REVIEW ARTICLE

# Molecular biological research on Foraminifera*

LI Baohua[1**], Kemal Topac ERTAN[2] and Christoph HEMLEBEN[2]

(1. Nanjing Institute of Geology and Paleontology, Chinese Academy of Sciences, Nanjing 210008, China; 2. Institute and Museum of Geology and Paleontology, University of Tuebingen, D-72076 Tuebingen, Germany)

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**Abstract** As one of the most important groups in micropaleontology, Foraminifera is traditionally described to have a membranous, agglutinated or carbonate shell according to its morphology, which resembles the marine granuloreticuloseans. However, recent molecular analyses on its ribosomal RNA gene have disclosed the existence of the naked, and also freshwater and terrestrial species. Foraminiferal SSU rDNA sequence suggests that this group is positioned at the base of the Eukaryotes phylogenetic trees between Euglenoida and Diplomonadida. Existence of a large amount of genetic types in planktonic foraminifera suggests an underestimation of the biodiversity for the nearly 50 species in world oceans and their close relationship with the ocean environment, such as bio-geographic distribution and water currents. This provides a more reliable proxy for future paleoenvironmental study.

**Keywords:** Foraminifera, ribosomal DNA, molecular biology, phylogeny, paleoceanography.

Foraminifera belongs to Sarcodina (Protozoa) and exhibits cytoplasmic organization and pseudopdial streaming characteristic broadly of amoeboid organism[1]. It mainly lives in the marine environment with a membranous, agglutinated or calcareous shell. As one of the most important groups in micropaleontology, Foraminifera has been widely used for the stratigraphic subdivision, correlation in petroleum wells and for the reconstruction of paleo-environments during the last several decades[2, 3]. Furthermore, since the 1960s with the start of Deep Sea Drilling Project and Ocean Drilling Program, Foraminifera has played a key role in the paleoceanographic research, such as $\delta^{18}O$ stratigraphy, AMS $^{14}C$ dating, and sea surface temperature reconstructions[4—6].

Though many biological investigations have been carried out on Foraminifera since the 1970s, the study on its taxonomy and evolution is still based on its morphology[7—9]. There are still some uncertainties on the classification and phylogeny, such as for *Ammonia beccarii* (var.). Since the 1990s, with the application of molecular methods to the study on Foraminifera, more and more work has been done on its molecular phylogeny, evolution history and the relationship with the environmental changes[10, 11].

Molecular analysis of Foraminifera is most on the ribosomal RNA (rRNA) gene (Fig. 1). Ribosomal RNA, a relatively stable gene, is composed of small subunit (SSU), large subunit (LSU), internal transcribed spacer (ITS) and inter-genetic spacer (IGS). In Foraminifera, there are characteristic insertions (such as F1, F2, and F3) in the conserved regions of rRNA gene sequence. These special insertions contribute much to the separation of foraminiferal DNA from other organisms. Inside the Eukaryotes, rRNA has 50—5000 copies of these components, and thus because of these duplications, research on Foraminifera in the laboratories can be easily carried out though this organism has only a single cell.

## 1 Foraminiferal DNA extraction, PCR, cloning and sequencing

Two methods to obtain foraminiferal DNA have been tried through direct extraction or after the culture of gametogenesis[11, 12]. Both ways can get valid foraminiferal DNA for experiment. Since the latter method is difficult to perform, most people are now adopting the direct extraction.

Direct extraction includes deoxycholate (DOC), guanidine or cetyltrimethylammonium bromide (CTAB) methods[11, 13—16]. Foraminiferal specimens are ground after being carefully cleaned. Then, the extraction buffer

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** To whom correspondence should be addressed. E-mail: bhli@public1.ptt.js.cn

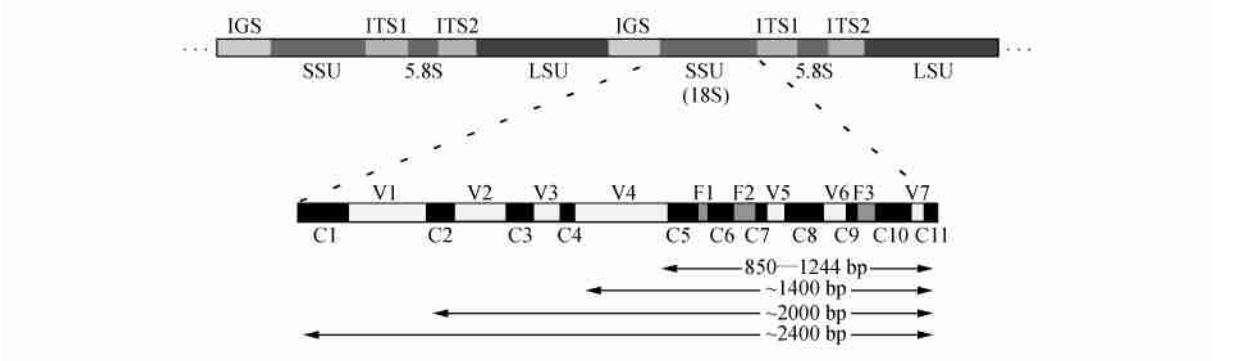


Fig. 1. The gene that encodes for ribosomal RNA in Foraminifera. Lower figure shows the enlarged structure of small subunit rRNA gene in Foraminifera (the approximate lengths of different fragments in the SSU rRNA gene are also shown in the figure). ITS, internal transcribed spacer; IGS, inter-genetic spacer; LSU, large subunit (such as 25S); SSU, small subunit (such as 18S). C1-C11, conserved regions; V1-V7, variable regions; F1-F3, special units in between conserved regions of Foraminifera.

is added and incubated at 60 ℃ for one hour. Sometimes special kits are also used, such as the Qiagen DNeasy Plant MiniKit[17—20]. Extracted foraminiferal DNA materials are stored at —20 ℃ for further analysis.

Many reports of the foraminiferal DNA sequence are those of the SSU fragments (C5-C11, about 1000 bp, Fig. 1). Special primer pairs (S14F1 and S21F1, for example) and annealing temperature (50—54 ℃) are designed during the polymerase chain reaction (PCR)[21, 22]. After the PCR products are purified, cloned and sequenced, SSU rDNA sequences are available for comparison and phylogenic analyses. As to other foraminiferal genes, such as LSU rRNA and RPB1, similar methods are used with the difference only in the design of primers[16, 23].

## 2 Molecular biological techniques used in the research on the taxonomy and evolutionary phylogeny of Foraminifera

Traditionally, foraminiferal taxonomy is based on the shape of their shells. Therefore, it is difficult to classify those species without shells or shells with large variety. The analysis of foraminiferal SSU rDNA sequence provides a more reliable method to identify them, for example, the erection of two new species, freshwater *Edaphoallogromia australica* Meisterfeld, Holzmann and Pawlowski and marine agglutinated *Toxisarcon synsuicidica* Cedhagen and Pawlowski[17, 18]. Based on molecular data, a new species *Ammonia catesbyana* (d'Orbigny) was separated from *Ammonia beccarii* (Linné)[15]; *Syringammina corbicula* Richardson was confirmed to be Foraminifera[24]; and freshwater *Reticulomyxa filosa* Nauss was suggested to be belonged to the naked foraminifera[25].

On account of the high morphologic variability, *Ammonia beccarii* var. has been one of the most difficult and confused groups in benthic foraminifera. These various morphologic "subspecies" are thought to be adapted to their different habitats. However, molecular studies displayed that *Ammonia beccarii* var. with different morphology does not belong to a single species[23, 26, 27]. With partial LSU rDNA sequences and morphometric analysis on living *Ammonia* specimens obtained from 30 localities in 17 countries, these *Ammonia* specimens belong to 12 different molecular types and each molecular type can be regarded as a separate species and distinguished in morphological characteristics (e.g. shell shape, chamber shape, porosity, prolocular diameter, folium shape, radial furrow length, umbilical diameter). Therefore, Hayward et al. suggested a new taxonomy for this group[28].

According to the phylogenetic analyses on the SSU and LSU rDNA sequences, Foraminifera was placed at the base of Eukaryotic tree, between Diplomonadida and Euglenoida[21] (Fig. 2). However, this early divergence of Foraminifera from the mitochrondriate lineage is inconsistent with its late fossil record since the Cambrian (~540 Ma)[29]. The discovery of the naked foraminifera partially solved the above problem[30]. With SSU rDNA sequences of the naked, thecate and agglutinated unilocular (single-chambered) foraminifera, molecular phylogeny revealed the major steps in the evolution of early Foraminifera[20].

The actin phylogenetic analysis suggested that Foraminifera might be closely related to Cercozoa among the eukaryotic "crown" groups[31]. Re-analyzing 54 foraminiferal SSU rDNA sequences, Berney et

al. proposed that Foraminifera is a sister group of Cercozoa in the Eukaryotic tree[32]. Sturdy of RPB1 gene sequences from Foraminifera and Cercozoa also confirmed such a conclusion[19].

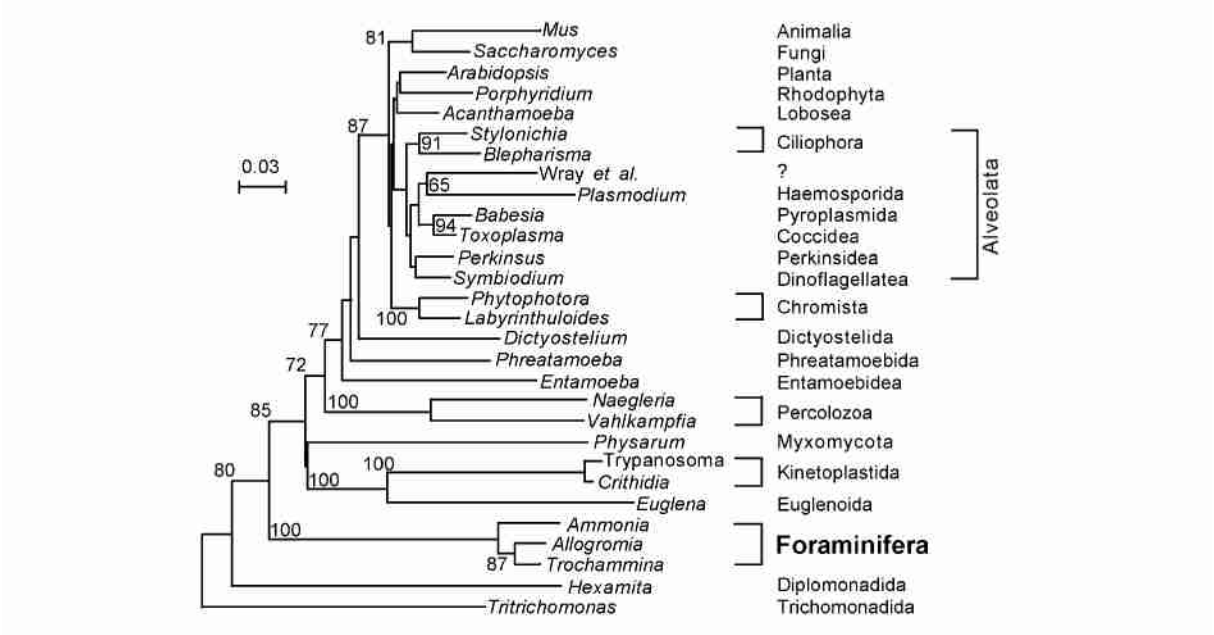


Fig. 2. Eukaryotic phylogeny inferred from 29 SSU rDNA sequences[22].

## 3 Biological characteristics of Foraminifera and their significance in the environmental and paleoceanographic studies

There are totally about 50 planktonic foraminiferal species in the world oceans[8], comparatively less than other plankton, such as Diatom and Radiolaria. However, planktonic foraminiferal rDNA analyses revealed that each species has several genetic types, while the biogeographic and biological analyses showed these different genetic types have their particular distributions. For example, in the Atlantic Ocean, *Globorotalia truncatulinoides* (d' Orbigny) has four genetic types, which colonize different latitudes and can be distinguished by coiling direction (Fig. 3, after Ref. [33]).

In the investigation of SSU rDNA sequence of *Globigerinella siphonifera* (Brady), two genetic types (type I and type II) were set up, with characteristic isotopes and shell microstructure. Type I has a relatively light $\delta^{18}O$ and $\delta^{13}C$, together with larger pore diameter[34,35]. Similarly, different genetic types of *Orbulina universa* (d' Orbigny), *Globigerinoides ruber* (d' Orbigny) and *Globigerina bulloides* d' Orbigny are also correlated to hydrographic provinces, but the gene communication could be realized by ocean currents[35,36].

Molecular evidence has shown that the foraminiferal trans-tropical gene flow could be associated with the seasonal upwelling in subtropical regions of the world oceans[37], while research indicated that there exists gene flow between the Indo-Pacific and Atlantic tropical-subtropical provinces through the prevailing global surface currents from the east to west round the South African Cape[35].

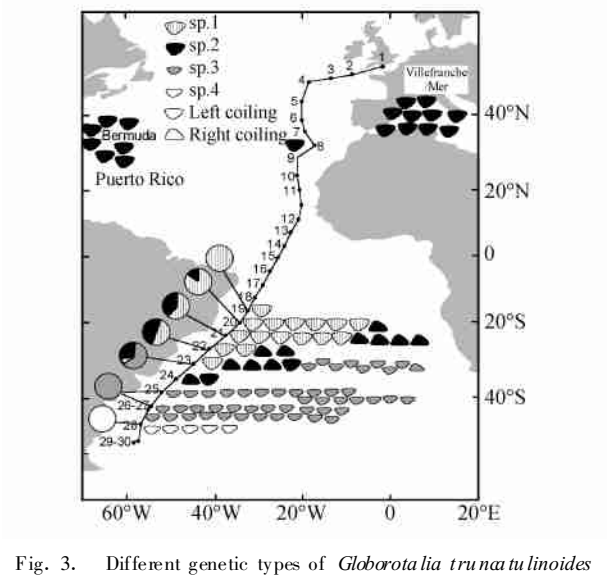


Fig. 3. Different genetic types of *Globorotalia truncatulinoides* (planktonic foraminifera), their contents and geographic distributions in the Atlantic Ocean (after Ref. [33]).

The ecophenotypes of planktonic foraminiferal species vary in different environments, which provides a basis for the paleoceanographic reconstruction. However, molecular phylogenetic analysis showed these various ecophenotypes represent different genetic types, such as right-, or left-coiled *Globorotalia truncatulinoides* and *Neogloboquadrina pachyderma* (Ehrenberg), and white or pink shell *Globigerinoides ruber*. Obviously, the existence of many genetic types for the planktonic foraminiferal species in different hydrological conditions implies the diversity of planktonic foraminifera is underestimated. Speciation of planktonic foraminifera may not display in their morphology. Therefore, the same species in different environments, or/and different ecophenotypes for one species in the same environment in traditional taxonomy, are possibly different species[13, 33].

Because of the difficulty in morphological separation between the various genetic types of planktonic foraminifera, these genetic types are often called "cryptic species". A lot of cryptic species with their characteristic distribution in the world oceans represent possibly particular planktonic foraminiferal ecophenotypes[36]. The using of cryptic species *Globigerina bulloides* in the reconstruction of paleo-sea surface temperature[38] could reduce the estimation error of up to 1 ℃, better than those of the traditional methods—transfer function, modern analogue technique (MAT) and artificial neural networks (ANNs).

## 4 Discussion

DNA sequence analysis combined with morphological observation provides a powerful method in the research of the origin and evolution of Foraminifera. As a taxonomic tool and molecular clock, the DNA sequence analysis can classify these species better and set up the history of foraminiferal evolution. The analysis can also estimate their precise time of speciation and the best hydrographic conditions, together with the morphological characteristics relevant to their genetic difference[33].

Though molecular analysis on Foraminifera can provide many genetic types and is helpful in the foundation of a more reliable foraminiferal proxy for the climatic reconstructions, only part of the genetic types can be clearly distinguished by their morphology[26, 27]. Therefore, the application of molecular analysis results to the micropaleontological, paleoenvironmental and paleoceanographic studies is quite restricted by the difficulty in correlating these genetic types to their morphological types.

Recently, a more detailed study revealed that the foraminiferal genetic types display an evident variation in the same region for different seasons, which implies the complex distribution of foraminiferal genetic types[39]. From the point of gene communication, there should not be such great variation for the free-exchangeable gene bank in the same environment. The laboratory analyses also show that no absolutely same sequences exist for the different clones (ITS rDNA) of one species[33]. Therefore, as a primeval single-celled organism, after the evolution for several hundred million years, does Foraminifera itself has a big variation in the different copies of their rRNA gene sequence (such as SSU or LSU)? And is part of this variation only intra-specific, instead of the species level? i.e. on the foraminiferal phylogeny, do the differences in the genetic types reach the level of "species" which is defined in the concept of common biology? At the genetic level, there is not strict standard for which variation could be considered as a new species. Thus, much more molecular work is required on Foraminifera.

Nevertheless, it is certain that more descriptions on the foraminiferal genetic types, their biogeographic distributions, ecological conditions, and recognition of their microstructure, chemical or stable isotopic characteristics, will distinguish between different genetic types in paleoceanography and will make Foraminifera much more precise in the paleo-environmental reconstruction.

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