New supraordinal classification of Foraminifera: Molecules meet morphology

Jan Pawlowski^a, Maria Holzmann^a, Jarosław Tyszka^b

- ^a Department of Genetics and Evolution, University of Geneva, Quai Ernest Ansermet 30, 1211 Geneva 4, Switzerland
- ^b ING PAN, Institute of Geological Sciences, Polish Academy of Sciences, Research Centre in Kraków, ul. Senacka 1, 31-002 Kraków, Poland

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ABSTRACT

The limitations of a traditional morphology-based classification of Foraminifera have been demonstrated by molecular phylogenetic studies for several years now. Despite the accumulation of molecular data, no alternative higher-level taxonomic system incorporating these data has been proposed yet. Here, we present a new supraordinal classification of Foraminifera based on an updated SSU rDNA phylogeny completed with the description of major morphological trends in the evolution of this group. According to the new system, multi-chambered orders are grouped in two new classes: Tubothalamea and Globothalamea. Naked and single-chambered Foraminifera possessing agglutinated or organic-walled tests are arranged into a paraphyletic assemblage of "monothalamids". The new system maintains some multi-chambered calcareous orders, such as Rotaliida, Miliolida, Robertinida and Spirillinida, although their definitions have been modified in some cases to include agglutinated taxa. The representatives of the planktonic order Globigerinida are tentatively included in the order Rotaliida. The agglutinated Textulariida are probably paraphyletic. The position of the order Lagenida is uncertain because reliable molecular data are only available for one species. The new classification system separates orders or families, which differ in basic chamber shapes, prevailing mode of coiling and distance between successive apertures. It appears that these features correspond better to the main evolutionary trends in Foraminifera than wall composition and structure, both used in traditional classification.

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1. Introduction

The classification of Foraminifera has a long history going back to the beginning of the 19th century and the work of d'Orbigny (1826) who established the order Foraminifera and proposed the first taxonomic system based on the growth plan of foraminiferal tests. d'Orbigny's successors have developed diverse systems based on the morphology of fossil and recent tests, differing principally by the importance given to form and chamber arrangement versus wall composition and structure (reviewed in Cifelli, 1990). The primary division of Foraminifera into single-chambered Monothalamia and multi-chambered Polythalamia (Schultze, 1854) was progressively replaced by a classification based on the presence or absence of pores (Reuss, 1861; Carpenter et al., 1862). In the 20th century, wall characteristics gained more and more importance and became the main criterion to distinguish higher-level groups in Foraminifera (Pokorny, 1963; Loeblich and Tappan, 1964; Hohenegger and Piller, 1975). In the seminal work of Loeblich and Tappan (1988, 1989, 1992), Foraminifera were divided into 12 suborders that mainly differ by mineralogical and ultrastructural features of the test wall. In the most recent modifications of this classification (Sen Gupta, 1999; Mikhalevich, 2004; Kaminski, 2005), the number of orders (or classes/subclasses) increased to 16, but the foundations of this system remained unchanged (Table 1).

A few important attempts were carried out to group suborders into higher level taxa (Hohenegger and Baal, 2004; Hohenegger, 2011). The noticeable classification proposed by Mikhalevich (1998, 2000, 2004) and Mikhalevich and Debenay (2001) was based on a "macrosystem" dividing Foraminifera into seven classes and

resting upon "the whole organization of the test" rather than composition and ultrastructure of the test wall. This system revealed some interesting tendencies in the evolution of Foraminifera mainly based on morphological test patterns. Many taxonomic studies of Foraminifera concerned revisions of lower-level taxa (e.g., Hottinger, 1980; Gudmundson, 1994; Revets, 1996) but only a few tried to build up a more general system, such as the classification of agglutinated foraminiferans, whose updated versions are published on a regular basis (Kaminski, 2004).

The lack of progress in higher-level classifications of Foraminifera was mainly due to difficulties in inferring evolutionary relationships between major groups defined exclusively by morphological features and the sheer number of taxa involved. Despite the excellent fossil record, phylogenetic schemes of foraminiferal evolution are limited to textural and morphologic characters of tests (e.g., Cushman, 1948; Grigelis, 1978; Tappan and Loeblich, 1988; Vachard et al., 2010). This situation has changed with the advent of molecular studies that shed new light on the evolution of Foraminifera (Pawlowski, 2000; Bowser et al., 2006). The majority of molecular phylogenies were based on analyses of partial SSU and LSU rDNA sequences (Holzmann and Pawlowski, 2000; Pawlowski, 2000, 2002a,b, 2003). Because of their unusual length (>3000 nucleotides) complete SSU sequences were only obtained for a few species, mainly representatives of the order Rotaliida (Pawlowski et al., 2005), tubulin (Habura et al., 2006) and RNA polymerase (Longet and Pawlowski, 2007), but the number of species analyzed in these studies was very small. Recent analysis of combined sequence data confirmed major trends in the evolution of Foraminifera from single gene phylogenies (Groussin et al., 2011). However, up to now no formal attempt has been made to modify the higher-level classification of Foraminifera by including molecular data.

Here, we propose a new higher-level system of Foraminifera, based on molecular data. We present an updated version of a SSU rDNA phylogeny based on complete sequences obtained from representatives of almost all foraminiferal orders, including 23 new sequences. We discuss this phylogeny with reference to other multigene studies and we describe the basic morphological features for new molecular groupings.

2. Material and methods

2.1. DNA extraction, amplification, cloning and sequencing

DNA was extracted using guanidine lysis buffer (Pawlowski, 2000), and each extraction was performed with a single specimen. The DNA collection numbers, collection sites and taxonomic references for all analyzed species are given in Table 2. PCR amplifications of the complete SSU rDNA were performed using several primer pairs (Table 3). The amplified PCR products were purified using High Pure PCR Purification Kit (Roche Diagnostics), cloned with the TOPO TA Cloning Kit (Invitrogen) following the manufacturer's instructions and transformed into competent *Escherichia coli*. Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and analyzed on a 3130XL Genetic Analyser (Applied Biosystems). The new sequences reported in this paper were deposited in the EMBL/GenBank data base and their accession numbers are listed in Table 2.

2.2. Phylogenetic analyses

The obtained sequences were aligned to an existing database using Seaview vs 4.3.3. (Gouy et al., 2010). After elimination of highly variable regions, 1904 sites were left for analysis. Based on MEGA5 (Tamura et al., 2011), a GTR + G model of evolutionary changes was selected. A phylogenetic tree was constructed using maximum likelihood (ML) method using RAxML as implemented in BlackBox (Stamatakis et al., 2008). Bayesian inference (BI) was performed with MrBayes 3.2.1 (Huelsenbeck and Ronquist, 2001). The analysis consisted of four simultaneous chains that were run for 10,000,000 generations, and 10,000 trees were sampled, 2000 of which were discarded as burn-in. Posterior probabilities at all nodes were estimated for the remaining trees.

The results of phylogenetic analyses were compared to morphological trends revealed from fundamental shell features characterized all analyzed taxa, including unilocularity vs. bi- and multilocularity, basic shape of chambers, and composition of the wall (organic, agglutinated and calcareous). These features were indicated in a phylogenetic tree and discussed based on recent knowledge on morphogenetic patterns responsible for the foraminiferal shell formation.

3. Results and discussion

3.1. Molecular phylogeny

The ML and BI analyses of complete SSU rDNA sequences show congruent results (Fig. 1). The foraminiferal tree comprises two large clades of multi-chambered species. The first multi-chambered clade, called here the Globothalamea, is composed of species belonging to the orders Rotaliida, Robertinida and Textulariida. Rotaliida form a relatively well-supported clade (81% BV, 0.96 PP) that also includes the sequences of planktonic Globigerinida. *Robertina arctica*, the only representative of the order Robertinida branches at the base of Rotaliida, together with *Leptohalysis scotti*. Another textulariid, *Reophax* sp. branches independently as sister to all Globothalamea, in both ML and BI analyses. The Globothalamea group together in all analyses, but their clade is not well supported. This is partly due to the genetic similarity between globothalamea is much stronger when the highly divergent sequences are removed and a larger number of sites are analyzed.

The second multi-chambered clade, called here the Tubothalamea, is composed of Miliolida, Spirillinida and Ammodiscidae, the latter two groups being represented by the genera *Spirillina* and *Ammodiscus*, respectively. *Spirillina* and *Ammodiscus* form a strongly supported (100%) clade branching as sister to Miliolida. The relations within Miliolida are well supported, except for the position of *Cornuspira*, that branches as sister to other species, but without a strong support in both ML and BI analyses. The agglutinated genus *Miliammina* was shown to be related to miliolids in previous studies (Fahrni et al., 1997; Habura et al., 2006) but has not been included in our analyses, as the authenticity of its SSU rDNA sequence could not be ascertained. Nevertheless, actin and tubulin molecular records (Fahrni et al., 1997; Habura et al., 2006), as well as a clearly tubular shape of chambers still support its close affinity to miliolids and the Tubothalamea.

All multi-chambered species could be placed in one of the two clades, except for the sequence of *Glandulina antarctica*, the only representative of the order Lagenida reliably documented so far. DNA amplification of lagenid specimens has very low success rates, even when attempting a fragment of the SSU rDNA that in general yields positive results for all other tested groups of Foraminifera. The lack of broader taxon sampling in Lagenida makes the accurate establishment of its phylogenetic position difficult. Yet, the sequence of G. antarctica is so different from other multi-chambered taxa that Lagenida possibly form a separate group that evolved independently from an unknown monothalamous lineage. This is also supported by a different morphology of lagenid chambers and the structure of their tests (see below).

The deep relationships of monothalamous lineages remain unresolved. A few well-supported clades such as *Bathysiphon argenteus* + *Micrometula* sp. (clade BM), or *Psammophaga* sp. + *Vellaria* sp. (clade E) emerged already in previous studies based on partial SSU rDNA sequences (Pawlowski et al., 2002a,b). As indicated above, clades A and C are always sister to the Globothalamea. Clade BM is positioned at the base of Tubothalamea, in agreement with multigene analyses (Groussin et al., 2011). However, the support for this relationship is very low. The remaining monothalamous clades and lineages branch at the base of the tree but their relationships are not supported. Based on previous protein-coding studies (Flakowski et al., 2005; Longet and Pawlowski, 2007), we selected *Allogromia* sp. + *Astrammina rara* to root the tree. However, a lack of protein coding genes for most of the monothalamous lineages and high divergence of foraminiferal SSU rDNA sequences makes the exact positioning of the root uncertain.

The foraminiferal phylogeny as presented in Fig. 1 is very similar to previously published trees based on sequences of partial SSU rDNA (Pawlowski et al., 2002a,b, 2003; Bowser et al., 2006), actin (Flakowski et al., 2005), tubulin (Habura et al., 2006), and RNA polymerase (Longet and Pawlowski, 2007). It differs from earlier SSU rDNA trees (Pawlowski et al., 1997; Pawlowski, 2000) by the placement of Miliolida in the center of the tree rather than at its base, shown to be an artifact due to low GC content in miliolid rDNA sequences (Bowser et al., 2006). The separation between the two multi-chambered clades Globothalamea and Tubothalamea has been already evident in previous analyses of partial SSU rDNA sequences (Pawlowski et al., 2003). Compared to these studies, the support for these multilocular clades clearly increased, especially in the case of Globothalamea, whose monophyly was not recovered when the fast evolving Tubothalamea were included. Yet, the phylogenetic signal in SSU rDNA is insufficient for resolving relationships between the two multi-chambered and single-chambered clades. Multigene analyses and increased taxon sampling for monothalamous lineages will be necessary to improve the resolution at the base of the foraminiferal tree (work in progress). However, molecular evidence for the presence of two multi-chambered clades is sufficiently well established to introduce them into the new classification of Foraminifera.

3.2. Morphological trends

The phylogenetic clades defined by molecular data were compared to fundamental shell features (represented by symbols in Fig. 1). The wall composition of the analyzed taxa shows a complex polyphyletic pattern that seems to be useful in defining intermediate rank taxonomic units. The multi-chambered clades of Globothalamea and Tubothalamea are primarily defined by globular and coiled tubular chamber shapes, respectively (Fig. 1). These important morphological characters are related to the relative distance between successive apertures and are associated with two different development patterns (Fig. 2). A summary of morphological and textural features characterizing Globothalamea and Tubothalamea is presented in Table 4.

3.2.1. Chamber shapes

According to Hottinger (2000), "in order to generalize the broad variety of shell architecture in foraminifera, two basic types of protoplasm compartment shapes can be distinguished, a tubular and a non-tubular, more or less isotropic, subspherical to subconic one" (p. 69). These two fundamental chamber patterns were retrieved in our two multichambered clades.

The class Tubothalamea is characterized by tubular chambers either throughout ontogeny or in early ontogenetic stages (Table 4). Such truly tubular chambers were also called longithalamous (sensu Brasier, 1980), emphasizing the elongation of chambers that are "longer than they are wide" (Armstrong and Brasier, 2005). Tubular chambers are best developed in small, simple bi-chambered and multi-chambered forms of Tubothalamea, which are either agglutinated, like *Ammodiscus, Miliammina*, or calcareous, like *Spirillina* or *Quinqueloculina*. In order to shorten axial elongation of tubular chambers, some large miliolids, such as *Alveolina* or *Praealveolina*, evolved low composite chambers composed of short tubular chamberlets. Other complex miliolids shortened the axial elongation of tubular chambers by formation of nearly equidimensional chamberlets. However, their early ontogenetic growth stage still preserves a single chamber or series of coiled, tubular chambers (see Loeblich and Tappan, 1988; Hottinger, 2006; Hohenegger, 2011).

Globothalamea possess multi-chambered tests with globular chambers that are often brevithalamous, meaning "wider than long" (Armstrong and Brasier, 2005). Small, simple globothalamid tests are always constructed by successive growth of globular chambers that are more or less overlapping preceding chambers. Chamber shape is species specific and varies from simple globular, more or less inflated, discoid, narrow or broad to elongate. Chamber shape also strongly depends on the ontogenetic stage, with early ontogenetic chambers being usually more globular. Larger, complex globothalamids exhibit very diverse geometries of chamberlets forming composite chambers.

3.2.2. Bilocular vs multilocular growth

Although both groups are characterized by multi-chambered tests, some primitive representatives of Tubothalamea are bilocular having a tubular second chamber either without or with only rudimentary partitions. Such simple bilocular forms appear not only in the order Spirillinida, including agglutinated (Ammodiscina) and calcitic (Spirillinina) wall textures but also in porcelaneous Miliolida (*Cornuspira*). Bilocularity is generally missing in Globothalamea because once built, a globular chamber cannot get bigger and thus their only way to grow is by adding a new chamber.

3.2.3. Apertures and their morphogenetic implications

Fundamentally different chamber shapes are linked to different patterns of aperture formation. In Tubothalamea with simple tubular chambers, apertures are located at the end of each chamber and therefore the distance between them is maximized (Fig. 2). The apertures are self-defined by the tube end, the latter one being determined by addition of a new chamber. Tubothalamea can decrease aperture distance by shortening their chambers and additionally either multiplying chamber number in a growth cycle or widening their diameter. Globothalamea have a different approach to aperture formation. A new aperture is created mostly at the shortest distance from the previous aperture (Topa and Tyszka, 2002; Łabaj et al., 2003; Tyszka and Topa, 2005). This minimizes the distance between proloculus and the last aperture and possibly presents some advantages for intracellular transportation (Hottinger, 1978; Brasier, 1982; Hohenegger, 1999).

The aperture is a moving reference for the formation of new foraminiferal chambers (Tyszka and Topa, 2005; Tyszka, 2006). The clade Globothalamea comprises Textulariida and Rotaliida that use similar morphogenetic mechanisms to create corresponding morphologies, dependent on the self-organization of foraminiferal cytoskeleton (Tyszka et al., 2005; Topa et al., 2012). Both orders use the same flabellate, radial pattern of

microtubules iteratively shaping every new chamber during its formation. In contrast, Tubothalamea seem to use streaming patterns of longitudinal microtubules to stimulate accretionary growth of their tests (Tyszka et al., 2005; Tyszka, 2006). This is a very different mode of growth adopted by ammodiscids, rzehakinids, and miliolids (see De Nooijer et al., 2009). In theory, this growth pattern can be simulated based on the accretionary growth models known from larger shells of ammonites, gastropods and bivalves (Raup and Michelson, 1965; Okamoto, 1988).

3.3. New classification of Foraminifera

Based on updated molecular data and their morphological interpretation, we propose a new higher-level classification presented in Appendix 1. In this new system, Foraminifera are considered as a phylum composed of three main groups:

- The class Globothalamea grouping multi-chambered species whose chambers are typically globular;
- The class Tubothalamea grouping multi-chambered species whose chambers are typically tubular;
- The paraphyletic assemblage of "monothalamids" grouping all single-chambered species having organic and agglutinated walls.

At the order-level, the new system is relatively conservative. Several traditional orders, such as Rotaliida, Miliolida, Spirillinida, and Robertinida are preserved, although their definition has sometimes been modified. In the case of Rotaliida, the distinction between Rotaliida and Buliminida (Sen Gupta, 1999), based principally on the presence/absence of an internal toothplate, is not justified in view of molecular data (Schweizer et al., 2008). Moreover, this previously exclusively benthic order also includes planktonic Foraminifera traditionally classified in the order Globigerinida. The grouping of benthic and planktonic species is supported by molecular evidence showing a triserial planktonic species branching among benthic rotaliids (Ujiié et al., 2008) and a conspecificity of some biserial planktonic species and benthic bolivinids (Darling et al., 2009). The position of other planktonic families is difficult to establish because of the heterogeneity of their evolutionary rates (de Vargas et al., 1997, 1998). As shown in Fig. 1 at least one planktonic family (Globorotaliidae) branches within Rotaliida. The family Globigerinidae, for which complete SSU rDNA sequences are not available, also branches within rotaliids in some but not all protein-coding gene analyses (Ujiie, unpublished data).

The orders Miliolida and Spirillinida also need to be redefined. Both orders are traditionally calcareous and monophyletic, but in the new system they include some agglutinated genera and therefore cannot be defined by wall composition and structure only. However, each of these orders could be divided into suborders characterized by different wall features. For example, in the case of Spirillinida, the agglutinated Ammodiscidae form a sister group to calcareous spirillinids, and therefore could be considered as a separate suborder Ammodiscina, as suggested by Mikhalevich (1992), as well as Mikhalevich and Debenay (2001). The situation is more complex in the case of porcelaneous Miliolida that comprise the agglutinated genus *Miliammina* (Fahrni et al., 1997; Habura et al., 2006). Although the position of this genus could not be established with certainty in SSU rDNA trees, proteincoding genes indicate that it branches between *Cornuspira* and other miliolids (Habura et al., 2006; Groussin et al., 2011). If we accept the hypothesis of a reversal from calcareous to agglutinated tests (Galloway, 1933; Habura et al., 2006), and if other agglutinated miliolids branch with *Miliammina*, the order Miliolida could be split into several suborders, each characterized by distinctive morphological features. Mikhalevich and Kaminski (2008) have included the agglutinated miliamminids and rzehakinids within the order Schlumberinida, which they considered ancestral to the order Miliolida.

In the new system, the two single-chambered orders Allogromiida and Astrorhizida have been replaced by a paraphyletic assemblage of monothalamids. The distinction of these two orders characterized by an organic or agglutinated wall, respectively, could not be maintained because the transition between organic and agglutinated walls takes place in different monothalamous lineages (Pawlowski et al., 2002a,b, 2003). The number of these lineages is rapidly growing, with many new phylotypes identified in environmental DNA samples (Lecroq et al., 2011; Pawlowski et al., 2011) and many new species described from high-latitude and deep-sea habitats, where monothalamous clades, including 8 environmental lineages have been identified in marine environments (Pawlowski et al., 2011) and additionally 4 clades have been described from freshwater and soil samples (Lejzerowicz et al., 2010). Most of these clades are represented by partial SSU rDNA data only. Their classification is therefore still in progress, but for sure their richness and genetic diversity by far exceed some of the better known multi-chambered orders.

Another order that possibly represents a paraphyletic assemblage is the Textulariida. Although most textulariids branch together at the base of Rotaliida (Fig. 1) their grouping is not well supported. The sequences of the genera *Reophax* and *Leptohalysis* often branch independently. This is also the case of some other genera, for which only partial SSU rDNA sequences have been analyzed (Bowser et al., 2006). There is certainly no support for splitting multi-chambered agglutinated Foraminifera into the orders Lituolida, Trochamminida and Textulariida, as proposed by Loeblich and Tappan (1989). The Lituolida as defined by them are a polyphyletic group, with some genera belonging to Miliolida (*Miliammina*) or Spirillinida (*Ammodiscus*). Moreover, the genera *Trochammina* and *Textularia* are so closely related in molecular phylogenies (Fig. 1) that it would be difficult to consider them as members of different orders. There are some simple transitional agglutinated Foraminifera, such as Trochamminoides, included by Loeblich and Tappan (1989) to the suborder Textulariina, which show a proloculus followed by spirally coiled and undivided tubular second chamber, switching later to numerous more or less globular chambers. Such transitional forms Grzybowski (1898) regarded to be descended from *Ammodiscus*. It is likely that these morphotypes should be included to Tubothalamea; nevertheless, this should be verified by molecular data.

Within the remaining orders, Robertinida and Carterinida have been placed in the Globothalamea, based on an analysis of *Robertina arctica* (Fig. 1) and unpublished molecular data obtained for Carterina. The Silicoloculinida have been included into Miliolida, based on the quinqueloculine chamber arrangement characterizing the genus Miliammellus, the unique representative of this order (Loeblich and Tappan, 1988).

Three orders (Lagenida, Fusulinida and Involutinida) remain as incertae sedis. Concerning Lagenida, there is only one complete SSU rDNA sequence available derived from *G. antarctica*, whose position is not well established. The partial SSU rDNA sequences of *Marginulopsis* and *Lenticulina* published previously (Bowser et al., 2006) could not be extended. Additional sequence data of lagenids are needed to decide whether this order forms a separate multichambered class or belongs to one of the two classes described here. Lagenida differ from them by mode of growth and terminal chamber formation. They partly maximize the distance between apertures as is the case in Tubothalamea. On the other hand, their chambers are rather globular. A common feature of Lagenida is an ontogenetically stable terminal aperture combined with more or less tapering chamber shapes. Such terminal apertures and chambers are known from uncoiling, rectilinear parts of some Globothalamea (*Ammobaculites, Spiroplectinata*, etc.). However, in Globothalamea terminal apertures are not ontogenetically stable and tend to appear during their final growth stages. Calcareous Lagenida have agglutinated homologs/analogs, constructing similar chamber and shell morphologies. Therefore, Mikhalevich (1992, 2005), Mikhalevich and Debenay (2001) included calcareous and agglutinated forms into the class Nodosariata. Due to lack of molecular data we prefer an *incertae sedis* status for this group.

Fusulinida are an extinct order, and therefore its placement can be based only on an analysis of morphological data. Mikhalevich and Debenay (2001) included them in Rotaliata, but later studies (Mikhalevich, 2006, 2009) based on comparative morphological analysis placed the superorder Fusulinoida in the class Miliolata. Some authors proposed a close relationship of certain fusulinids to Textulariida (Rigaud, 2012). A detailed analysis of fusulinid early chamber development and internal foraminal lines of communication would be necessary to verify these two hypotheses. The Involutinida have been considered as closely related to Spirillinida (Sen Gupta, 1999), however, as there is no molecular data available, we prefer to leave it as *incertae sedis*.

From an evolutionary perspective, the new system assumes that the major step in the evolution of Foraminifera is the transition from a single-chambered to a multi-chambered test. As shown in Fig. 1, this transition occurred at least twice, in the stem lineages of Globothalamea and Tubothalamea, and probably also in the stem lineage leading to Lagenida. The traditional view of successive evolution of Foraminifera from organicwalled to agglutinated and further to calcareous lineages (Cushman, 1935, 1948; Tappan and Loeblich, 1988) is challenged by molecular evidence for multiple origins of agglutinated and calcareous walls. The transformation from organic to agglutinated walls (brown symbol in Fig. 1) occurred several times in monothalamous lineages (Pawlowski et al., 2002a,b; Bowser et al., 2006). Many monothalamid clades comprise both organic and agglutinated species and some, e.g. Astrorhiza limicola, may even change the nature of their test wall depending on environmental conditions (Cedhagen and Tendal, 1989). However, the presence of an agglutinated wall seems to be a prerequisite to the formation of a multi-chambered (polythalamous) test. With respect to our available molecular data (Fig. 1), multi-chambered agglutinated tests evolved independently in lineages leading to Tubothalamea (Ammodiscus), and Globothalamea (Reophax), probably from an agglutinated monothalamous lineage. The calcareous wall appeared at least five times independently (blue rhomboid symbol in Fig. 1). Remarkably, each time a different type of calcareous wall was developed. The three orders with a calcareous bilamellar wall (Rotaliida, Buliminida and Globigerinida) form one clade. The other calcareous orders, including the aragonitic Robertinida, the calcitic monolamellar Lagenida, the imperforate Miliolida, and the monocrystalline Spirillinida form independent monophyletic groups.

In many respects, our system is complementary to previous classifications. The supra-ordinal groupings correspond well to the phylogenetic scheme presented by Tappan and Loeblich (1988). The distinction between single-chambered and multi-chambered foraminiferans has been proposed several times, starting with d'Orbigny (1826) who introduced the class Monostegua for all unilocular species, including planktonic *Orbulina* and organic-walled *Gromia*, considered at that time as a foraminifer. Avnimelich (1952) introduced a suborder Monothalamia for tubular foraminiferans, but included genera such as *Amphitrema* and *Diplophrys* that are no longer considered to be Foraminifera. Our system states the importance of the position and form of foraminiferal apertures and is therefore similar to the one developed by Mikhalevich (1998). The main differences concern our paraphyletic assemblage of monothalamids that replaces Mikhalevich's unilocular classes Lagynata and Astrorhizata, and the Tubothalamea that are regrouped by Mikhalevich and Debenay (2001) in the classes Spirillinata and Miliolata. Moreover, although the composition of our class Globothalamea is almost identical to Mikhalevich's class Rotaliata, her subdivision into superorders and orders does not correspond to the relationships inferred from molecular data.

The most important fact that distinguishes our system from all previous classifications is that it is solidly anchored in molecular phylogenetic data. Phylogenetic analyses based on sequences of SSU rDNA, actin, betatubulin and RNA-polymerase genes show a relatively congruent view of foraminiferal macroevolution. In all phylogenetic trees, monothalamous species appear as a basal paraphyletic group, from which multi-chambered lineages emerged two or three times independently. Although this system still lacks molecular data for many taxa and genes, the major groups are well identified and the molecular view of the macroevolution of Foraminifera as presented here will not undergo substantial changes with the addition of further sequences.

There are several taxonomic issues that remain still open. The phylogenetic position of Lagenida remains uncertain due to a very limited taxon sampling and lack of agglutinated polythalamous lineages that associate to them. The polyphyly of Globigerinida as suggested by some earlier studies (Darling et al., 1997; Pawlowski, 2000) has to be tested. Finally, the paraphyly of Textulariida shall be confirmed by a more detailed analysis of multi-chambered agglutinated species. All these studies will require a much larger taxon sampling, especially in the case of Textulariida, Miliolida and Robertinida. New phylogenetic markers are also necessary to resolve the deep phylogenetic relationships at the base of multi-chambered clades. We expect that further development of phylogenomic studies of Foraminifera (Burki et al., 2010; Sierra et al., 2013) will generate new genomic data that could be used to test the phylogenetic foundations of the present classification.

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Appendix 1. New high-rank phylogenetic classification of Foraminifera

Phylum Foraminifera (d'Orbigny, 1826)

Monothalamids

Single chamber (monothalamous) test with an organic or agglutinated wall; the group comprises all genera traditionally included into the orders Allogromiida and Astrorhizida, as well as the deep-sea giant Xenophyophorea; it also includes freshwater and marine "naked" amoeboid species and environmental clades with unknown morphology (see Pawlowski et al., 2011); the diversity of this mainly unfossilized group is poorly known and has been largely overlooked in micropaleontologically oriented foraminiferal research.

Remarks: The group is paraphyletic, i.e. it comprises the ancestor of all Foraminifera including monothalamous and polythalamous groups. Further phylogenetic analyses are needed to subdivide it into independent monothalamous lineages.

Class Tubothalamea classis nov

Diagnosis: Bi- or multi-chambered test with tubular chambers at least in the juvenile stage; wall agglutinated or calcareous; in ancestral forms the test is composed of a spherical proloculus followed by a spirally enrolled tubular chamber; more evolved forms have multi-chambered tests;

Molecular characteristics: relatively short SSU rRNA gene (2289-2866 nt) and very low GC content (b30%).

Stratigraphic range: Lower Cambrian — recent.

Order Miliolida (Delage and Hérouard, 1896) emend

Test bi- or multi-chambered, chambers tubular or elongate, some with complex internal structures adapted to host algal endosymbionts; wall generally imperforate, calcareous of high magnesium calcite with randomly oriented crystals refracting light in all directions and resulting in a porcelaneous appearance of the test; may be agglutinated or siliceous in some taxa (*Miliammina*, *Miliammellus*).

Order Spirillinida (Hohenegger and Piller, 1975) emend

Test composed of proloculus followed by an enrolled tubular chamber, aperture terminal; wall of low magnesium calcite, optically a single crystal in Spirillinidae and Patellinidae; wall agglutinated in Ammodiscidae.

Class Globothalamea classis nov

Diagnosis: Test multi-chambered, typically trochospirally enrolled but may be triserial, biserial or uniserial; chambers globular or crescent-shaped in early stage; wall agglutinated or calcareous.

Molecular characteristics: SSU rRNA gene length averaging 3500 nt (3000–4000 nt) and GC content of about 40%; a conserved motive in the variable region 41f of the SSU rDNA (Pawlowski and Lecroq, 2010).

Stratigraphic range: Lower Cambrian - recent.

Order Rotaliida (Delage and Hérouard, 1896)

Wall of low magnesium calcite, optically radial, bilamellar, perforate; some with internal canal system; possibly include all or most of planktonic globigerinids.

Order Robertinida (Loeblich and Tappan, 1984)

Wall of hyaline, perforate, optical radial aragonite; chambers with internal partitions in modern taxa.

Order "Textulariida" (Delage and Hérouard, 1896) (P)

Wall agglutinated, with foreign particles attached to organic lining or cemented by low magnesium calcite; possibly paraphyletic group. The term "Textulariida" is here used only partially in the sense of the definition given by Loeblich and Tappan (1988), and includes the most of the Lituolida, the Loftusiida, and the Textularida (sensu stricto), of Kaminski (2004).

Order Carterinida (Loeblich and Tappan, 1981)

Wall composed of rodlike spicules of low magnesium calcite held in organic lining; chambers numerous, trochospirally coiled.

Incertae sedis orders

Order Lagenida (Delage and Hérouard, 1896)

Test single- or multi-chambered, chambers uniserial, biserial, or planispirally coiled; wall of low magnesium calcite, monolamellar; possibly an independent class.

Order Fusulinida (Wedekind, 1937)

Extinct; test multi-chambered wall microgranular, calcareous; possibly could be partly attached to Globothalamea and Tubothalamea.

Order Involutinida (Hohenegger and Piller, 1977)

Test composed of proloculus and spirally coiled tubular second chamber; wall aragonitic; possibly belong to Tubothalamea.

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Table 1

Recent morphology-based high-rank classifications of Foraminifera.

| Loeblich and Tappan (1988) | Sen Gupta (1999) | Mikhalevich (2004) | Kaminski (2005) |
|-------------------------------|------------------|--------------------|------------------|
| Order | Order | Order | Order |
| Foraminiferida | Foraminifera | Foraminifera | Foraminifera |
| Suborders | Orders | Classes | Orders |
| Allogromiina | Allogromiida | Astrorhizata | Allogromiina |
| Textulariina | Astrorhizida | Lagynana | Astrorhizida |
| Fusulinina | Lituolida | Astrorhizana | Lituolida |
| Involutinina | Trochamminida | Spirillinnata | Loftusiida |
| Spirillinina | Textulariida | Ammodiscana | Textulariida |
| Carterinina | Fusulinida | Spirillinana | Fusulinida |
| Miliolina | Miliolida | Miliolata | Miliolida |
| Silicoloculinina | Carterinida | Miliamminana | Silicoloculinida |
| Lagenina | Spirillinida | Miliolana | Involutinida |
| Robertinina | Lagenida | Nodosariata | Robertinida |
| Globigerinina | Rotaliida | Hormosinana | Favusellida |
| Rotaliina | Buliminida | Nodosariana | Spirillinida |
| | Globigerinida | Rotaliata | Lagenida |
| | Involutinida | Textulariana | Buliminida |
| | Robertinida | Rotaliana | Rotaliida |
| | Silicoloculinida | Globigerinana | Globigerinida |

Table 2

Details about sampling and sequencing of investigated specimens.

| Species | Accession no. ^a | Isolate | Sequence length (bp) | GC content | Sampling locality | Taxonomic references |
|-----------------------------|----------------------------|---------------|----------------------|------------|---------------------------|----------------------------|
| Allogromia sp. | X86093 | Not specified | 3042 | 32.63 | Turkey, Antalya | Pawlowski (2012) |
| Allogromiid | HE998678 | 3132 | 3826 | 37.25 | Antarctica, McMurdo Sound | Not published |
| Ammodiscus sp. | HE998691 | 9952 | 2452 | 28.59 | Andaman Sea | Not published |
| Ammodiscus sp. | HE998690 | 1406 | 2468 | 28.16 | Israel, Eilat | Not published |
| Amphisorus hemprichii | AJ404314 | 720 | 2185 | 30.48 | Japan, Sesoko | Holzmann et al. (2001) |
| Astrammina rara | AJ318223 | 111 | 4140 | 28.38 | Antarctica, McMurdo Sound | Bowser et al. (1995) |
| Astrammina triangularis | AJ318224 | 118 | 4066 | 28.86 | Antarctica, McMurdo Sound | Bowser et al. (2002) |
| Bathysiphon argenteus | HE998681 | 1780 | 3323 | 36.32 | Sweden, Gullmard Fjord | Höglund (1947) |
| Borelis schlumbergeri | AJ404295 | 191 | 2744 | 27.53 | Bermuda | Holzmann et al. (2001) |
| Bulimina marginata | DQ408646 | 3599 | 3462 | 42,77 | Norway, Oslo Fjord | Schweizer et al. (2008) |
| Cassidulinoides parkerianus | DQ408639 | 3924 | 3348 | 37.03 | Antarctica, Terranova Bay | Ward and Webb (1986) |
| Cibicides lobatulus | DQ408650 | C120 | 3632 | 40.91 | Sweden, Skagerrak | Schweizer et al. (2009) |
| CladeY_allogromiid | HE998686 | 12323 | 2996 | 43.88 | Denmark, Aarhus | Not published |
| Cornuspira sp. | HE998689 | 7349 | 2080 | 27.50 | Antarctica, McMurdo Sound | Pawlowski (2012) |
| Cribrothalammina alba | AJ318225 | 226 | 2709 | 37.42 | USA, Sapelo Island | Goldstein and Barker (1988 |
| Eggerelloides scaber | AJ318228 | 139 | 3553 | 36.42 | France, Bretagne | Foram Barcoding website |
| Eggerella sp. | HE998674 | 13132 | 3491 | 32.94 | Israel, Eilat | Not published |
| Epistominella exigua | HE998671 | 3623 | 3403 | 41.61 | Weddell Sea | Lecroq et al. (2009a) |
| Glandulina antarctica | HE998680 | 1015 | 3886 | 36.93 | Antarctica, McMurdo Sound | Ward and Webb (1986) |
| Globorotalia inflata | EU199447 | VB11 | 3327 | 40.94 | France, Villefranche | de Vargas et al. (1997) |
| Heterostegina depressa | AJ879132 | 308 | 3413 | 42.34 | Japan, Sesoko | Holzmann et al. (2003) |
| Hyalinea baltica | DQ408645 | 3604 | 3631 | 39.91 | Norway, Oslo Fjord | Foram Barcoding website |
| Islandiella norcrossi | DQ408638 | 2643 | 3278 | 37.87 | Svalbard | IOPAN website |
| Leptohalysis scotti | HE998676 | 12288 | 3138 | 34.77 | Denmark, Aarhus | Höglund (1947) |
| Marginopora vertebralis | AJ404312 | 499 | 2213 | 29.64 | Australia, Lizard Island | Holzmann et al. (2001) |
| Melonis pompilioides | DQ408657 | 1400 | 3556 | 40.15 | Sweden, Tjärno | Schweizer et al. (2008) |
| Micrometula sp. | HE998682 | 6832 | 3251 | 39.00 | Norway, Bergen | Pawlowski et al. (2008) |
| Nemogullmia sp. | HE998685 | 12024 | 4206 | 53.90 | Israel, Eilat | Not published |
| Neogloboquadrina dutertrei | EU199449 | B47 | 3412 | 37.84 | Bermuda | De Vargas et al. (1997) |
| Nummulites venosus | AJ318226 | 301 | 3366 | 42.05 | Japan, Sesoko | Holzmann et al. (2003) |
| Oridorsalis umbonatus | EF534075 | 5410 | 3373 | 41.45 | Northern Atlantic | Pawlowski et al. (2007) |
| Pararotalia nipponica | AI879137 | 862 | 3180 | 41.07 | lapan | Not published |
| Peneroplis pertusus | AJ132368 | 69 | 2464 | 29.44 | France, St. Cyr | Holzmann et al. (2001) |
| Psammophaga sp. | HE998683 | 12208 | 3255 | 41.90 | UK, Bangor | Not published |
| Pullenia subcarinata | DQ408655 | 1850 | 3472 | 40.86 | Antarctica, McMurdo Sound | Foram Barcoding website |
| Quinqueloculina seminulum | HE998688 | 9680 | 2724 | 27.06 | Barents Sea | Not published |
| Reophax sp. | HE998675 | 1879 | 3113 | 40.67 | Antarctica, McMurdo Sound | Not published |
| Reticulomyxa filosa | A[132367 | 613 | 3347 | 32.58 | Germany, culture | Pawlowski et al. (1999) |
| Rhizammina algaeformis | EU649779 | 156 | 3213 | 39.43 | Weddell Sea | Foram Barcoding website |
| Robertina arctica | HE998677 | 2632 | 3533 | 40.50 | Svalbard | Foram Barcoding website |
| Shinkaiya lindsayi | EU649778 | not specified | 4054 | 32.21 | Japan, Sanriku | Lecroq et al. (2009b) |
| Sorites sp. | AI132369 | 206 | 2264 | 29.56 | Egypt, Safaga | Holzmann et al. (2001) |
| Spirillina sp. | HE998692 | 10451 | 2502 | 26.82 | France, Porquerolles | Pawlowski (2012) |
| Spirotextularia sp. | HE998673 | 13195 | 3369 | 36.72 | Israel, Eilat | Foram Barcoding website |
| Stainforthia fusiformis | HE998670 | 3965 | 3469 | 43.01 | Sweden, Skagerrak | Schweizer et al. (2008) |
| Syringammina corbicula | HE998679 | 2270 | 3297 | 34.09 | Cape Verde | Richardson (2001) |
| Textularia sagittula | HE998672 | 6792 | 3437 | 35.73 | Norway, Bergen | Höglund (1947) |
| Trifarina earlandi | DQ408640 | 2187 | 3671 | 40.29 | Antarctica, McMurdo Sound | Foram Barcoding website |
| Triloculina sp. | HE998687 | 12961 | 3247 | 32.31 | Italy, Naples | Not published |
| Trochammina hadai | DQ408637 | 95 | 3475 | 35.77 | Japan | McGann et al. (2012) |
| Trochammina sp. | X86095 | not specified | 3341 | 36.80 | France, St. Cyr | Not published |
| Uvigerina phlegeri | DQ408641 | U239 | 3579 | 40.98 | Portugal, Nazaré Canyon | Schweizer et al. (2005) |
| | | | | | | |
| Vellaria sp. | HE998684 | 12192 | 3222 | 41.09 | UK, Southampton | Not published |

Foram Barcoding website=http://forambarcoding.unige.ch.

IOPAN=http://www.iopan.gda.pl/projects/biodaff/Foram_Pawl/foram_pawl.htm. ^a New sequences are marked in bold.

Table 3

PCR primers used for amplification (1st PCR) and reamplification (2nd PCR) of complete SSU rRNA genes.

| Name | Sequence | Forward | Reverse | 1st PCR | 2nd PCR |
|-------|---------------------------|---------|---------|---------|---------|
| sA10 | ctcaaagattaagccatgcaagtgg | х | | х | х |
| s17 | cggtcacgttcgttgc | | х | х | |
| s13 | gcaacaatgattgtataggc | | х | | х |
| s6f | ccgcggtaataccagctc | x | | х | х |
| newB | tgccttgttcgacttctc | | х | х | х |
| 20r | gacgggcggtgtgtacaa | | х | | х |
| s14F3 | acgcamgtgtgaaacttg | x | | х | |
| s14F1 | aagggcaccacaagaacgc | x | | | х |

Table 4

Characteristics of new classes of Foraminifera.

| | Tubothalamea | Globothalamea |
|---------------------------------|---|--|
| Chamber basic shape | Tubular and related | Globular and related |
| Chambers in large complex tests | Proloculus followed by undivided coiled tubular chamber | Proloculus followed by globular chamber |
| Number of chambers | Bilocular or multilocular | Multilocular |
| Chambers | Never or slightly overlapping | Overlapping in various degree |
| Aperture | End of tube (areal marginal in large complex forms) | All types: e.g. basal, areal, terminal, etc. |
| LCPa (foraminal distance) | Maximized | Minimized |
| Wall composition | Agglutinated with organic matrix | Agglutinated with organic or organic/calcareous low Mg matrix |
| | Calcareous Porcelaneous high Mg calcite Mono-, polycrystalline low Mg | Calcareous Calcite/aragonite Hyaline low Mg with accessory high Mg |
| Calcite/aragonite secretion | • Not in situ - in vacuoles transported to wall | • In situ |
| (De Nooijer et al., 2009) | • Non-lamellar | • Bilamelar |
| - | No secondary laminas | Secondary laminas |
| Mode of life | • Benthic | Benthic Planktonic Benthic/Planktonic |
| Stratigraphic range | Cambrian — recent | Cambrian — recent |

^a LCP — local communication path, as a distance between successive foramina.

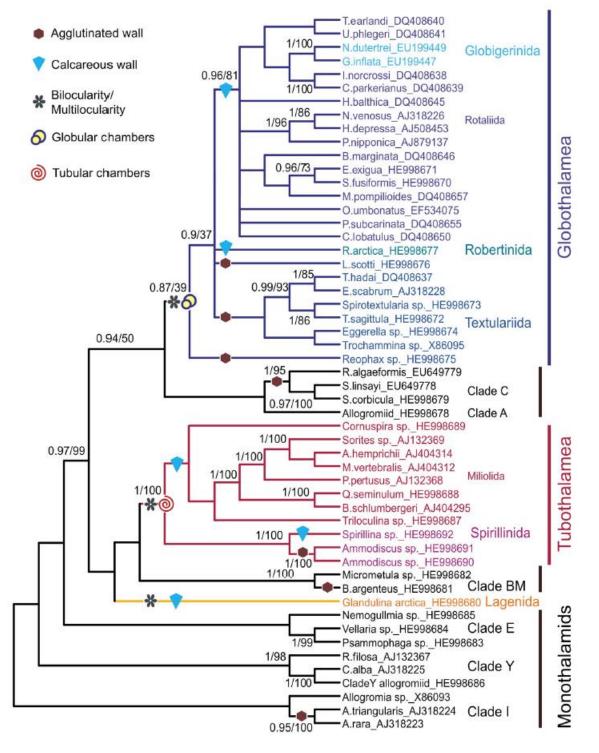


Fig. 1. Bayesian phylogenetic tree (GTR + G model) showing the phylogeny of Foraminifera inferred from 53 complete SSU rDNA sequences. Numbers at nodes indicate (from left to right) posterior probabilities (BI) and bootstrap values (ML). The tree was rooted with *Allogromia* sp., *A. triangularis* and *A. rara*, as suggested by protein phylogenies. Color symbols indicate stem lineages of Globothalamea and Tubothalamea, as well as groups having agglutinated and calcareous wall.

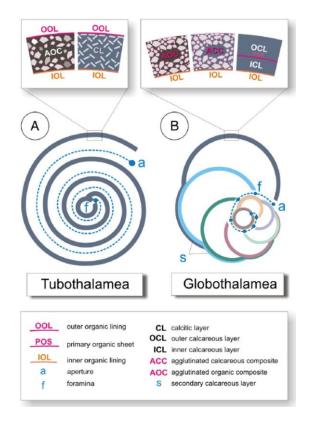


Fig. 2. Fundamental morphogenetic features identified within Tubothalamea and Globothalamea. A. Tubothalamea have elongated, tubular chamber(s) constructed either from the agglutinated organic composite (AOC) or the calcitic layer (CL) composed of calcareous–organic composite (porcelaneous tests). The foraminal distance (f–a) between an aperture (a) and the last foraminum (f) is maximized; B.Globothalamea show globular or semi-globular chambers constructed either from AOC or agglutinated calcareous composite (ACC) or calcitic/aragonitic layer (CL). The foraminal distance (f–a) between an aperture (a) and the last foraminum (f) is minimized. Secondary calcitic layer (s) is limited to calcareous tests. "Purple" organic structures (OOL, POS, AOC, ACC) serve as organic matrix, partly responsible for shaping chambers.