Protistology 5 (4), 281–302 (2008) Protistology

The twilight of Sarcodina: a molecular perspective on the polyphyletic origin of amoeboid protists [1]

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> "All known eukaryotes are the sarcodines"
>
> (Kirill A. Mikrjukov)

Summary

For convenience, the traditional classifications placed all amoeboid protists in the group Sarcodina, subdivided based on the type of pseudopodia into Rhizopoda and Actinopoda. Early molecular phylogenies provided evidence for the polyphyly of Sarcodina, but the relationships between different amoeboid taxa remained largely unresolved. It was only recently that a more congruent view of the phylogeny of amoeboid protists emerged from multigene analyses. According to this view, the majority of amoeboid protists are placed in the supergroups Amoebozoa and Rhizaria. Amoebozoa include almost all lobose amoebae and mycetozoans, while Rhizaria comprise the majority of protists bearing filopodia, reticulopodia and axopodia. Both supergroups also contain a variety of free-living flagellates and some parasitic lineages. The few amoeboid protists that did not find their place within these two supergroups have been placed by molecular phylogenies either among excavates (Heterolobosea), opisthokonts (*Nuclearia*), and stramenopiles (Actinophryida, some Filosea), or as independent lineages (Centrohelida, Breviata). Although the molecular data clearly indicate that the pseudopodial structures have been developed several times in the history of eukaryotes, they also show that a large diversification of amoeboid protists occurred only twice, in lineages leading to Amoebozoa and Rhizaria. The evolution of different types of pseudopodia within these supergroups constitutes a new challenge for future phylogenomic studies of amoeboid protists.

Introduction

Reconstructing the tree of life is one of the main objectives of evolutionary biology since Ernst Haeckel's legendary "Staumbaum des Lebens" (1866). Yet, neither Haeckel nor his direct successors could produce an accurate representation of the tree of life, because of limited access to the phylogenetic characters shared by all living organisms available at that epoch. The reconstruction of the evolutionary history of protists constituted a particularly difficult case. For years the macrosystem of protists was based on gross morphological and life cycle features. The application of electron microscopy to study the ultrastructure of the protistan cell provided a new set of characters that could resolve some particular taxonomic problems but offered no resolution of the global phylogeny of eukaryotes (Patterson, 1994). For more than a century the higher ranks in classification of protists were based on convenience (Corliss, 1984)

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[1] Materials presented on the V European Congress of Protistology (July 23–27, 2007, St. Petersburg, Russia).

or “matter of taste” (Margulis and Schwartz, 1988) rather than on testable phylogenetic hypotheses.

This situation has radically changed with the advent of molecular phylogenies, which offer the possibility to compare homologous DNA sequences across the whole living world. Early phylogenetic reconstructions based on ribosomal DNA sequences have totally modified the systematics of eukaryotes (Cavalier-Smith, 1993, 1998). However, after an enthusiastic period of the pioneering molecular studies, it became obvious that the first ribosomal phylogenies of eukaryotes were strongly biased by long branch attraction artefacts (Philippe and Adoutte, 1998; Philippe and Germot, 2000). New methods were developed to correct these biases (Philippe et al., 2000) and the single gene phylogenies were progressively replaced by multigene and phylogenomic approaches (Delsuc et al., 2005). Based on these approaches, novel phylogenetic groupings have emerged (Cavalier-Smith, 2002; Baldauf, 2003; Simpson and Roger, 2004; Keeling et al., 2005; Burki et al., 2007), providing the basis for a new macrosystem of eukaryotes (Adl et al., 2005).

Placement of amoeboid protists in this emerging macrosystem has been considerably delayed mainly due to the lack of sequence data for most amoeboid taxa and biased analyses of existing data (Philippe and Adoutte, 1998; Pawlowski et al., 1996; Amaral-Zettler et al., 1997). Amoeboid protists were globally underrepresented in early molecular trees, particularly in multigene phylogenies (Baldauf et al., 2000). Their molecular database began to grow rapidly only recently (Amaral-Zettler et al., 2000; Bolivar et al., 2001; Fahrni et al., 2003; Cavalier-Smith and Chao 2003; Nikolaev et al., 2004, Kudryavstev et al., 2005; Fiore-Donno et al., 2007; Brown et al., 2007; Tekle et al., 2007, and many others). Although in many cases these data are still limited to SSU rDNA sequences, their analyses combined with studies of a few available protein coding genes revealed new phylogenetic relationships among amoeboid protists and prompted their grouping in two new supergroups of eukaryotes: Amoebozoa and Rhizaria. This paper presents a brief review of molecular studies that led to the formation of these new supergroups, discussing the current position of all taxa traditionally included in Sarcodina.

The concept of Sarcodina

In traditional, morphology-based classifications, the protists were subdivided into four major types: amoebae, ciliates, flagellates and sporozoa. This system, founded by Bütschli (1880), remained in use, with some modifications, for more than a century (Levine et al., 1980; Lee et al., 1985). Its different variants consequently included the amoeboid protists in the class or phylum Sarcodina (Schmarda, 1871). Although the term “sarcode” was originally applied by Dujardin (1835) to designate the material forming the protoplasm of all protists, it soon came to designate mainly the protoplasm of amoeboid protists. According to the classical definition, the Sarcodina included all protists possessing pseudopodia or locomotive protoplasmic flow, with flagella usually restricted to developmental or other temporary stages (Levine et al., 1980).

Depending on the type of pseudopodia, the Sarcodina were further subdivided into the Rhizopodea von Siebold 1845 and Actinopodea Calkins 1901 (Levine et al., 1980). The superclass Rhizopodea comprised mainly protists having lobopodia, filopodia and reticulopodia, and included eight classes: Lobosea, Acarpomyxea, Acrasea, Eumycetozoea, Plasmodiophorea, Filosea, Granuloreticulosea and Xenophyophorea. The superclass Actinopodea was composed of all axopodia-bearing protists and included four classes: Acantharea, Polycystinea, Phaeodarea and Heliozoea (Table 1). This dichotomy of Sarcodina, introduced by Calkins (1901) and postulated already by Haeckel (1894), was not followed by all taxonomists (Jepps, 1956); nevertheless, the taxonomic composition of Sarcodina varied little from one classification to the other (Kudo, 1954; Honigberg et al., 1964; Levine et al., 1980).

The concept of Sarcodina and its classification was widely accepted, firstly because it was extremely convenient, and secondly because there were no data available to construct an alternative system. The inadequacy of the traditional system was strongly criticized based on results of ultrastructural studies (Patterson, 1994). However, the distinction of the major lineages of eukaryotes based on common patterns of cell organization was of little help in resolving the relations among amoeboid protists (Patterson, 1999). It is therefore not surprising that the amoeboid groups were classified in alphabetic order (Lee et al., 2000), with some classical amoeboid macrotaxa remaining in use, albeit always with clear indication of their polyphyletic character (Hausmann et al., 2003).

Early eukaryotic phylogenies

The first molecular phylogenies, mainly based on the SSU (18S) rDNA sequences, unfortunately brought more confusion than resolution to the phylogeny of amoeboid protists. Only very few lobose amoebae were sequenced, displaying a patchy dis-

Table 1. Classification of amoeboid protists: comparison of traditional (Levine et al., 1980) and modern (Adl et al., 2005) versions

Levine et al. 1980	Adl et al. 2005
Subphylum Sarcodina	AMOEBOZOA
Superclass Rhizopodea	• Tubulinea
Class Lobosea	• Flabellinea
Subclass Gymnamoebia	• Stereomyxida
Order Amoebida	• Acanthamoebidae
Order Schizopyrenida	• Entamoebida
Order Pelobiontida	• Mastigamoebidae
Subclass Testacealobosia	• Pelomyxa
Order Arcellinida	• Eumycetozoa
Order Trichosida	
Class Acarpomyxea	RHIZARIA
Order Stereomyxida	• Cercozoa
Order Leptomyxida	• Haplosporidia
Class Acrasea	• Foraminifera
Class Eumycetozoea	• Gromia
Subclass Protosteliia	• Radiolaria
Subclass Dictyosteliia	
Subclass Myxogastria	OPISTHOKONTA
Class Plasmodiophorea	• Nucleariida
Class Filosea	
Order Achonchulinida	CHROMALVEOLATA
Order Gromiida	• Actinophryidae (Actinophrys)
Class Granuloreticulosea	
Order Athalamida	EXCAVATA
Order Monothalamida*	• Heterolobosea
Order Foraminiferida	
Class Xenophyophorea	Incertae sedis EUKARYOTA
Superclass Actinopodea	• Centrohelida
Class Acantharea	
Class Polycystinea	
Class Phaeodarea	
Class Heliozoea	
Order Actinophryida	
Order Desmothoracida	
Order Taxopodida	
Order Centrohelida	

tribution across the phylogenetic tree, strongly suggesting a polyphyletic origin (Clark and Cross, 1988; Sogin, 1991; Hinkle et al., 1994). The basal position of pelobionts and entamoebids led to their placement in a separate phylum, Archamoebae, postulated to be early branching amitochondriate eukarytotes (Cavalier-Smith, 1991). When it was demonstrated that the entamoebids lost their mitochondria secondarily (Clark and Roger, 1995), the phylum Archamoebae was abandoned and almost all lobose amoebae, with exception of the Heterolobosea, were placed in the phylum Amoebozoa (Cavalier-Smith, 1998).

The position of the remaining Sarcodina (i.e. Filosea, Granuloreticulosea and Actinopoda) remained unclear for a much longer time. The first analyses of filosean SSU rDNA showed that the euglyphid testate amoebae and the photosynthetic chlorarachniophytes with reticulate pseudopodia formed a clade (Bhattacharya et al., 1995). This clade was later enlarged to include also the plasmodiophorids and some free-living flagellates (Cavalier-Smith and Chao, 1996/1997), leading to the creation of the phylum Cercozoa (Cavalier-Smith, 1998). On the other hand, early phylogenetic analyses of Foraminifera (Pawlowski et al., 1994, 1996) and Radiolaria (Amaral-Zettler et al., 1997) showed that these groups branched separately in the SSU trees, suggesting that they may represent independent phyla (Cavalier-Smith, 1998). It was only after the publication of actin-based evidence for the possible relationships between Cercozoa and Foraminifera (Keeling, 2001) that the infrakingdom Rhizaria was proposed (Cavalier-Smith, 2002).

There are several reasons explaining why it was so difficult to find the correct phylogenetic position for most amoeboid protists in early eukaryotic phylogenies. Firstly, as mentioned above, the early ribosomal trees suffered from a general lack of molecular data representing amoeboid taxa. Secondly, many amoeboid protists show an extreme heterogeneity of substitution rates in ribosomal genes, often leading to a misinterpretation of their phylogenetic position. The exemplary case is the spectacular acceleration of the stem lineage leading to foraminifera and the extreme variations of rDNA rates between planktonic and benthic foraminiferal groups (Pawlowski et al., 1996; Pawlowski et al., 1997; Pawlowski and Berney, 2003). Unusually rapid evolutionary rates are also observed in Polycystinea (Amaral-Zettler et al., 1997), as well as in most pelobionts, entamoebids and mycetozoans (Bolivar et al., 2001; Edgcomb et al., 2002). Their fast evolving rRNA genes are often much longer than the usual (Hinkle et al., 1994; Pawlowski et al., 1996; Milyutina et al., 2001) and contain lineage-specific insertions (Habura et al., 2004), long introns (Lundblad et al., 2004) or additional variable regions that further complicate their accurate phylogenetic placement.

The problem of rate heterogeneity of ribosomal genes was partially resolved with the introduction of new evolutionary models correcting for among-site heterogeneity and invariable sites (GTR + G + I), as well as by progressive replacement of distance and parsimony methods by probabilistic approaches that are much less sensitive to rate variations (Lewis, 2001). However, a veritable revolution in the global view of eukaryotic phylogeny was brought about by the use of multigene dataset that permitted challenging the phylogenetic hypotheses based solely on the SSU sequences (Baldauf et al., 2000).

Phylogenomics and a new classification of eukaryotes

The first large-scale analysis of 123 genes from EST libraries of *Dictyostelium*, *Mastigamoeba* and *Entamoeba* provided well supported evidence for the monophyly of the Conosa (Bapteste et al., 2002). Monophyly of Amoebozoa and Rhizaria was later suggested by combined SSU and actin sequence data (Fahrni et al., 2003; Nikolaev et al., 2004). Several multigene studies demonstrated a relationship between Amoebozoa and Opisthokonts, unifying them into the super-assemblage of Unikonts (Cavalier-Smith, 2002; Keeling et al., 2005). On the other hand, the analysis of 85 genes from the chlorarachniophyte *Bigellowiella* and the foraminiferan *Reticulomyxa* confirmed the monophyly of Rhizaria (Burki and Pawlowski, 2006). The analysis of 16 genes indicated a relationship between Rhizaria and Stramenopiles (Hackett et al., 2007). Further analyses of larger sampling of taxa and genes confirmed the close relationship of Stramenopiles, Alveolates and Rhizaria, suggesting the existence of a new super-assemblage of eukaryotes (SAR), which comprises the largest diversity of protists (Burki et al., 2007).

A new view of global phylogeny of eukaryotes is emerging based on the rapidly growing genomic database (Embley and Martin, 2006). According to this view, the majority of eukaryotes are distributed between several major divisions, called supergroups or "kingdoms". The number of these supergroups is progressively decreasing, from eight (Baldauf, 2000) to six (Simpson and Roger, 2004), five (Keeling et al., 2005) and four (Burki et al., 2007). Six of these supergroups form the backbone for a new classification of eukaryotes (Adl et al., 2005). The majority of amoeboid protists is placed in the supergroups of Amoebozoa and Rhizaria (Table 1). Only five amoeboid lineages branch outside these supergroups, either within excavates (Heterolobosea), stramenopiles (Actinophryida), opisthokonts (Nucleariidae), or independently (Centrohelida, Breviatea). The composition and phylogeny of the two amoeboid supergroups as well as the phylogenetic position of the remaining amoeboid taxa are discussed below.

Amoebozoa

The taxon Amoebozoa (Lühe, 1913) was emended by Cavalier-Smith (1998) to unify in a single taxonomic group the naked and testate lobose amoebae, pelobionts, entamoebids and mycetozoans as well as some flagellates (*Multicilia*). In Cavalier-Smith's (1998) classification, the Amebozoa had the rank of a phylum subdivided into the subphyla Lobosa and Conosa. The subphylum Lobosa included all protists traditionally classified in the class Lobosea (Page, 1987). The subphylum Conosa included the Archamoebae, previously classified among the Archeozoa (Cavalier-Smith, 1983), and Mycetozoa, shown to be related to lobose amoebae, by analysis of actin and actin-related proteins (Kelleher et al., 1995; Bhattacharya and Weber, 1997) as well as by the fusion between cox1 and cox2 genes and the similar order of ribosomal protein genes in the mitochondrial genomes of *Acanthamoeba* and *Dictyostelium* (Iwamoto et al., 1998; Gray et al., 1999).

The first molecular evidence for the monophyly of Amoebozoa was presented independently by Bolivar et al. (2001) and Milyutina et al. (2001). The monophyletic grouping of gymnamoebae, mycetozoans, entamoebids, and pelobionts in the SSU rDNA trees was later confirmed by analyses of actin and SSU rDNA with a much larger taxon sampling of loboseans (Fahrni et al., 2003). In the meantime the monophyly of *Dictyostelium discoideum* and two amitochondriate amoebae (*Entamoeba histolytica* and *Mastigamoeba balamuthi*) was inferred from combined analysis of the SSU rDNA and translation elongation factors (EF-1alpha and EF-2) sequences (Arisue et al., 2002), as well as the analysis of 123 genes from EST libraries of these species (Bapteste et al., 2002). Later, the close relationship between *Dictyostelium* and *Entamoeba* was demonstrated by comparison of their completely sequenced genomes (Song et al., 2005). Simultaneously, the monophyly of Amoebozoa was suggested by myosin II phylogeny (Richards and Cavalier-Smith, 2005).

The composition of the Amoebozoa has hardly changed since its emendation (Cavalier-Smith, 1998). The only significant modification was to include the uniciliate zooflagellate *Phalansterium solitarium* of unclear phylogenetic position (Cavalier-Smith et al., 2004). The analyses of SSU rDNA sequences obtained from Arcellinida (Nikolaev et al., 2005), *Cochliopodium* (Kudryavtsev et al., 2005) and *Multicilia* (Nikolaev et al., 2006) confirmed their placement among Amoebozoa. Very recently, the amoebozoan origin has also been confirmed for the genus *Trichosphaerium* (Pawlowski and Fahrni, 2007; Tekle et al., 2008), representing the last unsequenced order of Lobosea. On the other hand, the recent multigene analysis of *Corallomyxa tenera* sp. n. (Tekle et al., 2007) refuted its placement within the Amoebozoa, suggesting that the order Stereomyxida, which traditionally included *Corallomyxa* and *Stereomyxa* (Levine et al., 1980), may belong to the supergroup Rhizaria instead of Amoebozoa.

While it is reasonable to think that the composition of Amoebozoa is now well established, the relationships within this supergroup still comprise many unresolved or controversial points (Fig. 1). The initial division of Amoebozoa into Lobosa and Conosa (Cavalier-Smith 1998) can be retained only if the root is placed between these two groups, as has been proposed by Nikolaev et al. (2006) and if Conosa is emended to include some loboseans, such as *Filamoeba*, *Acramoeba*, and *Multicilia*. Such rooting is interesting from the point of view of the evolution of flagellated amoebae because it leads to a considerable reduction in the number of flagella losses by separating most aflagellate lobose amoebae from ancestrally flagellated Conosa. However, most analyses using various outgroups place the root between Tubulinea and other amoebozoans (Fahrni et al., 2003; Cavalier-Smith et al., 2004; Nikolaev et al., 2005; Smirnov et al., 2005). Such rooting would make Lobosa paraphyletic, which was used as an argument to abandon this taxon in recent classifications (Smirnov et al., 2005).

The majority of taxa traditionally included in the Lobosa are now grouped into two classes: Tubulinea and Flabellinea (Smirnov et al., 2005). The Tubulinea represent a strongly supported clade grouping the orders Tubulinida (Amoebidae and Hartmanellidae), Leptomyxida, Arcellinida, and some species of unknown affinities (*Echinamoeba* spp. and *Hartmannella vermiformis*). The synapomorphies for this clade are tubular pseudopodia and monoaxial cytoplasmic flow (Smirnov et al., 2005). The Flabellinea, defined by the flattened shape of the cells and polyaxial cytoplasmic flow or cytoplasmic flow without pronounced axis (Smirnov et al., 2005), include Vannellida and Dactylopodida and correspond to the order Glycostylida (Cavalier-Smith et al., 2004). This clade, revealed by Peglar et al. (2003), is almost always recovered by molecular phylogenies, albeit with weaker support than Tubulinea.

Among other monophyletic groupings within Amoebozoa, the clade *Acanthamoeba* + *Balamuthia*, characterized by a trilaminated cytoplasmic MTOC, consistently appeared in all molecular phylogenies since Stothard et al. (1998). There is also a relatively good support for the Archamoebae, the taxon composed of amitochondriate amoebae, including *Pelomyxa*, entamoebids and mastigamoebids (Cavalier-Smith, 1998). Weak evidence for a sister group relation between entamoebids and mastigamoebids shown in the SSU rDNA analyses (Silberman et al., 1999; Bolivar et al., 2001; Milyutina et al., 2001, and others) was later reinforced by multigene analyses (Arisue et al., 2002; Bapteste et al., 2002).

The Eumycetozoa are another possible monophyletic clade, grouping Dictyostelia + Myxogastria and some Protostelia. The monophyly of this group, represented by *Dictyostelium*, *Physarum* and *Planoprotostelium*, is strongly supported in the EF1A phylogenies (Baldauf and Doolittle, 1997; Arisue et al., 2002). However, because the EF1A trees usually include very few other amoebozoans, these analyses are of weak relevance for the question of mycetozoan monophyly. In the SSU phylogenies, the dictyo-

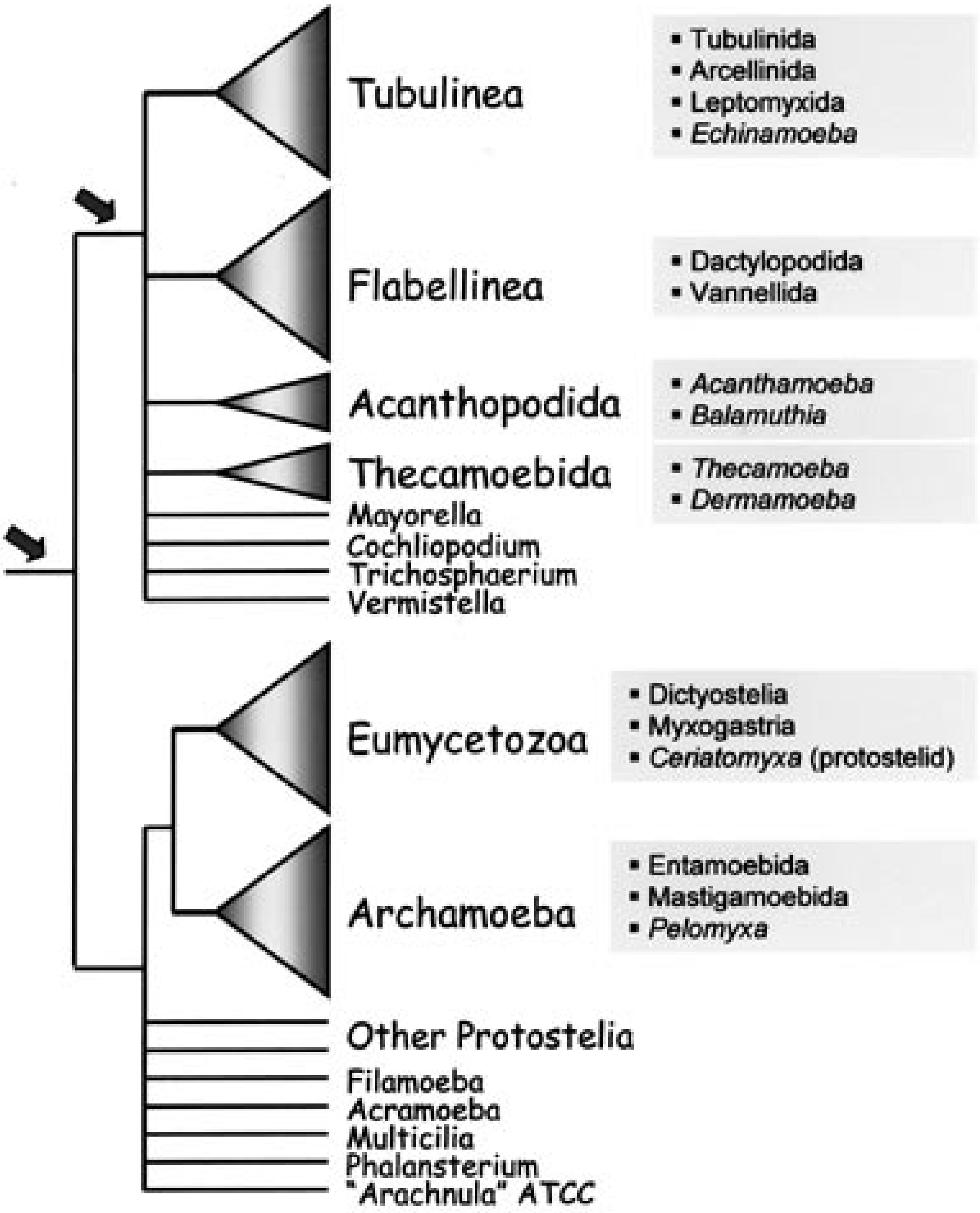

Fig. 1. Hypothetical consensus tree of Amoebozoa. Two possible positions of the root are indicated by arrows.

stelids and myxogastrids branch either separately or in a weakly supported clade (Silberman et al., 1999; Bolivar et al., 2001). This can be explained by the extremely divergent SSU rDNA sequence of *P. polycephalum* as well as by its strong nucleotide composition bias (Baldauf and Doolittle, 1997). Addition of new sequences of myxogastrids breaking the long stem branch allows the recovery of the monophyly of Eumycetozoa in some (Nikolaev et al., 2006) but not all (Tekle et al., 2008) SSU analyses. Recent SSU and EF1A phylogenies show that the clade of Eumycetozoa also includes some Protostelida (*Ceratiomyxa*), but most of them branch separately within Conosea (Brown et al., 2007; Fiore-Donno et al., subm.).

A hypothetical consensus tree of Amoebozoa based on the currently available molecular data is illustrated in Fig 1. The tree is presented with a basal dichotomy as suggested by Nikolaev et al. (2006) in unrooted form with possible positions of the root indicated by an arrow. Five well-supported clades in the published molecular trees, as well as the Thecamoebida clade - thecamoebids s.s. (*Thecamoeba*, *Sappinia*, *Stenamoeba*) and *Dermamoeba* suggested by unpublished analysis of myosin II sequences (Berney, pers. comm.), are represented by triangles. Other taxa that could not be confidently placed in any of these clades are represented by separate branches; the environmental sequences of uncultured amoebozoans are not included. The clade of Conosea, grouping Archamoebae, Mycetozoa and several independently branching lineages (Smirnov et al., 2005; Tekle et al., 2008; Fiore-Donno et al., subm.), is indicated. However, the support for this clade is not very strong and the position of some genera (*Phalansterium*) varies depending on the type of analyses.

The main weakness of the present amoebozoan phylogeny is its almost exclusively single-gene (SSU rDNA) character. Actin is the only protein gene available for larger taxon sampling of Amoebozoa (Fahrni et al., 2003; Nikolaev et al., 2005, 2006; Tekle et al., 2008). However, the phylogenetic information of this relatively conserved gene is of limited value for inferring intra-group relationships (Fahrni et al., 2003). The work in progress on myosin II provides a promising insight into their phylogeny (Berney and Cavalier-Smith, 2007; Berney, pers. comm.,). Among other molecular markers, the EF1A was extensively used in the study of Mycetozoa (Baldauf and Doolittle, 1997; Fiore-Donno et al., 2005; Schaap et al., 2006; Fiore-Donno et al., subm.), but there are almost no EF1A sequences for other amoebozoans. The EST and genome projects have focused mainly on Conosea (*Entamoeba*, *Mastigamoeba*, *Dictyostelium*, *Physarum*), whereas only two species of lobose amoebae *Acanthamoeba castellanii* and *H. vermiformis* were analyzed (Table 2).

Rhizaria

The term Rhizaria was introduced by Cavalier-Smith (2002) to define a group of protists characterized by “commonly root-like reticulose or filose pseudopodia”. The group was given a taxonomic status of infrakingdom and included four phyla: Apusozoa, Cercozoa, Retaria and Heliozoa (Centrohelida). The diagnosis of this infrakingdom proposes that the members of the Rhizaria are “ancestrally and typically bikonts with tubular mitochondrial cristae, the centrioles ancestrally with a single root of a microtubular band or fan, and the extrusomes are generally in the form of kinetocysts”. The initial description of Rhizaria was rather intuitive and based on actin evidence for relationship between Cercozoa and Foraminifera (Keeling, 2001), as well as the weakly supported branching of included phyla in some SSU-based trees. In fact, the Apusozoa as well as the Heliozoa (Centrohelida) were later removed from Rhizaria, and in the following classification, the Rhizaria comprises only two phyla: Cercozoa and Retaria, composed of the subphyla Filosa + Endomyxa, and Radiozoa + Foraminifera, respectively (Cavalier-Smith, 2003). The Apusozoa were considered as incertae sedis of the subkingdom Biciliata, while the Heliozoa (Centrohelida) were included in the kingdom Chromista (Cavalier-Smith, 2003).

Rhizaria were shown for the first time to be a strongly supported supergroup in combined analysis of SSU and actin genes, which included the representatives of all main groups of “Actinopoda” (Nikolaev et al., 2004). The authors of this study provided a multigene evidence for the polyphyly of “Heliozoa”, suggested previously by ultrastructural studies (Smith and Patterson, 1986; Mikryukov et al., 2000)

Table 2. Currently available molecular data for Amoebozoa and Rhizaria

Group	Genera (species)	Genes	References
		AMOEBOZOA	
Tubulinida	Amoeba spp.	SSU, actin	Bolivar et al. 2001, Fahrni et al. 2003
	Chaos spp.	SSU, actin	Bolivar et al. 2001, Fahrni et al. 2003
	Hartmannella cantabrigiensis	SSU, actin	Fahrni et al. 2003
	Hartmannella abertawensis	SSU	Kuiper et al. 2006
	Glaeseria mira	SSU, actin	Fahrni et al. 2003
	Nolandella spp.	SSU, actin	Tekle et al. 2008
	Saccamoeba limax	SSU	Amaral Zettler et al. 2000

Leptomyxida	Leptomyxa reticulata	SSU	Amaral Zettler et al. 2000
	Rhizamoeba saxonica	SSU	Smirnov et al. 2007a
	other Leptomyxida	SSU	Amaral Zettler et al. 2000, Tekle et al. 2008
Arcellinida	Arcella spp.	SSU	Nikolaev et al. 2005, Tekle et al. 2008
	Heleopera sphagni	SSU, actin	Nikolaev et al. 2005, Lara et al. 2008
	other Arcellinida	SSU	Nikolaev et al. 2005, Lara et al. 2008
Dactylopodida	Korotnevella sp.	SSU	Peglar et al. 2003
	Vexillifera spp.	SSU	Fahrni et al. 2003, Peglar et al. 2003
	Neoparamoeba spp.	SSU	Dykova et al. 2005b, Moran et al. 2007, Wong et al. 2004
	Pseudoparamoeba pagei	SSU	Wong et al. 2004
Vannellida	Vannella spp.	SSU, actin	Fahrni et al. 2003
		SSU	Dykova et al.2005a, Smirnov et al. 2007b, Moran et al. 2007
	Clydonella sp.	SSU	Peglar et al. 2003
	Lingulamoeba leei	SSU	Peglar et al. 2003
	Pessonella sp.	SSU, actin	Tekle et al. 2008
Thecamoebida	Thecamoeba similis	SSU, actin	Fahrni et al. 2003
	Thecamoeba quadrilineata	SSU	Michel et al. 2006
	Dermamoeba algensis	SSU, actin	Fahrni et al. 2003
	Sappinia spp.	SSU	Michel et al. 2006, Brown et al. 2007
	Stenamoeba ("Platyamoeba") stenopodia	SSU	Fahrni et al. 2003, Smirnov et al. 2007b
Acanthamoebida	Acanthamoeba castellanii	genome	in progress
		mt genome	Burger et al. 1995
	Acanthamoeba spp.	SSU	Stothard et al. 1998 and others
	Protacanthamoeba	SSU	Dykova et al. 2005d
	Balamuthia mandrillaris	SSU	Stothard et al. 1998
Entamoebida	Entamoeba histolytica	genome	Loftus et al. 2005
	other Entamoeba (4 sp)	genome	in progress
Mastigamoebida	Mastigamoeba balamuthi	SSU + EST	Hinkle et al. 1994, Bapteste et al. 2002
		EF1, EF2	Arisue et al. 2002
	Mastigella	SSU	Edgcomb et al. 2002
	Endolimax	SSU	Silbermann et al. 1999
Pelobiontida	Pelomyxa	SSU, actin	Fahrni et al. 2003
Protostelia	Planoprotostelium	SSU, EF1	Baldauf & Doolittle 1997, Brown et al. 2007
	Ceriatomyxa	SSU, EF1	Fiore-Donno et al. (subm)
	other Protostelia	SSU, EF1	Fiore-Donno et al. (subm)
	Protostelium	SSU	Brown et al. 2007
Dictyostelia	Dictyostelium discoideum	genome	Eichinger et al. 2005
	other Dictyostelium spp.	genome	in progress
	Polysphondylium	genome	in progress
	Acytostelium	SSU, a-tubulin	Schapp et al. 2006
Myxogastria	Physarum polycephalum	genome	in progress
	other Myxogastria	SSU, EF1	Fiore-Donno et al. 2005, Fiore-Donno et al. subm.
		SSU	Horton & Landweber 2000, Wikmark et al. 2007, Fiore-Donno et al. 2008
Incertae sedis	Trichosphaerium sp.	SSU, actin	Tekle et al. 2008, Pawlowski & Fahrni 2007
	Cochliopodium spp.	SSU	Kudryavtsev et al. 2005
	Mayorella sp.	SSU, actin	Fahrni et al. 2003

	Echinamoeba spp.	SSU	Amaral Zettler et al. 2000, Baumgartner et al. 2003
	"Hartmannella" vermiformis	EST	PEP
	Filamoeba spp.	SSU	Amaral Zettler et al. 2000, Dykova et al. 2005c
	Acramoeba dendroida ("Gephyramoeba" sp. ATCC)	SSU	Amaral Zettler et al. 2000, Smirnov et al. 2007a
	Multicilia marina	SSU	Nikolaev et al. 2006
	Phalansterium solitarium	SSU	Cavalier-Smith et al. 2004
	Vermistella antarctica	SSU	Moran et al. 2007
	"Arachnula" sp. ATCC	SSU, actin	Tekle et al. 2008
		RHIZARIA	
Cercomonadida	Cercomonas sp.	EST	PEP
	other Cercomonadida	SSU	Zaman et al. 1999
		SSU	Cavalier-Smith & Chao 2003
		LSU	Moreira et al. 2007
Cryomonadida	Cryothecomonas spp.	SSU	Kuhn et al. 2000
Heteromitida	Heteromita globosa	SSU	Cavalier-Smith and Chao 1996/1997
		LSU	Markmann & Tautz 2005
	other Heteromitida	SSU	Cavalier-Smith and Chao 2003
Thaumatomonadida	Thaumatomonas seravini	SSU	Cavalier-Smith & Chao 2003
	Thaumatomastix sp.	LSU	Moreira et al. 2007
	others	SSU	Cavalier-Smith & Chao 2003
Euglyphida	Euglypha rotunda	SSU	Bhattacharya et al. 1995
	Paulinella chromatophora	SSU	Bhattacharya et al. 1995
	Assulina muscorum	SSU, LSU	Wylezich et al. 2002, 2007
	Cyphoderia spp.	SSU	Wylezich et al. 2002, Hegger, in prep
	other Euglyphida	SSU	Wylezich et al. 2002, Lara et al. 2007
Chlorarachniophyta	Bigellowiella natans	EST	PEP
		genome	in progress
	Chlorarachnion reptans	SSU	McFadden et al. 1994
		genome	in progress
	Lotharella amoeboformis	SSU,actin,pub	Keeling 2001, Archibald et al. 2003
		genome	in progress
	Gymnochlora stellata	SSU	Ishida et al. 1999
Phaeodarea	Coelodendrum ramosissimum	SSU	Polet et al. 2004
	Aulosphaera trigonopa	SSU	Polet et al. 2004
	Aulacantha scolymantha	SSU	Polet et al. 2004
	other Phaeodarea	SSU	Yuasa et al. 2006
Desmothoracida	Hedriocystis spinifera	SSU	Nikolaev et al. 2004
	Clathrulina elegans	SSU	Nikolaev et al. 2004
Ebriidea	Ebria tripartita	SSU	Hoppenrath & Leander, 2006
Plasmodiophorida	Plasmodiophora brassicae	multigene	Bulman et al. 2001,2006, 2007
	other plasmodiophorids	SSU	Bulman et al. 2001
		actin,pub	Archibald & Keeling 2004
Phagomyxida	Phagomyxa	SSU	Bulman et al. 2001
Haplosporidia	Haplosporidium louisiana	SSU, actin	Flores et al. 1996, Reece et al. 2004
	Haplosporidium spp.	SSU	Stokes and Burreson 1995
	Minchinia teredinis	SSU	Stokes et al. 1995
	other Haplosporidia	SSU, actin	Flores et al. 1996, Reece et al. 2004

Paramyxida	Marteilia refringens	SSU	Berthe et al. 2000
	Marteilioides chungmuensis	SSU	Itoh et al. 2003
Gromiida	Gromia spp.	SSU	Burki et al.2002, Aranda da Silva et al.2006
		LSU	Pawlowski et al. 1994
		actin, RPB1	Longet et al. 2004
Foraminifera	Reticulomyxa filosa	EST	Burki et al. 2006, Burki & Pawlowski 2006
	Quinqueloculina sp.	EST	Burki et al. 2007
	Xenophyophorea	SSU	Pawlowski et al. 2003b
	other foraminifera	LSU	Pawlowski et al. 1994, Moreira et al. 2007
		SSU	Pawlowski et al. 1996, 2002, 2003a
			Habura et al. 2004
		actin	Flakowski et al. 2005, 2006
		polyubiquitin	Archibald et al. 2003, Bass et al. 2005
		RPB1	Longet & Pawlowski 2007
		a-, b-tubulin	Takishita et al. 2005
Collodaria	Collozoum inerme	SSU, actin	Polet et al. 2004, Nikolaev et al. 2004
	Thalassicolla pellucida	SSU, actin	Polet et al. 2004, Nikolaev et al. 2004
	Sphaerozoum punctatum	SSU	Amaral Zettler et al. 1997
	Rhaphidozoum acuferum	SSU	Amaral Zettler et al. 1999
Nassellarida	Pterocorys zancleus	SSU	Kunitomo et al. 2006
	Eucyrtidium hexagonatum	SSU	Kunitomo et al. 2006
Spumellarida	Dictyocoryne profunda	SSU	Takahashi et al. 2004
	Spongodiscus biconcavus	SSU	Kunitomo et al. 2006
	other Polycystinea	SSU	Amaral Zettler et al. 1997, 1998, 1999
			Takahashi et al.2004, Kunitomo et al.2006
Acantharea	Acanthometra sp.	SSU	Amaral Zettler & Caron 2000
	other Acantharea	SSU	Amaral Zettler et al. 1997
			Amaral Zettler & Caron 2000
Taxopodida	Sticholonche zanclea	SSU	Nikolaev et al. 2004
Incertae sedis	Gymnophrys cometa	SSU	Nikolaev et al. 2003
	Lecythium sp.	SSU, actin	Nikolaev et al. 2003, Nikolaev et al. 2004
	Massisteria marina	SSU	Atkins et al. 2000
			Cavalier-Smith & Chao 2003
	Pseudodifflugia gracilis	SSU	Wylezich et al. 2002
	Dimorpha-like	SSU	Cavalier-Smith & Chao 2003
		LSU	Moreira et al. 2007
	Spongomonas spp.	SSU	Cavalier-Smith & Chao 2003
	Metopion fluens	SSU	Cavalier-Smith & Chao 2003
	Metromonas simplex.	SSU	Bass and Cavalier-Smith 2004
	Pseudopirsonia mucosa	SSU	Kühn et al. 2004
	Cercobodo agilis	SSU	Bass et al. 2005
	Aurigamonas solis	SSU	Vickerman et al. 2005
	"Corallomyxa" tenera	SSU, actin	Tekle et al. 2007

and SSU analyses (Cavalier-Smith and Chao, 2003). The examined heliozoan taxa branched within stramenopiles (Actinophryida), among cercozoans (Desmothoracida) or as an independent eukaryotic lineage (Centrohelida). This study also provided further evidence for the polyphyly of "Radiolaria" confirming that Pheaodarea group within the core Cercozoa, as suggested by Polet et al. (2004). Acantharea and Polycystinea form a monophyletic clade, as shown by Lopez-Garcia et al. (2002), branching together with Taxopodida (*Sticholonche*), as a sister group to other Rhizaria.

The relationships within the Rhizaria have been extensively studied (Cavalier-Smith and Chao, 2003; Bass and Cavalier-Smith, 2004; Bass et al., 2005), but the branching order, in particular between the basal groups, remains unresolved. The supergroup has been divided into three phyla: Cercozoa, Foraminifera and Radiozoa (Cavalier-Smith, 2004). The phylum Cercozoa comprised two subphyla: Filosa and Endomyxa (Cavalier-Smith, 2003). Filosa correspond to core Cercozoa (Nikolaev et al., 2004) and include the filose testate amoebae (Euglyphida, *Pseudodifflugia*), the chlorarachnean algae (*Bigellowiella*, *Lotharella*) and a large array of various zooflagellates, which are often able to generate filopodia (*Cercomonas, Heteromita, Thaumatomonas, Proleptomonas, Massisteria, Cryothecomonas* and others). Further studies showed that Cercozoa also include the radiolarians of the class Phaeodarea (Polet et al., 2004; Yuasa et al., 2006), the heliozoans of the class Desmothoracida (Nikolaev et al., 2004) and the flagellates Ebriidae (Hoppenrath and Leander, 2006). The phylogenetic analyses usually recover the monophyly of the Filosa, albeit not always with strong support (Cavalier-Smith and Chao, 2003; Bass and Cavalier-Smith, 2004). It has been demonstrated that most Filosa possess a two amino acids insertion at the monomer-monomer junction of the polyubiquitin gene, with the exception of the chlorarachniophytes (*Lotharella, Bigelowiella*) and some flagellates (*Metopion, Helkesimastix* and *Cercobodo*), which possess a single amino acid insertion (Bass et al., 2005).

Compared to a relatively well supported Filosa clade, the relationships between the cercozoan subphylum Endomyxa and other rhizarian phyla (Foraminifera and Radiozoa) are much more controversial. The Endomyxa have originally been defined as plasmodial endoparasites of other eukaryotes, and included the class Phytomyxea (Plasmodiophorida + Phagomyxida) and the class Ascetosporea (Haplosporida + Paramyxida) (Cavalier-Smith, 2002). Later, the group was extended to include the gromiids (class Gromiidea) (Cavalier-Smith and Chao, 2003) and some uncultivated environmental clades (Bass and Cavalier-Smith, 2004; Bass et al., 2005). However, this definition of Endomyxa was based on non-exhaustive SSU analyses, which did not include the Foraminifera and Radiozoa. When these sequences are added, the SSU phylogenies give a much more complex image of relationships at the base of the Rhizaria.

Foraminifera either branch with Haplosporidia and Gromida (Nikolaev et al., 2004; Berney et al., 2004) or as a sister group to the Polycystinea (Cavalier-Smith and Chao, 2003). The branching of Foraminifera with the Haplosporidia was considered as a long branch attraction artifact caused by the fast evolving stem lineage of foraminiferal SSU rDNA (Cavalier-Smith and Chao, 2003). However, even if the haplosporidian SSU sequences evolve faster than other Cercozoa, this is certainly not the case of *Gromia*. Its position close to Foraminifera has been suggested by some analyses of the SSU (Berney and Pawlowski, 2003), as well as the analyses of the largest subunit of the RNA polymerase II (Longet et al., 2003, 2004) and actin (Longet et al., 2004; Flakowski et al., 2005, 2006), although the presence of two actin paralogues in Foraminifera complicates its interpretation in the latter case. A recent multigene analysis of the reticulate plasmodial protist *Corallomyxa tenera* placed this new species in a clade that includes *Gromia*, Haplosporidia and Foraminifera, hence reinforcing the phylogenetic hypotheses of a close relationship among these taxa (Tekle et al., 2007)

An alternative hypothesis concerning the phylogenetic relationships at the base of the Rhizaria suggests that Foraminifera are closely related to the Radiozoa. Branching of both groups in some SSU rDNA trees led to the creation of an infrakingdom or phylum Retaria (Cavalier-Smith 1999). In fact, this relationship seems to be strongly influenced by rapid evolutionary rates in ribosomal genes of Foraminifera and some Radiozoa, particularly the Polycystinea. Foraminifera branch with the Radiozoa, as a sister group to the Polycystinea, in some SSU trees (Cavalier-Smith and Chao, 2003 a,b), and in combined analyses of SSU and LSU data with limited taxon sampling (Moreira et al., 2007). However, this relationship is absent from many other broadly sampled SSU trees (Berney et al., 2004; Nikolaev et al., 2004; Tekle et al., 2007).

The main difficulty in resolving the relationships between Radiozoa and other Rhizaria is the lack of radiolarian protein sequences. Only three actin sequences for two species of Polycystinea are available (*Collozoum inerme* and *Thalassicolla pellucida*). These three fast evolving sequences usually (Nikolaev et al., 2004; Longet et al., 2004), but not always (Flakowski et al., 2005), branch as sister groups to foraminiferan actin paralogue II. A recent analysis of broad taxon sampling of rhizarian actins shows that the polycystinean and foraminiferan paralogue II actins form a clade with one paralogue of haplosporidian actins; the other haplosporidian paralogue groups with Plasmodiophoridae, as a sister group to the clade grouping *Gromia*, *Corallomyxa* and the

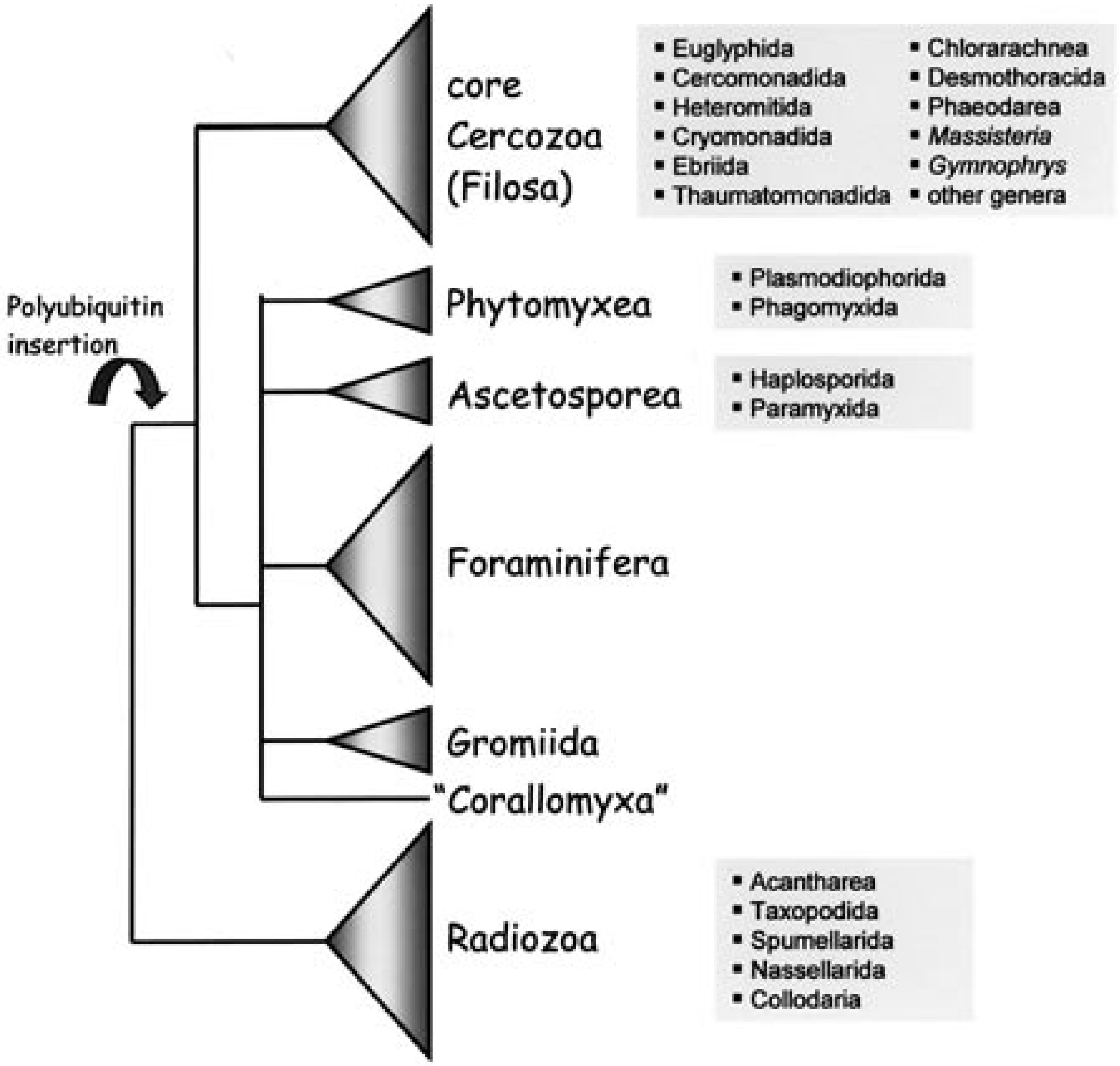

Fig. 2. Hypothetical consensus tree of Rhizaria.

foraminiferan paralogue I (Tekle et al., 2007). This suggests that two actin paralogues exist not only in Foraminifera but also in Haplosporidia. Whether this is the case also in Radiozoa is unknown. The unclear position of polycystinean actins and the apparent absence of amino acid insertion in polyubiquitin of Polycystinea and Acantharea (Bass et al., 2005) provide evidence against the taxonomic grouping of Radiozoa and Foraminifera; however, further protein coding genes analyses are necessary to test the Retaria hypothesis.

A hypothetical consensus tree of the Rhizaria is illustrated in Fig. 2. This supergroup has been tentatively divided into three major groups: the core Cercozoa (Filosa), the clade Phytomyxea (Plasmodiophorida, Phagomyxida) + Ascetosporea (Haplosporida, Paramyxida) + Gromiida + *Corallomyxa* + Foraminifera, and the Radiozoa. At least nine clades have been distinguished among core Cercozoa, modified from the classification of Cavalier-Smith and Chao (2003). Many other taxa belonging to core Cercozoa have been presented as independent lineages. There is one monospecific lineage (*Corallomyxa*) next to Gromiida, but the number of lineages branching in this part of the rhizarian tree is certainly much larger (Bass, pers. comm.; Lecroq, work in progress). Finally, the Radiozoa are represented by five clades (Acantharea, Taxopodida, Spumellarida, Nassellarida, Collodaria), in agreement with the polyphyly of the Polycystinea suggested by Kunitomo et al. (2006).

As in the case of Amoebozoa, the main weakness of rhizarian phylogeny is its basically single-gene character. Until now, the protein-coding gene data were mainly used to confirm the monophyly of the group. Apart from actin and ubiquitin, no protein sequences are available for most of the groups, particularly the Radiolaria. The EST data are currently available only for two Foraminifera (*Reticulomyxa*, *Quinqueloculina*), the chlorarachniophyte *Bigelowiella natans* and two cercozoans *Gymnophrys* and *Cercomonas*. The sequencing of the genome of *Bigelowiella* is in progress and that of *Reticulomyxa* is in preparation (Table 2).

Other amoeboid lineages

There are at least five groups of amoeboid protists, traditionally included in Sarcodina, that now branch outside the supergroups of Amoebozoa and Rhizaria. Among them, there are two orders of Heliozoa (Actinophryida and Centrohelida), one class of Lobosea (Heterolobosea), one genus of Filosea (*Nuclearia*) and the species *Breviata anathema* (ex. *Mastigamoeba invertens*) initially considered as Lobosea and then reclassified as the only representative of the new class Breviatea (Cavalier-Smith et al., 2004). In molecular phylogenies, the Heterolobosea, Actinophryida and Nucleariidae have been placed within excavates, stramenopiles and opisthokonts, respectively, while the Centrohelida and Breviatea branch independently.

HETEROLOBOSEA

This group is characterized by eruptive flow of cytoplasm in the amoeboid stage, discoidal mitochondrial cristae and the absence of typical dictyosomes. Based on these ultrastructural features, the Heterolobosea were removed from the Lobosea and placed in their own class (Page and Blanton, 1985; Page, 1987). The independent origin of Heterolobosea was confirmed by early ribosomal phylogenies (Clark and Cross, 1988). Further molecular studies increased the taxon sampling of Heterolobosea by including other Vahlkampfiidae (Hinkle and Sogin, 1993) and *Acrasis* (Keeling and Doolittle, 1996). They also revealed a weakly supported relationship between Heterolobosea and Euglenozoa in the SSU rDNA trees (Simpson, 2003), a cluster that is more strongly supported by analyses of combined protein data (Baldauf et al., 2000). Both Heterolobosea and Euglenozoa share the unusual "discoidal" mitochondrial cristae and have been grouped in the taxon Discicristata, based on rDNA and protein sequence data (Keeling and Doolittle, 1996; Cavalier-Smith, 2002; Baldauf et al., 2003). However, the accuracy of this grouping was recently questioned by multigene phylogenies, which suggest that the Heterolobosea are closely related to jakobids possessing typical tubular or flattened cristae (Simpson et al., 2006).

ACTINOPHRYIDA

This order of heliozoans is composed of only two genera (*Actinosphaerium* and *Actinophrys*) that possess a typical heliozoan morphology. Their ultrastructure shows important differences compared to other heliozoans, and some cellular structures are similar to those found in pedinellids (Smith and Patterson, 1986; Mikrjukov and Patterson, 2001). The first molecular evidence confirming the affinities between actinophryids and stramenopiles was based on SSU and actin gene sequences (Nikolaev et al., 2004). However, because of an extremely fast evolving SSU sequence of *Actinosphaerium eichornii* and limited taxon sampling of stramenopile actin genes, these data could not firmly establish the position of the Actinophryida. A recent SSU phylogeny of Chromista suggested that actinophryids are sister group to Opalozoa (Cavalier-Smith and Chao, 2006), but the support for this relationship was very weak and could be the result of a long branch attraction artefact.

NUCLEARIIDAE

The nucleariids are filose amoebae traditionally classified within the Filosea (Levine et al., 1980). Unexpectedly, the first SSU-based molecular analyses found them to branch among the Opisthokonts (Amaral-Zettler et al., 2001). This position was confirmed by multigene analyses, including EF1α, actin, HSP70, α-tubulin, and β-tubulin (Steenkamp et al., 2006). In all these analyses, the Nucleariidae appear as a sister group to the Fungi, and this relationship is confirmed by the presence in *Nuclearia* of a well conserved insertion in EF1A, typical for all Opisthokonta.

CENTROHELIDA

This group differs from other heliozoans by the presence of flat mitochondrial cristae and a centroplast from which the axopodial microtubules arise (Febvre-Chevalier, 1990). The phylogenetic position of the Centrohelida has been investigated by analyses of SSU (Cavalier-Smith and Chao, 2003), SSU + actin (Nikolaev et al., 2004), actin + α-tubulin + β-tubulin (Sakaguchi et al., 2005; Takishita et al., 2005), SSU + LSU (Moreira et al., 2007), and a multigene analysis including actin + α-tubulin + β-tubulin + EF2 + HSP70 + HSP90 genes (Sakaguchi et al., 2007). In spite of this considerable sequencing effort, the phylogenetic position of the Centrohelida remains unclear. There is no evidence for their affinity to any of the recognized eukaryotic supergroups. In a seven gene analysis, the Centrohelida are placed as a sistergroup to the clade uniting Chromalveolates and Plantae (Sakaguchi et al., 2007). A similar position was found in combined SSU and actin analysis (Nikolaev et al., 2004), while the SSU alone placed them as a sister group to Haptophytes (Cavalier-Smith and Chao, 2003) or as a sister group to the Rhodophyta (Sakaguchi et al., 2005). None of these positions is statistically supported. Most probably, the Centrohelida are an independent lineage of eukaryotes, a hypothesis that should be tested with larger scale multi-gene analyses and larger taxon sampling.

BREVIATEA

The class Breviatea was introduced by Cavalier-Smith et al. (2004) for the enigmatic free-living amoeboflagelate *Mastigamoeba invertens.* A lack of phylogenetic affinity between *M. invertens* and other mastigamoebids (Bolivar et al., 2001; Milyutina et al., 2001) suggested that the species was misidentified (Edgcomb et al., 2002; Cavalier-Smith et al., 2004). It has been recently redescribed as *Breviata anathema*, gen. n., sp. n., based on a light-microscopical and ultrastructural study (Walker et al., 2006). The phylogenetic position of *B. anathema* at the base of Amoebozoa in some SSU trees (Bolivar et al., 2001), and its putative single basal body prompted its inclusion in the Amoebozoa (Cavalier-Smith et al., 2004). However, most SSU analyses placed *B. anathema* among Bikonts, often as a sister group to some Apusozoa (Cavalier-Smith and Chao, 2003; Simpson, 2003; Nikolaev et al., 2004; Walker et al., 2006), while RNA polymerase II trees placed it at the base of all other eukaryotes (Stiller et al., 1998; Longet et al., 2003).

Concluding remarks

The demise of the traditional system of Sarcodina is a logical consequence of the replacement of a "convenient" protist classification by a new macrosystem based on molecular phylogenetic hypotheses. There is certainly nothing unexpected in this taxonomic reshuffling. The polyphyly of sarcodinids was suggested by many protistologists from the pre-molecular era. The macrotaxa such as Sarcodina, Rhizopoda, Actinopoda and Heliozoa survived for more than a century mainly because there was no alternative to the convenient morphology-based system. The advances of molecular phylogenies not only confirmed their obsolete character but also provided an opportunity to replace them by new groupings of eukaryotes.

It is interesting to notice, however, that the traditional grouping of amoeboid protists based on their pseudopodial characteristics might be partially right. Although practically all traditional macrotaxa have been shown to be polyphyletic, many amoeboid groups persist in a slightly modified form. For example, the majority of lobosean amoebae remain grouped within the Amoebozoa, while the filosean and reticulosean amoebae are mainly found among the Rhizaria. Even if these supergroups also contain some flagellated species, many of them possess the capacity to develop pseudopodia. Although almost all other major eukaryotic groups, with the notable exception of plants and alveolates, include at least one amoeboid lineage. In most cases these lineages represent minor taxonomic groups of one or a few genera and their phylogenetic position is often difficult to establish.

The ability of protists to produce pseudopods is certainly a universal feature of eukaryotes. However, it seems that this ability was fully expressed only in Amoebozoa and Rhizaria, which contain the most diversified assemblage of amoeboid protists. Despite a certain criticism of the taxonomic status of these two supergroups (Parfrey et al., 2006), the rapidly accumulating phylogenomic data continuously reinforce the support for these, *par excellence*, amoeboid assemblages. The monophyly of Amoebozoa and Rhizaria seems indisputable, even if there are still some uncertainties concerning their final composition, in particular the phylogenetic position of some basal lineages and some yet unsequenced taxa.

Today, the principal challenge is to establish the phylogenetic relationships within Amoebozoa and Rhizaria and to understand the genomic basis for the development of different patterns of pseudopodial form and movement. This can be achieved only by substantially increasing the genomic data on major components of both supergroups. New EST and genome sequencing projects are necessary to search for genomic signatures that could help to infer the root of each supergroup and to determine the evolutionary steps leading to the formation of different types of pseudopods. In particular, a more extensive study of actin, myosins, tubulins and other proteins involved in pseudopodial movement and their interactions may help to better understand the evolution of both amoeboid supergroups.

Another challenge is to describe more accurately the diversity of Amoebozoa and Rhizaria. The study of uncultured isolates and environmental samples showed an unexpectedly high diversity of some amoeboid groups, such as monothalamous foraminifera (Pawlowski et al., 2002, 2003), Cercozoa (Bass and Cavalier-Smith, 2004), Euglyphida (Lara et al., 2007) or Radiozoa (Not et al., 2007). However, many amoeboid groups, for example, the lobose amoebae and foraminifera, are rarely recovered from environmental sampling (Berney et al., 2004). This is partly due to the difficult amplification of their highly divergent ribosomal genes, and can be overcome by using specific PCR primers (Habura et al., 2004). More specific search for amoebozoans and rhizarians in environmental DNA surveys, as well as more extensive molecular studies of uncultured amoeboid taxa, will certainly reveal many new, undescribed species, which may change our view on their diversity and evolution.

Acknowledgements

This paper is dedicated to the memory of Kirill A. Mikrjukov, who performed an extensive research on the phylogeny of “heliozoa” and inspired my interest in the evolution of this group. I thank Cédric Berney, Fabien Burki, Anna Maria Fiore-Donno, José Fahrni, Alexander Kudryavtsev and Robert Peck for comments and discussion. The Swiss National Science Foundation is acknowledged for the generous support to my research, through grants 3100-064073.00 and 3100A0-112645, and SCOPES Joint Research Projects (7SUPJ062342 and IB73A0-111064).

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