Molecular phylogeny of Rotaliida (Foraminifera) based on complete small subunit rDNA sequences

Magali Schweizer a,b,⁎, Jan Pawlowski c, Tanja J. Kouwenhoven a, Jackie Guiard c, Bert van der Zwaan a,d

a Department of Earth Sciences, Utrecht University, The Netherlands
b Geological Institute, ETH Zurich, Switzerland
c Department of Zoology and Animal Biology, University of Geneva, Switzerland
d Department of Biogeology, Radboud University Nijmegen, The Netherlands

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Abstract

The traditional morphology-based classification of Rotaliida was recently challenged by molecular phylogenetic studies based on partial small subunit (SSU) rDNA sequences. These studies revealed some unexpected groupings of rotaliid genera. However, the support for the new clades was rather weak, mainly because of the limited length of the analysed fragment. In order to improve the resolution of the phylogeny of the rotaliids, 26 new complete SSU rDNA sequences have been obtained. Phylogenetic analyses of these data, together with seven sequences obtained previously, confirm with stronger statistical support the presence of three major clades among the Rotaliida. The first clade comprises members of the families Uvigerinidae, Cassidulinidae and Bolivinidae. The second clade includes all analysed Discorbidae, Rosalinidae, Planulinidae, Planorbulinidae, Rotaliidae, Elphidiidae, Nummulitidae and one of the Nonionidae. Finally, the third clade comprises the Cibicididae, Pseudoparreliidae, Oridorsalidae, Stainforthiidae, Buliminidae and part of the Nonionidae. The clades 1 and 3 are strongly supported by analyses of the complete SSU rDNA, while the monophyly of clade 2 is less certain, probably due to the rapid evolutionary rates of some lineages included in this clade. These results clearly contradict the classical separation of rotaliid foraminifera into two orders: Rotaliida and Buliminida. Relatively good agreement has been found between molecular data and the morphological definition of the families for which more than one genus was sequenced. However, larger taxon sampling will be necessary for a better definition of the three major clades.

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1. Introduction

Benthic foraminifera are important elements of the meiofaunal community; their abundance data as well as the chemical composition of their fossil tests are extensively used as proxies for environmental changes in the geological past (Van der Zwaan et al., 1999). Together with their potential use for monitoring recent environments, this has led to an increasing interest in their biological functioning. Since the second half of the last century research has focused on the use of foraminifera for environmental and pollution issues (e.g. Bandy et al.,...
perforate foraminifera in the suborder Rotaliina which included ten superfamilies (i.e. Nodosariacea, Buliminacea, Discorbacea, Spirillinacea, Rotaliacea, Globigerinacea, Orbitoidacea, Cassidulinacea, Carterinacea and Robertinacea) distinguished on the basis of the wall microstructure as well as coiling and aperture characteristics. In 1981, Haynes retained the wall structure as the primary basis of subdivision; however, more emphasis was given to the shape of the aperture. In his classification, the superfamilies were raised to orders, and the Nodosariida, Robertinida, Buliminida and Globigerinida were separated from the Rotalida. The Buliminida comprised the hyaline perforate foraminifers with a toothplate and included the following superfamilies: Buliminacea, Bolivinitacea and Cassidulinacea, whereas the Rotaliida contained the Spirillinacea, Discorbacea, Asterigerinacea and Orbitoidacea (Haynes, 1981).

In their monumental work, Loeblich and Tappan (1988) defined supplementary suborders for the hyaline perforate calcareous foraminifers in addition to their former classification (1964): Involutinina, Spirillinina, Carterinina, Silicoloculinina, Lagenina, Robertinina and Globigerinina. The suborder Rotaliina was divided into 24 superfamilies; the criteria used were the number of chambers, the presence or absence of perforations, canals and cavities in the test, and the aperture. In 1992, to solve some of the inconsistencies reported by Haynes (1981, 1990), Loeblich and Tappan raised the foraminifera from an order to a class (the foraminifer suborders were thus given order status) and recognized the order Buliminida Fursenko, 1958. In the most recent classification, Sen Gupta (2002) followed the classification of Loeblich and Tappan (1992) with slight modifications (Fig. 1). According to this view, Buliminida are distinguished from Rotaliida by the presence of a toothplate, a loop-shaped aperture and a high trochospiral coil.

Since the mid-90s, the molecular approach has shed new light on foraminiferal phylogeny. The first molecular results showed a clade combining the Textulariida and the Rotaliida (Pawlowski et al., 1997; Pawlowski, 2000). This mixed clade was explained either by a radiation occurring in a relatively short time, or by slow rates of evolution of both groups (Pawlowski et al., 1997, 2003). More recent analyses were able to separate the rotaliids and textulariids, albeit without strong statistical support (Holzmann et al., 2003; Ertan et al., 2004; Bowser et al., 2006). Within the rotaliids, Holzmann et al. (2003) examined the links between the Nummulitidae and seven other rotaliid families, Ertan et al. (2004) explored the relationships of eleven genera of Rotaliida, whereas Schweizer et al. (2005) investigated the position of uvigerinids.
All these studies were based on a fragment of about 1000 base pairs (bp) long, and situated at the 3′ end of the SSU rDNA. The phylogenetic information contained in this fragment seems to be insufficient to resolve rotaliid phylogeny, as shown by low statistical support for the deep nodes in the analyses of Ertan et al. (2004) and Schweizer et al. (2005). For this reason, we decided to sequence the complete SSU of selected rotaliid species which would result in a three-fold increase in the number of analysed sites. Therefore, 26 new complete sequences belonging to 12 of the 22 extant superfamilies of Rotaliida (according to the classification of Sen

Fig. 1. Diagram showing the taxonomic positions of the genera studied in this paper and in Schweizer et al. (2005) with the classifications of Sen Gupta (2002). The first column represents the 22 extant superfamilies with the ones represented in phylogenetic analyses in bold. The second column represents the studied families and the third one the studied genera.
Gupta, 2002) were obtained. These new data, along with seven previously obtained sequences, were analysed together to investigate the phylogeny of the rotaliids.

2. Materials and methods

2.1. Collection of the samples

Live specimens of rotaliids were collected during several different expeditions between 1995 and 2006 (see Table 1 for details). Sediment samples were either taken by hand with a scraper at the shallow sites or collected by boxcoring and multicoring at the deeper sites. The top few centimetres of sediment were collected from the cores with a spoon and immediately sieved using water from the same environment as the sampling site (fractions 500/250/125 \( \mu \text{m} \)). The different fractions were stored at temperatures close to that of the collection site. Specimens were selected alive, imaged and extracted as explained in Schweizer et al. (2005).

2.2. DNA extraction, PCR amplification, cloning and sequencing

Extraction of DNA from single or multiple specimens was done with DOC lysis buffer, CTAB or guanidine buffer (Pawlowski, 2000), and occasionally with DNeasy Plant Mini Kit (Qiagen) for multi-specimen samples. The SSU was amplified in three steps with fragments between 800 and 1600 bp long. The primers sA10 and s13 were used to amplify the 5' end fragment (sA-s6), the primers s6F and s17 for the middle fragment (s6-s14) and the primers s14F3 and sB for the 3' end fragment (s14-sB). A reamplification of the PCR products (nested PCR) was often necessary and was performed using the following primers: sA10-s6rA for the sA-s6 fragment, s6F-s15rot for the s6-s14

Table 1

<table>
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<th>Superfamily</th>
<th>Species</th>
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<th>DNA isolate</th>
<th>SSU length</th>
<th>Access number</th>
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S. fusiformis and N. labradorica SSU sequences are hybrids of two genetically close samples. Concerning the uvigerinids, it was shown earlier (Schweizer et al., 2005; Schweizer, 2006) that the two species attributed to the genera Rectuvigerina (R. phlegeri) and Trifarina (T. earlandi) are in fact closer to Uvigerina peregrina than U. mediterranea and U. elongatastriata. It was therefore decided to give all these species the same genus name (see discussion in Schweizer, 2006, Ch. 6).
fragment and s14F1-sB for the s14-sB fragment. The positions and sequences of the primers are summarized in Fig. 2. The PCR conditions were the following: total volume of 50 μl, denaturation at 94 °C for 30 s, annealing for 30 s at 50 °C for the amplification and at 52 °C for the reamplification, extension at 72 °C for 2 min with 40 cycles for the amplification and 35 cycles for the reamplification, final elongation for 5 min at 72 °C. The positive PCR products were purified using High Pure PCR Purification Kit (Roche Diagnostics). A few amplifications of the s14-sB fragment were sequenced directly; all the others were cloned. Purified products were ligated in the pGEM-T Vector system (Promega) or the Topo Cloning vector (Invitro Gene), and cloned using ultracompetent cells XL2-Blue MRF’ (Stratagene). Sequencing reactions were prepared using an ABI-PRISM Big Dye Terminator Cycle Sequencing Kit and analysed with an ABI-377 DNA sequencer or an ABI-PRISM 3100 (Applied Biosystems), all according to the manufacturer’s instructions.

2.3. Phylogenetic analysis

The new sequences presented here were deposited in the EMBL/GenBank Nucleotide Sequence Database; their accession numbers are reported in Table 1. To extend the data set, other complete SSU sequences from the EMBL/GenBank database have been added (deposited by M. Holzmann (unpublished) and Pawlowski et al. (1996, 1999)). Out-group sequences have been chosen among textulariids, the closest relatives of rotaliids (Bowser et al., 2006). Presently, there are only two sequences available for the complete SSU and they are used in our analyses. Sequences were aligned manually by using Seaview software (Galtier et al., 1996), and the regions which were too variable to be properly aligned were removed. From an alignment of 5565 sites, 1766 sites were removed to obtain a final alignment of 3799 sites, 1745 of them containing no gap.

The GTR + I + Γ model was chosen by Modeltest (Posada and Crandall, 1998). However, we preferred to also use another model (HKY + I + Γ) to make comparisons, as Modeltest tends to favour complex models (Kelchner and Thomas, 2007). Therefore, the maximum likelihood (ML) trees were obtained using PhyML 2.4.4 (Guidon and Gascuel, 2003) with the HKY (Hasegawa, Kishino, Yano) model (Hasegawa et al., 1985) allowing transitions and transversions to have potentially different rates, and the GTR (General Time Reversible) model allowing all types of substitution to have different rates (Lanave et al., 1984; Rodriguez et al., 1990). To correct for the among-site rate variations, the proportion of invariable sites (I) and the alpha parameter of gamma distribution (Γ), with eight rate categories, were

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**SSU primer** | **Sequence** | **Orientation** | **Specificity**
--- | --- | --- | ---
sA-s6 | CTC AAA GAT TAA GCC ATG CAA GTG G | Forward | Forams
s13 | GCA ACA ATG ATT GTA TAG GC | Reverse | Forams
s6rA | GCA CCA GAC TTG CCC | Reverse | Universal
s6F | CCG CGG TAA TAC CAG CTC | Forward | Forams
s17 | CGG TCA CGT TCG TTG C | Reverse | Forams
s15rot | CAT ATT CAT GAA AGG ACT AGC | Reverse | Rotaliida
s14F3 | ACG CAA GTG TGA AAC TTG | Forward | Forams
s14F1 | AAG GGC ACC ACA AGA ACC C | Forward | Forams
sB | TGA TCC TCT TGC AGG TTC ACC TAC | Reverse | Universal

**Fig. 2.** Localization and sequences of the primers used to amplify the three fragments of the complete SSU rDNA.
estimated by the program and taken into account in all analyses. Non-parametric bootstrapping (BS) (Felsenstein, 1985) was performed with 100 replicates to assess the reliability of internal branches.

The Bayesian analysis was performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001), using the GTR+I+Γ model. Two independent analyses were performed at the same time with four simultaneous chains (one cold and three heated) run for 1,000,000 generations, and sampled every 100 generations with 2500 of the initial trees discarded as burn-in. The posterior probabilities (PP) were calculated at the same time.

Fig. 3. Localization of the inserts found in Rotaliida (in black) compared to the schematised SSU secondary structure of *Chlamydomonas reinhardtii* (in grey, redrawn from Wuyts et al., 2004) with the names of the helices (in grey, Wuyts et al., 2001). F1–F6 are inserts found only in foraminifers, whereas V1–V9 are variable regions recognized in all eukaryotes (Wuyts et al., 2000). The minimum and maximum numbers of nucleotides (=nt) observed in rotaliids for each insert are indicated, with the number found in *C. reinhardtii* between brackets for the variable regions (V2–V9).
3. Results

3.1. Sequence data

The 26 complete SSU sequences presented here have a length ranging from 2772 (Elphidium) to 3768 nucleotides (Nonionella); the four shortest sequences of which belong to the fast evolving taxa Elphidium, Ammonia and Haynesina (see Table 1 for details).

The GC content of the studied rotaliids is between 37 and 46%, with the lowest value observed in Cassidulinoides and the highest one in Ammonia. These values are higher than those recorded for Miliolida and Astrorhizida (29–32%), but comparable to the value of 45% found in Globigerinida (Pawlowski, 2000).

The typical insertions observed in other foraminifera (e.g. Pawlowski et al., 1999) are also present in the rotaliid sequences (Fig. 3). Some of these insertions are
in regions of the SSU which show variations among eukaryotes (V1–V9), whereas others are only present in certain eukaryotes (8/e1, 23/e15–23/e17, 45/e1), or exclusively in foraminifers (F1–F6). The insertions F4–F6 belong to the s14-sB fragment and have been described elsewhere with different names (e.g. Darling et al., 1997 (regions F1–F3); de Vargas et al., 1997 (regions I, II and V)).

3.2. Phylogenetic analyses

The phylogenetic analysis of the 26 complete SSU sequences of rotaliid foraminifera and the seven sequences from GenBank is presented in Fig. 4. Two textulariids (Trochamminia sp. and Eggerelloides scabrum) are used as an outgroup. Analyses performed with PhyML (ML/HKY and ML/GTR) and MrBayes (GTR) give exactly the same topology. Three major clades emerge within the Rotaliida.

Clade 1 comprises Bolivina, Cassidulinoides, Islandiella and Uvigerina. These genera commonly have an elongate test (Islandiella being an exception) and an aperture with a toothplate. The statistical support is 89% BS (ML/HKY) or more (96% BS for ML/GTR and 1.00 PP for Bayesian inference (BI)).

Clade 2 includes Rosalina, Discorbis, Planorbulinella, Hyalinea, Pararotalia, Cycloclypeus, Heterostegina, Operculina, Nummulites, Ammonia, Elphidium and Haynesina, genera with a trochospiral to planispiral test. The statistical support of this clade is 77% BS (ML/HKY) or higher (ML/GTR: 83% BS, BI: 1.00 PP). This second clade can be divided in three subgroups. The first subgroup comprises species with a trochospiral to planispiral test and an interiomarginal arch-shaped aperture, sometimes with the presence of a second identical aperture (Planorbulinella) or secondary openings (Discorbis). Genera included in this first subgroup are Rosalina, Discorbis, Planorbulinella and Hyalinea. The statistical support is rather good (88% BS or higher and 1.00 PP). The second subgroup includes Pararotalia, a genus with a low trochospiral test and an imperforate toothplate and the Nummulitidae, a family with planispiral tests and a complex canal system. This subgroup is less well supported statistically: 77% for ML/HKY, 83% for ML/GTR and 0.93 PP. The third subgroup is composed of three shallow-water and fast evolving genera: Ammonia, Elphidium and Haynesina. The test is a low trochospiral (Ammonia) or planispiral coil (Elphidium and Haynesina) with primary and secondary apertures. This is the best-supported subgroup of clade 2 (95% BS or higher).

Clade 3 includes Nonionella, Bulimina, Stainforthia, Epistominella, Oridorsalis, Pullenia, Melonis and Cibicides, and combines species with elongate and lenticular tests. This clade has a high statistical support (ML/HKY+ML/GTR: 99% BS, BI: 1.00 PP). A first subgroup comprises elongate triserial tests with a loop-shaped aperture (Bulimina, Stainforthia) and low trochospiral ones with an interiomarginal arch-like aperture (Nonionella, Epistominella). The statistical support of this subgroup is at least 83% BS. A second subgroup includes species with a planispiral (Pullenia, Melonis) or low trochospiral (Oridorsalis, Cibicides) test and a slit-like interiomarginal aperture. The support of this subgroup is even better with 97% BS or higher.

4. Discussion

4.1. Ribosomal RNA secondary structure and foraminiferal insertions

The secondary structure of the s14-sB fragment has been studied by Ertan et al. (2004) and Habura et al. (2004). There is evidence that the three foraminiferal insertions found in this fragment (here referred to as F4, F5 and F6, see Fig. 3) are not introns and are retained in the final rRNA inside the ribosome (Habura et al., 2004). However, at the same time it has been suggested by Pawlowski et al. (1996) that about 1000 nt are removed from the SSU of Ammonia during the maturation process of RNA. Ertan et al. (2004) have used the variable regions (here referred to as V7–V9, see Fig. 3) and the foraminiferal insertions (see above) to improve the alignment and for taxonomic purposes, but it was noticed that these regions were not always diagnostic and that convergent evolution could occur.

In the data presented here, representing the complete SSU, half of the insertions present similar patterns in rotaliids, textulariids (Trochamminia sp. and Eggerelloides scabrum) and globigerinids (Globorotalia inflata, G. hirsuta, Neogloboquadrina dutertrei) showing that these groups are closely related.

If all the foraminiferal insertions are retained in the rRNA, as could be the case for F4–F6 (Habura et al., 2004), there are certainly evolutionary constraints to modelling their DNA sequences. Therefore, the use of these insertions for taxonomic purposes, as was initiated by Ertan et al. (2004), is promising. However, constructing the secondary structure of foraminiferal rRNA is rather difficult, because of all the original insertions and the rate heterogeneity, and therefore any conclusions based on rRNA structure should be treated with caution.
4.2. Complete versus partial SSU phylogeny

A question hotly debated is whether adding more taxa, or more sites will improve phylogenetic accuracy (e.g. Hedtke et al., 2006 and references therein). It has been shown that increasing the taxon sampling could improve the accuracy of phylogenetic inferences, particularly in cases of whole genomes sequenced for a few taxa (Geuten et al., 2007 and references therein). In the case of foraminifers, however, the situation is rather different. For the moment, there is a wide taxon sampling on a portion of the SSU and we are therefore in the situation described by Hillis et al. (2003), where a few characters are obtained from the taxa analysed. The authors argue that in this case, it is better to add more characters per taxon and we followed this suggestion by sequencing the complete SSU for some taxa, for which the partial SSU sequences were obtained previously.

The 24 rotaliid genera present in the tree based on the complete SSU (Fig. 4) represent 65% of the rotaliid genera for which partial SSU rDNA sequences were deposited in GenBank. The general topology of this tree is relatively congruent with previous studies based on partial SSU sequences (Holzmann et al., 2003; Ertan et al., 2004; Schweizer et al., 2005). However, important differences exist in relations between higher taxa and the support for particular groupings.

The three clades found in the complete SSU tree were also identified in our previous analysis based on the s14-sB fragment (Schweizer et al., 2005, Fig. 7). However, the statistical support of the lower nodes is particularly improved with the analysis of the complete SSU compared to our former analysis, where the clade 1 branched as a sister-group of clade 3 instead of clade 2, albeit the relations between these clades were not supported (33% for the grouping of clades 1 and 3 in the partial SSU). The structure of particular clades is similar both in the present analyses and those in Schweizer et al. (2005). In clade 1, the relations between Bolivina, Cassidulinidae and Uvigerinidae are the same, but this clade comprised also Globobulimina in analyses of the partial SSU. In clade 2 the two subgroups are present with similar topology, except for the first subgroup, where Hyalinea and Planorbulinella grouped together in the partial SSU phylogeny with a good support (75% BS with PhyML and HKY). The second subgroup comprises the Nummulitidae, which always forms a homogeneous and highly supported clade (>90% BS) and Pararotalia, which in the partial SSU phylogeny branched with a high support close to the Calcarinidae (a family not included in the complete SSU analysis). Ammonia, Elphidium and Haynesina were not represented in the s14-sB analysis. Clade 3 includes the same two subgroups in both analyses, but with slightly different topologies. Bulimina and Nonionella were also closely related in the partial SSU analysis. However, Virgulina and Virgulinella, two taxa not represented in the complete SSU phylogeny, branched closer to Nonionella. Epistominella and Stainforthia were sister-groups in the partial SSU phylogeny, albeit with a low statistical support (31% BS with PhyML and HKY). In the second subgroup, Chilostomella (not included in the present analysis) was the closest relative of Pullenia with a reasonable support (74% BS with PhyML and HKY), and Cibicides was branching with that subgroup instead of with Melonis, albeit with no statistical support.

The global topology of the second clade also appeared in an analysis performed by Holzmann et al. (2003) on the s14-sB fragment and focusing on Nummulitidae. The Nummulitidae were equally well supported (87% BS or higher) and branched together with Pararotalia and the Calcarinidae, despite a low support with the ML analysis (51% BS). The general topology of the other subgroup was similar to the one observed in the present analysis. However, the Cassidulinidae and Bolivinidae (belonging to the first clade in this study) appeared as a sister-group of the sub-clade Planorbulina–Rosalina, with Hyalinea less closely related. Moreover, the branching of Cassidulinidae–Bolivinidae inside this subgroup in Holzmann et al. (2003) was not statistically supported.

The main discrepancy between our present study and partial SSU analysis of Ertan et al. (2004) concerns the relations between members of our clade 3. In Fig. 7 of Ertan et al. (2004), Bulimina and Virgulinella, belonging to the first subgroup in our clade 3 branched as a sister-group of taxa belonging to our clade 1, whereas Chilostomella and Melonis formed a clade sister to all the other rotaliid sequences. These relations were interpreted as evidence for partition between the orders Rotaliida and Buliminida, however, the support of these two deeper nodes was very weak (respectively 0.19 and 0.38 PP). Despite this discrepancy, the relations between other rotaliids were quite congruent with the present study. The study of Ertan et al. (2004) confirmed with good support (0.88 PP) the grouping between members of our clade 2 (Rosalina, Haynesina, Ammonia, Elphidium). Representatives of clade 1 (Bolivina, Uvigerina, Globobulimina) equally branched together with a good support (0.80 PP), but with a different topology than in Schweizer et al. (2005): Globobulimina branched closer to Bolivina instead of Uvigerina in the study of Ertan et al. (2004).
4.3. Molecular phylogeny and morphology-based classification of Rotaliida

Because there is a good congruence between the trees based on complete and partial SSU sequences, an analysis has been performed including the complete SSU sequences from the present study and the partial sequences (s14F-sB) from Schweizer et al. (2005, Fig. 7) (Fig. 5). This recapitulative tree is compared to the mainstream morphological classifications, with the one of Sen Gupta (2002) as a summary (Fig. 1).

All genera represented by more than one species were monophyletic, except Rosalina (Fig. 5). Four morphologically defined families for which different taxa could be sampled, i.e. Cassidulinaeidae, Uvigerinidae, Calcarinidae and Nummulitidae, received a high statistical support in the phylogenetic analyses. On the other hand, the Nonionidae (Melonis, Pullenia, Nonionella, Haynesina), the Rotaliidae (Pararotalia, Anmonia) and the Buliminidae (Bulimina, Globobulimina) appeared polyphyletic, questioning the morphological basis of their grouping and asking for further, preferably integrated morphological and genetic studies.

At a higher taxonomic level, the superfamilies Buliminacea, Nonionacea and Planorbilinacea appear polyphyletic. The polyphyly of the first two superfamilies is already clear at the family level. The Planorbilinacea, which include Hyalinea, Planorbilinella, Planorbulina, and Cibicides, are split between clades 2 and 3 (Fig. 5). This is quite surprising given that Hyalinea has always been grouped with Cibicides (e.g. Loeblich and Tappan, 1964, 1988; Haynes, 1981) and that Planorbulina was thought to be a stage in the life cycle of Cibicides (Nyholm, 1961).

Some clades observed in the molecular analyses were proposed by Haynes (1981) based on morphological studies. The genera Pullenia and Chilostomella were classified in the family Chilostomellidae and the genera Bulimina, Stainforthia, Virgulina and Virgulinella in the family Buliminidae, superfamily Buliminacea (Haynes, 1981, 1990). Haynes (1981) also included Epistominella in the Buliminacea, but in a different family (Turrilinidae). Our results partially corroborate Haynes’ subdivision, although other Buliminacea (Uvigerina and possibly Globobulimina) branch well separated from this group.

Our data do not support the separation between the orders Buliminida and Rotaliida as proposed in the latest classifications (Haynes, 1981; Loeblich and Tappan, 1992; Sen Gupta, 2002). Although the two main groups observed in the phylogenetic analyses of the complete SSU (clade 1 and clades 2+3, see Fig. 4) roughly correspond to these orders, several buliminid genera (Bulimina, Stainforthia, Virgulina, Virgulinella), branch within clade 3 together with some of the rotaliids. The position of these genera questions the pertinence of the criteria used to separate the two orders. Apparently, the presence of a toothplate, which is the main feature to distinguish the two orders, is not as important as was previously thought (e.g., Hofker, 1951, 1956; Mikhalevich and Debenay, 2001) and was already questioned by Haynes (1981, p. 24). Moreover, the fact that Cassidulina, a genus without a toothplate, is traditionally placed in the Cassidulinidae (position confirmed by the molecular analyses, see Schweizer et al., 2005), a family where other genera represented in this study do possess a toothplate, shows that the taxonomic value of this character is questionable. Another representative feature of the Buliminida is the high trochospiral coil. However, this characterizes only 70% of the members of this order (Haynes, 1981).

In view of (paleo-) ecological applications of foraminifera it is interesting to note that Nonionella, which appears closely related to Bulimina, includes species that are able to denitrify nitrate, as well as Stainforthia and Globobulimina (Risgaard-Petersen et al., 2006), a process which was formerly recognized only in prokaryotes. The results presented here indicate that the denitrification pathway exists in at least two rotaliid clades: clade 1 with Globobulimina (some Uvigerina and Bolivina species are also possible candidates but further tests need to confirm this (N. Risgaard-Petersen and E. Geslin, pers. comm.) and clade 3 with the pair Nonionella–Stainforthia.

5. Conclusions and perspectives

Currently, our results based on complete and partial SSU rDNA sequences favour the existence of a unique order Rotaliida. This order is subdivided into three clades which could be considered as suborders. However, further molecular studies are needed to confirm this partition and to better define the three clades morphologically. Sequencing of the complete SSU rDNA clearly improved the phylogenetic signal contained in analyses of the s14-
sB fragment. By multiplying the number of analysed sites by a factor three, very strong statistical support was obtained for most of the clades. Still, problems persist with some clades composed of fast evolving species (i.e. Ammonia, Elphidium, Haynesina), which are more sensitive to the long branch attraction (LBA) phenomenon. Analysis of protein-coding genes could resolve these problems. However, it is already known that at least two genes for which data are available (actin, β-tubulin), are too conserved to resolve the phylogeny of Rotaliida (Flakowski et al., 2005; Ujiie and Pawlowski, in preparation). Although it is expected that some mitochondrial genes could be helpful, the data are not yet available. At the same time, to revise the classification of Rotaliida it will be compulsory to obtain complete SSU sequences for taxa belonging to the families that have not yet been examined.

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Appendix A. Taxonomic notes

**Bulimina marginata** d’Orbigny, 1826, Ann. Sci. Nat., sér. 1, 7, p. 269, pl. 12, figs 10–12.


*Cibicides lobatulus* (Walker and Jacob): *Nautilites lobatulus* Walker and Jacob, 1798, in Kammacher, Adam’s Ess. Micr., p. 642, pl. 14, Fig. 36.

*Cibicides pachyderma* (Rzehak): *Truncatulinina pachyderma* Rzehak 1886, Naturf. Ver. Brünn, 24, p. 87, pl. 1, Fig. 5a–c.


*Hyalinea balthica* (Schroeter): *Nautilus balthicus* Schroeter, 1783, Einleitung in die Conchylienkenntniss nach Linné, 1, p. 20, pl. 1, Fig. 2.


*Nonionella labradorica* (Dawson): *Nonionina labradorica* Dawson, 1860, Canadian Nat. Geol., 5, p. 192, Fig. 4.

*Oridorsalis unbonatus* (Reuss): *Rotalina unbonata* Reuss, 1851, Deutsch. Geol. Ges. Zeitschr., 3, p. 75, pl. 5, Fig. 35.

*Pullenla subcarinata* (d’Orbigny): *Nonionina subcarinata* d’Orbigny, 1839, Voyage dans l’Amérique Méridionale; Foraminifères, 5, pt. 5, p. 28, pl. 5, Figs. 23–24.


*Uvigerina phlegeri* (Le Calvez): *Rectuvigerina phlegeri* Le Calvez 1959, Rec. Trav. Inst. Pêches Maritimes, 23, p. 263, pl. 1, Fig. 11.

References


