Diversity of Polar Monothalamous Foraminifera –
Morphological and Molecular Approach

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Introduction

In many marine areas, Foraminifera are a dominant meiofaunal group in terms of both numerical abundance and biomass. Monothalamous (single-chambered) species with either agglutinated or organic-walled shells (‘allogromiids’ in the broad sense) are an important component of marine foraminiferal assemblages, particularly in fine-grained sediments, for example in the deep sea and in high latitudes regions (Gooday, 2002). Monothalamous Foraminifera also occur in freshwater environment (Holzmann et al. 2002, 2003) and have even been discovered to live in a damp terrestrial habitat (Meisterfeld et al. 2001). These delicate Foraminifera often account for 10-20% of individuals and species in deep-sea samples (Gooday, 2002). In Polar coastal regions, influenced by turbid glacial melt water, and in some estuaries, allogromiids (including saccamminids) represent an even higher proportion of live Foraminifera, in some cases >90% (Gooday, 2002).

Despite of their high abundance and ecological importance, the diversity of monothalamous Foraminifera is very poorly known. Relatively few species have been described and most of these come from easily accessible, intertidal environments. Deeper sea samples routinely yield ten or more undescribed species (Gooday et al. 1998). Several new morphotypes of allogromiids have also been reported from Antarctic coastal waters (Gooday et al. 1996). Several new species of polar and subpolar monothalamous Foraminifera have been described (Gooday et al. 1995; DeLaca et al. 2002; Wilding 2002) or redescribed (Bowser et al. 2002) during the past few years. Because the morphological characters are not always sufficient to establish the phylogenetic relationships among allogromiids, many recent species descriptions include the analysis of molecular data (Pawlowski et al. 2002a; Cedhagen and Pawlowski, 2002; Gooday et al. in press; Gooday and Pawlowski, submitted; Sabbatini et al., 2004).

Molecular phylogenetic studies challenged the traditional, morphology-based classification of Foraminifera, prompting a profound revision of higher-level taxonomy and species identification (Pawlowski 2000, Pawlowski and Holzmann 2002). Phylogenetic analysis of molecular data, based mainly the small subunit ribosomal RNA and actin gene sequences, revealed that the Foraminifera include not only testate marine species but also the naked freshwater amoeboid protists (Pawlowski et al. 1999). Molecular studies also show that there is no clear boundary between allogromiids in the ‘traditional’ sense (i.e. organic-walled species) and single-chambered agglutinated Foraminifera, traditionally considered as astrorhizids, suggesting that all of them form a paraphyletic group of monothalamous Foraminifera (Pawlowski et al., 2002a, 2003). A particularly high taxonomic diversity
of allogromiid Foraminifera, represented in majority by undescribed species, was revealed by molecular study of Antarctic Foraminifera (Pawlowski et al. 2002b).

At the beginning of project the main objective was to describe new Antarctic monothalamous Foraminifera. Many of these Foraminifera were isolated and molecularly characterized in preceding study (Pawlowski et al. 2002c) but could not be attributed to any known species. During the year there was possibility to collect other samples but in a different site and environment (Porcupine Abyssal Plain, NE Atlantic Ocean). The morphological analyses carried out on board on superficial sediment samples revealed an interesting monothalamous foraminiferal fauna composed especially by specimens belonging to Komokiacea group. These specimens are really poor known and the most recent studies are by Schroder et al. (1989), Gooday (1982, 1983), Gooday & Cook (1984), Tendal (1972, 1979), Tendal & Hessler (1977), Kamenskaya (1990, 1993).

Since there are no sequences available of Komokiacea yet, and several samples of them were available also from Antarctica to study, new aim was describe and identify a larger number of komokiacean specimens from these two sites and where possible correlate them. Important goals are 1) molecular data of Komokiacea group from the Atlantic Ocean in order to reveal them taxonomic diversity and phylogenetic position within the Foraminifera 2) a comparison of species present in the Antarctic and NE-Atlantic areas of study 3) morphological description of new species. I focused on the above points 1 and 2.

Komokiacea (Textulariina, Foraminiferida, Protista) are agglutinated Foraminifera with test consisting of a complex system of fine, branching tubules of even diameter. The test wall is simple, with argillaceous particles. Stercomata accumulate within the tubules. The group has worldwide distribution, particularly in the abyssal zone. The greatest relative abundances have been found in abyssal oligotrophic areas and in hadal trenches. Tendal & Hessler (1977) divided Superfamily Komokiacea in two families Komokiidae (Tendal & Hessler, 1977) and Baculilelidae (Tendal & Hessler, 1977). First have test bushy or arborescent constructing widely spaced branching cylindrical tubules and second test variously shaped, sparsely branched or dense clump. Branching is the basic feature controlling body form. In most cases branching is dichotomous, although tri- or polychotomy also exists. In the tube wall two layers can be distinguished: the inner layer is generally very thin and frequently laminated while the outer part is composed by agglutinated sediment. Komokiacea are devoid of real apertures. The pseudopodia must penetrate the tube wall through minute pores having possibly temporary character (Tendal & Hessler, 1977). The Komokiacea vary in according to the amount and type of particles in the agglutinated layer.

**Material and Methods**

*Specimen collection*

The material for this study was collected in the Weddel Sea (Antartica) during R/V Polarstern cruise ANT-XIX/4 (ANDEEP II, February 28th to April 1st, 2002) (Figure 1).
The other material was collected in the Porcupine Abyssal Plain (NE – Atlantic Ocean) in June 2004 (Figure 2). The Foraminifera (not only Komokiacea for a total of 80 samples) for molecular work have been isolated and stored in guanidine. Sediment samples for morphological studies were sieved on 300 micron mesh and preserved in formalin 10%. All monothalamous soft-walled Foraminifera (organic and agglutinated) will be sorted by hand, under a stereoscopic microscope, from the floated residue. Individuals will be placed in glycerol on a cavity slide, described, photographed and classified.

DNA extraction, amplification and sequencing

DNA was extracted from single or several cells using the guanidine method as described in Tkach & Pawlowski (1999). PCR amplifications were performed in a total volume of 50 µl with an amplification profile consisting of 40 cycles of 30s at 94°C, 30s at 48°C and 120s at 72°C, followed by 5 min at 72°C for final extension. A fragment of the SSU rRNA gene was amplified by PCR with the primer pair s14F3 (5’ACG CA(AC) GTG TGA AAC TTG) and sB (5’ TGA TCC TTC TGC AGG TTC ACC TAC). When the first PCR was unsuccessful, the PCR products were re-amplified using the nested primer s14F1 (5’ AAG GGC ACC ACA AGA ACG C), with an amplification profile consisting of 25 cycles and 52°C for annealing time. A second series of analyses were carried out using the primer pair s14F3 (5’ACG CA(AC) GTG TGA AAC TTG) and s17 (5’CGG TCA CGT TCG TTG C) for the PCR and the same nested primer (5’ AAG GGC ACC ACA AGA ACG C) for the re-amplification. The amplified PCR products were purified using High Pure PCR Purification Kit (Roche Diagnostics), then either sequenced directly or ligated into pGEM-T Vector system (Promega) and cloned in XL-2 Ultracompetent Cells (Stratagene). Sequencing reactions were prepared by using ABI-PRISM Big Dye Terminator Cycle Sequencing Kit and analysed with an ABI 3100 DNA sequencer (Perkin-Elmer), all according to the manufacturer’s instructions.

Sequence analysis

Sequences were aligned manually to the large database of foraminiferal sequences, using the Seaview software of Galtier et al. (1996) and Bioedit program (2004). A fragment of 462 sites was used for the analysis with sB primer and a fragment of 100 sites was used for analysis with s17 primer. Phylogenetic analyses were performed with the neighbor joining method (NJ) using Kimura 2 distance algorithm.

Result Morphological work

Here I present a short taxonomic survey of some of the must abundant larger (>300 micron) rhizopod species, representing a wide range of taxa, from populations on both the Porcupine Abyssal Plain and Weddel Sea.

The species and morphotypes from both study sites were divided into taxonomic categories in most cases genera such as Ipoa spp., Komokia spp., Lana spp., Normanina spp. and Septuma spp. but also indeterminate groups, e.g. Chain spp.

“Chain-like” komokiaceans:

These are common in the Porcupine Abyssal Plain and it is possible distinguish 7 morphotypes. The “chains” often consist of a branched or occasionally branching chain of small spherical chambers
connected by long, narrow, gently tapering necks. Occasionally globigerinacean tests of varying size are incorporated within the chamber wall. Other types are organized as a rigid chain of irregularly-shaped, elongate chambers connected by narrow necks. The chambers are angular; long narrow tubules of even width may extend from the end of the chamber. The test wall consists of globigerinacean tests of variable size. Similar morphotypes are distinguished from these forms by the irregularly-shaped chambers as well as by chambers separated by rigid, short, narrow necks. Other “Chain-like” forms consist of a series of spherical/ cylindrical chambers which adjoin directly; the chambers have a bumpy exterior surface, incorporating globigerinacean tests of varying size incorporated. Occasional long, single filaments may extend from the surface. Sometimes irregularly shaped chains of chambers are densely encrusted with globigerinacean tests which obscure the basically organic test wall and make individual chambers difficult to distinguish. Also in this case occasional short, fine, transparent fibres, and long, white filaments, extend from the surface. In other species, a branching chain of triangular, claw or arrow-head shaped chambers joined together at their pointed ends to produce a chain with an elegant appearance. Finally, a distinctive morphotype comprises pear-shaped chambers joined to form a branching chain which undergoes characteristic angular changes of direction. The chambers are joined head-to-toe (Plate 1).

All chain-like morphotypes are quite delicate, they break easily and are typically found in a fragment state.

**Komokia-like group**

This group is dominant in samples from Porcupine Abyssal Plain area; some specimens Komoki-like were found also in the Antarctic study area.

According to Tendal & Hessler (1977), Komokia morphotypes have generally bush –like body consisting of mostly dichotomously branching tubules without anastomoses, the branching points occurring at any place along the tubules. Ramification increases toward periphery of body. All tubules are the same diameter (more less), and are nonseptate (Tendal & Hessler, 1977).

In our material the interstices between the tubules are sometimes filled with abundant sediment and the general body form is elongate, pear or club-shaped body. Long, unbranched, empty filaments of varying length, frequently extend from the surface. Specimens often differ from classical representatives of the genus is consisting of only a few tubules. Globigerinacean tests are occasionally attached to branching tubules (Plate 2).

**Genus Lana and Reticulum**

Species of the closely-related genera Lana are common in both study areas. Lana species have the form of a loose mass of branching and anastomosing tubules with no centre of organization or pattern of growth. The tubules are parallel sided and non-septate (Tendal & Hessler, 1977). In Reticulum the tubules anastomose more extensively than in the case of Lana. It is possible to recognize a large number of morphotypes and classification is based on form of the clump, on the frequency of anastomoses, and on the degree of filling of interstices with sediments (Gooday & Cook, 1984).

The species of Lana and Reticulum studied vary from irregularly-shaped clump of fine, or very fine,
branching and anastomosing tubules of even diameter to a pear-shaped, tightly packed clump of tubules of even diameter. There are also morphotypes represented by coherent, spongy, irregularly to spherically-shaped masses of regularly anastomosing tubules or loose network of branching tubules (sometimes fine) anastomosing, and not always of even diameter. The interstices of the Lana/Reticulum tubule system are either devoid of sediment or sediment-filled; the tubules may be transparent and typically contain dark masses of stercomata. Globigerinacean tests are often incorporated within the tubules network or throughout tubule clump (Plate 3).

**Ipoa group**
Morphotypes belonging to Ipoa group are better represented in the Antarctic environment. Most specimens are tree-like fragments treelike with a basal stem followed by burst of tightly spaced multiple branching; diameter of tubules decreases with each branching. Tubules are avoid of septa (Tendal & Hessler, 1977) (Plate 4).

**Normanina group**
Also specimens belonging to this group are also more common in the Antarctic although there are some PAP representatives. They have a base or center composed of tubules branching in complex, irregular pattern. Tubules radiate outward from this base and terminate in globular or clublike chambers (Tendal & Hessler, 1977) (Plate 4).

**Septuma group**
Species of Septuma group in the Antarctic samples are represented by specimens with bushlike body, sparse branching, dichotomous and nonastomosing at base. All tubules, as suggested by the group name, are divided internally by septa with foramina (Tendal & Hessler, 1977) (Plate 4).

Edgertonia spp. and Komoki mudball spp. these are the dominant benthic Foraminifera in the Porcupine Abyssal Plain samples. In addition to the above mentioned Komokiacea, a number of other monothalamous Foraminifera (Muddy Tube spp., Lagenammina spp., Rhizammina spp.) and polythalamous (Reophax spp.) Foraminifera were studied in the Antarctic samples.

**Result Molecular work**
Sixty-seven samples from Porcupine Abyssal Plain (NE Atlantic Ocean) and 24 samples from Weddel Sea (Antarctica) were analysed using molecular approach. It was possible obtain only DNA sequences from fragment of the SSU rRNA gene of monothalamous agglutinated Foraminifera (Astrorhizina) and polithalamous agglutinated and calcareous Foraminifera (Textulariina and Rotaliina). Only 7 DNA sequences from komokiacean morphotypes were obtained and results are represented in figure 3 (a-b). We obtained also DNA sequences of monothalamous and polithalamous agglutinated Foraminifera (Lagenammina spp., Reophax spp. and Rhizammina spp.) from the PAP; this is an important goal because all data of this genera come from shallow water or polar settings. In this case we have the first
DNA sequences from deep Atlantic Ocean.

Discussion and Conclusion

Komokiacea are an important group of macrofaunal sized agglutinated Foraminifera. This group of protists eluded, until recently, description because fragility of many species means that they were often lost during sample processing or their identification and classification were difficult. Even when present in sample residues, they are difficult to recognize as organisms (Tendal, 1979), looking instead like non descrip organic detritus, poorly washed balls of sediment, or minor fragments from the surface of some unidentified larger organisms (Tendal & Hessler, 1977) (Gooday & Cook, 1984).

Very few observations have been made on live cells of Komokiacea although they are classified within the Foraminifera, granulo-reticulate pseudopodia have not been observed in any true komokiacean organisms, causing some workers to doubt their assignment to this group. The group has non clear ultrastructural identity and is distinguished by the unusual gross morphology consisting of a system of branching of tubules.

Usually foraminiferal species are identified exclusively on the basis of morphological characters, such as the number and form of the chambers, the form of test periphery and the type of ornament. However, the paucity of morphological characters and their relatively large variability renders species identification in Foraminifera and in Komokiacea particularly difficult. In this project we therefore made the first attempts to investigate what Komokiacea are and their phylogenetic position between Foraminifera group based on molecular data. Our results are based principally on analysis of ribosomal DNA sequences.

During the course of this project we met problems with PCR analyses and on primers use. PCR is a tool of unrivalled power, but as is so often the case, this power is linked to unrivalled complexity. The source of this complexity is the PCR reaction itself—a myriad of ionic interactions, kinetic constants, and enzymatic activities, all taking place repeatedly and, hopefully, perfectly, within the space of a few hours. Because each DNA (template) and each primer pair is different, and because molecular systematics tend to use a wide variety of taxa or primers in a single research program, PCR reactions need to be carefully optimised. This means that a certain amount of trial and error is an integral part of the PCR experience. Apart from PCR conditions attention has been focused on the primers and how strongly they anneal to the template DNA. A first series of analyses have been made using foraminiferal primers and in particular primers with monothalamous foraminiferal “target”. We obtained few results associated to specimens belonging to polythalamous agglutinated (Reophax) and calcareous (Epistominella) Foraminifera. A second series of analyses were made using universal eukaryotic primers obtaining only contaminant sequences of animal and fungi. Other studies will be made using other foraminiferal primers. There is no evidence yet, but the possibility exists that these results could lead us to not consider Komokiacea as Foraminifera.

On the other hand morphological features, wall composition and test structure all suggest that Komokiacea are Foraminifera.

The test of Komokiacea are partially formed organically but it also include a varying proportion
of extraneous matter as the typical foraminiferal agglutinated tests. The foreign particles utilized in test construction could reflect to some extent the local environment; material commonly utilized includes quart grains, various heavy minerals, clay or carbonate fragments or grains, and organic debris, including tests of smaller Foraminifera, radiolarians, coccoliths, fragments of molluscan shells, and sponge spicules (Loeblich & Tappan, 1964). However Komokiacea are known to accumulate very fine particles within tubule lumen where they are presumably digested (Gooday & Cook, 1984). Many aspects of the ecology and biology of the Komokiacea are poorly understood: protoplasmic body, nucleus, pseudopodia, gamets, cysts and in particular symbionts and parasites life history and feeding habits are still unknown.

Little is known also about the distribution of komokiacean species (Schroder et al., 1989; Gooday & Cook, 1984; Kamenskaya, 1993).

Systematic tree obtained from molecular data (figure 3) revealed the distribution of several “komokiacean DNA sequences” with environmental DNA sequences or “not common foraminiferal DNA sequences” in a clade of monothalamous agglutinated Foraminifera from several habitats. These DNA sequences could belong to parasite organisms living in the sediment particles agglutinated by Komokiacea. This strategy is common to several protozoans and from the literature we know that the coccidian Trophosphaera planorbulinae (LE CALVEZ) (Loeblich and Tappan, 1964) lives in the larger chambers of Elphidium crispum extending throughout the test and destroying it eventually. Nematode worms may also be parasitic on them. An other condition is represented by bryozoan colonies that inter grow as networks with the komokiacean tubules (Lana and Edgertonia genera) (Gooday & Cook, 1984) forming a symbiotic relation between ctenostome peristomes of Bryozoa and the tubule mass of Komokiacea. These kinds of relationships complicate the study of komokiacean molecular identification.

Our findings suggest the following conclusions 1) to find DNA sequences of Komokiacea is necessary perform the PCR analyses with new foraminiferal primers or other universal primers; 2) we need to improve our knowledge of ultrastructural morphology in order to demonstrate that Komokiacea are really Foraminifera; 3) we need more information about the natural history of Komokiacea in general.

**Figure Captions**

**Fig. 1** – Location Map Antarctic Samples

**Fig. 2** – Location Map NE Atlantic Samples

**Fig. 3** – Molecular data a) 462 sites were used for the analysis with sB primer; phylogenetic analyses were performed with the neighbor joining method (NJ) using Kimura 2 distance algorithm; b) 100 sites were used for analysis with s17 primer; phylogenetic analyses were performed with the neighbor joining method (NJ) using Kimura 2 distance algorithm.
Plates of Komokiacean Foraminifera from NE-Atlantic

Plate 1 – “Chain-like” komokiaceans: 1) Chain sp.1 - 2) Chain sp.2 - 3) Chain sp.3 - 4) Chain sp.4 – 5) Chain sp.5 – 6) Chain sp.6 – 7) Chain sp.7

Plate 2 - Komokia-like group: 1) Komoki sp.1 – 2) Komoki sp.2 – 3) Komoki sp.3 – 4) Komoki sp.4 – 5) Komoki sp.5 – 6) Komoki sp.5-bis – 7) Komoki sp.6 – 8) Komoki sp.7

Plate 3 - Lana group: 1) Lana mudball – 2) Lana sp.1 or Cerebrum (Schroder) – 3) Lana sp.2 – 4) Lana sp.3 – 5) Lana sp.4 – 6) Lana sp.5 – 7) Lana sp.5a – 8) Lana sp.6

Plate 4 – Other Komokiacean Foraminifera: 1) Ipoa spp. – 2) Septuma spp. – 3) Reticulum sp.1 – 4) Edgertonia argillipsfherula – 5) Edgertonia floccula – 6) Mudball Komoki sp.2 – 7) Mudball Komoki sp.3 – 8) Mudwall Crithionina spp

Plate of Komokiacean Foraminifera from Weddel Sea


References


Figure 1. - Location Map
Plate 1 - “Chain-like” komokiaceans
Plate 2 - Komokia-like group
Plate 4 - Other Komokiacea Foraminifera
Plate 5 - Komokiacean Foraminifera from Weddel Sea