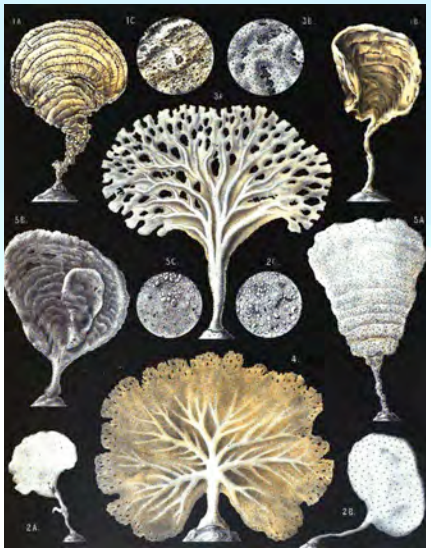
17

Xenophyophora

Endopolyploidy & large cells

- **Multinucleate cells**: the cytoplasm contains numerous nuclei
  - Mitosis (nuclear division) takes place during cell cycle, but cytokinesis doesn't occur (**endomitosis**)
  - *Shinkaiya lindsayi*: DAPI staining of cell body shows xeno contains 1.5 million nuclei per mm^3 of cytoplasm
- **Uninucleate stage** hasn't been observed in xenophyophores;
  - In other forams, the haploid stage is uninucleate; however, the nucleus undergoes **endocyling** (increased # chromosomes; M phase is dropped from cycle cycle (DNA replication occurs in S-phase, but mitosis doesn't occr)

Species of *Stannophyllum*



Species of *Stannophyllum* illustrated in Pl. I of Haeckel, 1889

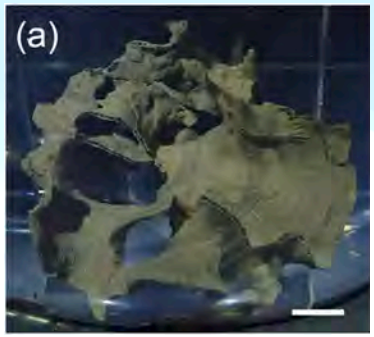
18

Xenophyophora

Episodic growth

- Gooday et al. (2011) recorded episodic growth in *Reticulammina labyrinthica* using time lapse photos of deep sea floor; episodic growth not reflected in test structure
- Tsuchiya & Nomaki (2021) used in situ labeling of xenos with ^{13}C-labelled glucose & ^{15}N-labelled diatoms to show that xenos take up both DOM (glucose) & POM (diatoms) relatively quickly; i.e., over 2 days of experiment

Unidentified xenophyophore



Unidentified xenophyophore collected by Tsuchiya & Nomaki (2021: Fig. 3). NOTE: Growth lines are visible on the specimen.

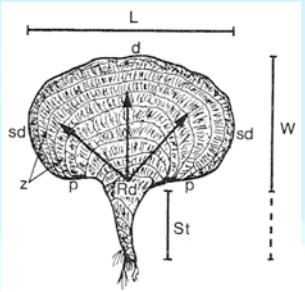
19

Xenophyophora

Episodic growth

- Taxa with "growth lines": growth lines recognizable on test surface, but not reflected in organization of test interior
  - *Galatheammina intersincta*
- Taxa with "growth zones": chamber-like modules added to test with growth; interior walls may delimit zones; no obvious apertures occur between zone
  - *Psammina* spp.
  - *Semipsammina licheniformism*
  - *Stannophyllum zonarium, S. radiolarium*

Growth zones in *Stannophyllum*



Tendal, 1972

***Stannophyllum radiolarium* in Kamenskaya et al., 2015**

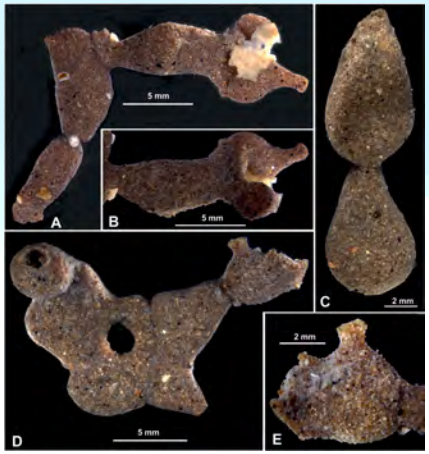
20

Xenophyophora

Episodic growth

- Taxa with discrete chambers add chambers as test grows:
  - *Aschemonella* spp.: Irregularly shaped chambers are connected by narrow necks
- Taxa that add polygonal modules to test with growth, but don't show zones:
  - *Tendalia reteformis*
  - *Ocultammina profunda*
- Taxa with tests of reticulated tubes show "radial growth zones"
  - *Syringammina limosa*
- Taxa with tree-like tests have "bifurcation zones"; i.e., bifurcation of granellare & test elements occur simultaneously across
  - *Spiculammina* delicata
  - *Stannophylum* species

Aschemonella monile



Aschemonella monile Gooday et al., 2017
(Gooday et al., 2020: Fig. 2)

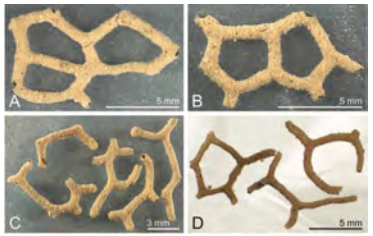
Test with bifurcation zones

Spiculammina delicata Kamenskaya et al., 2015

Test with radial growth zones

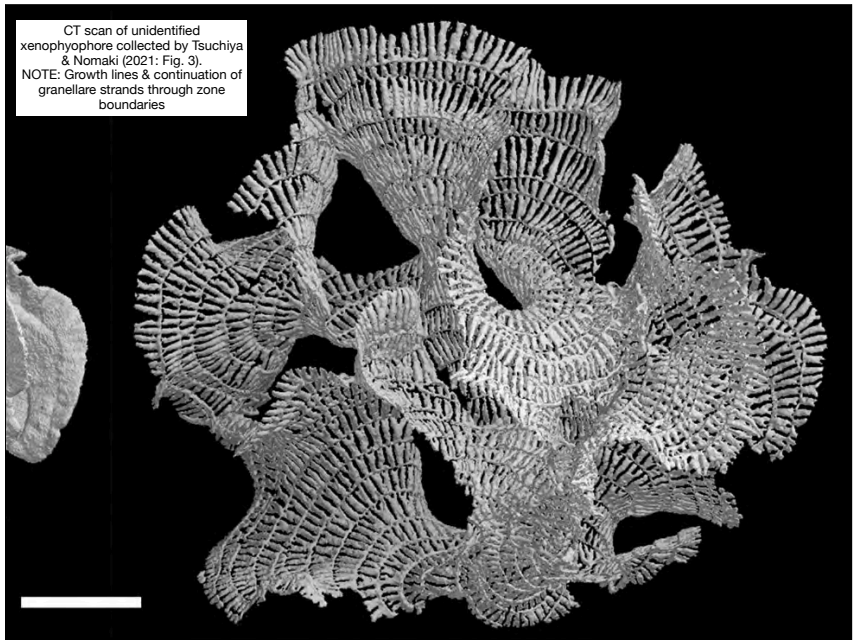
Syringammina limosa Voltski et al., 2018

Test with polygonal modules



Tendalia reteformis Gooday & Holzmann, 2017

CT scan of unidentified xenophyophore collected by Tsuchiya & Nomaki (2021: Fig. 3).
NOTE: Growth lines & continuation of granellare strands through zone boundaries

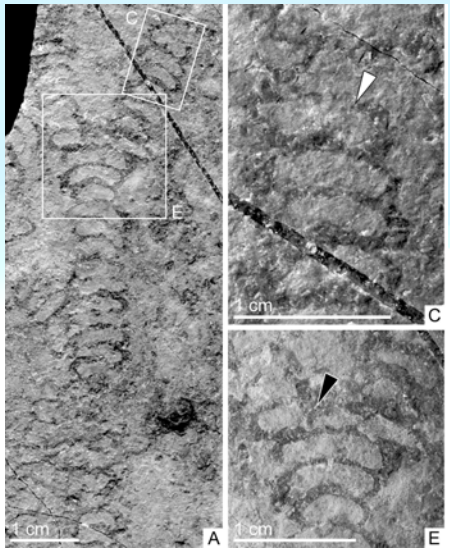


Xenophyophora

Fossils

- *Aschemonella bastillensis* McClellan, 1973: Silurian (Wenlock) Bastille Ls, Nevada: multichambered fragments
- *Bencovacina concentrica* Seilacher & Mrinjek, 2011: Eocene Benkovac Ls, Croatia: specimen with concentric growth zones, no xenophyae preserved, preserved on surface of microbial mat
- *Palaeopaschichnus linearis* Kolesnikov et al., 2017: relatively abundant Ediacaran fossil interpreted as having a multichambered agglutinated wall; maybe a stem xeno?

Stem xenophyophore?



Fossils of *Palaeopascichnus linearis* Kolesnikov et al., 2018
(Fig. 5) Khatyspyt Fm, Siberia

Evolutionary Trends in Foram Test Morphology

Summary

- Basal lineages with single-chambered tests that grow by expansion; shift to episodic growth results with addition of new test material at one end of cell; evolution of tests with discrete chambers
- Endopolyploidy in forams allows for larger cell sizes
- Not clear whether multi-chambered tests arose only once during evolution of Foraminifera, or multiple times?
  - Need more DNA sequences from Lagenida to help resolve molecular trees; also need to include more genes in molecular analyses, not just SSU rDNA
  - Need to integrate morphological data into trees, so that can include fossil taxa
- Xenophyophores have more compartmentalized tests & a greater diversity of ways to increase test size compared to Globothalamea & Tubothalamea clades
- Future studies should continue investigation of involvement of cytoskeleton (actin filaments & microtubules) in chamber morphogenesis, as well as genetic regulation of growth (TOR)

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