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Amoeboid protist systematics: A report on the "Systematics of amoeboid protists" symposium at the VIIIth ECOP/ISOP meeting in Rome, 2019

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Abstract

Amoeboid protists are extremely abundant and diverse in natural systems where they often play outstanding ecological roles. They can be found in almost all major eukaryotic divisions, and genomic approaches are bringing major changes in our perception of their deep evolutionary relationships. At fine taxonomic levels, the generalization of barcoding is revealing a considerable and unsuspected specific diversity that can be appreciated with careful morphometric analyses based on light and electron microscopic observations. We provide examples on the difficulties and advances in amoeboid protists systematics in a selection of groups that were presented at the VIIIth ECOP/ISOP meeting in Rome, 2019. We conclude that, in all studied groups, important taxonomical rearrangements will certainly take place in the next few years, and systematics must be adapted to incorporate these changes. Notably, nomenclature should be flexible enough to integrate many new high level taxa, and a unified policy must be adopted to species description and to the establishment of types.

Keywords: Cercozoa; Lobose amoebae; Myxomycetes; Taxonomy; Testate amoebae

Introduction

Amoeboid protists (=amoebae) designate those unicellular eukaryotes that use pseudopods, mobile extensions of the cytoplasm supported by acto-myosin or tubulin cytoskeletal elements, for feeding and locomotion. Such organisms can be found in practically all environments on Earth that are accessible to eukaryotic life, from deep sea to desert soil including hydrothermal, hyperacidic, anoxic and endobiotic habitats. They are mostly phagotrophic, and are generally associated with substrates such as sediment and soil aggregates where they use their pseudopodia for anchoring and foraging on diverse prey items. They can be very abundant, especially in soil where they reach abundances up to 10^5 individuals per gram of soil (Adl and Gupta, 2006). Their predatory action on bacteria and other eukaryotes is crucial in the cycling of nutrients in soil ecosystems and impacts *in fine* on fertility and plant production (Geisen et al., 2018). Environmental molecular diversity surveys suggest the existence

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of an immense diversity in some groups at least (Geisen et al., 2014a; Harder et al., 2016; Lecroq et al., 2011; Lejzerowicz et al., 2010). This contrasts with the relatively limited number of species described, that has been estimated to about 13,500 (Adl et al., 2007); interestingly, the groups that host the largest organisms, that is, Foraminifera, Arcellinida and Myxomycetes/Dictyostelids represent together 94% of all described species. The difficulty of observation and the lack of obvious external morphological features in many taxa prevented for a long time the establishment of a taxonomic frame for amoebae (Smirnov et al., 2011). Nowadays, however, the combination of barcoding and genomic data with ultrastructural traits is currently overcoming this obstacle both for deep nodes and fine, species level taxonomy. It is well-known now that environmental diversity of protists is immense and certainly overtakes that of animals and plants in many terrestrial (Fiore-Donno et al., 2019a; George et al., 2019; Mahé et al., 2017) and marine habitats (de Vargas et al., 2015). Classifying the immense, "nearly imponderable" diversity of protists (including amoebae) (Foissner, 1999) appears therefore as a prerequisite to characterize the organisms that take part in the many ecosystemic processes that are responsible for the maintenance of the biosphere. It is also necessary to characterize the evolutionary pathways followed by the eukaryotes that make up the bulk of the current diversity (Burki et al., 2020).

Amoeboid protists had been grouped within the Superclass Rhizopoda (lobose, heterolobose, filose, and reticulose amoebae, and mycetozoans), which, together with the Superclass Actinopoda (radiolarians and heliozoans), formed the Subphylum Sarcodina (Levine et al., 1980). But the onset of molecular data, from single gene to genome-wide have completely changed this classification (Pawlowski and Burki, 2009) as it is now known that amoebae are scattered almost all over the whole eukaryotic tree.

Integrating the newly described diversity into the system

These changes in amoeba classification reshuffled eukaryotes systematics, revealing new clades (see Kang et al., 2017 for instance) which often shifted hierarchical ranks and caused the redefinition of taxa. A practical solution was offered by the using the Phylocode, which defines taxa based on phylogenetic position and thus avoids name changes and rank shifting when a new clade is defined (Cantino and de Queiroz, 2020). The use of a rankless nomenclature has been favored by protistologists (Adl et al., 2005, 2012) due to the instability of eukaryotic phylogeny, although attempts of ranking taxa have been made recently because of a stabilization of deep eukaryotic nodes (Adl et al., 2019). The establishment of ranks depends therefore on the state of knowledge in the groups; in the case of amoeboid protists, researchers adopted a consensual attitude, which integrates linnean and phylogenetic nomenclatures. As an example, the Phylocode was used for higher hierarchical levels within Amoebozoa (Kang et al., 2017) while Arcellinida were treated as an order, followed by nested hierarchical levels such as suborders and infraorders (Lahr et al., 2019), thus keeping a maximum of nomenclatural stability.

Another element that has been subject to debate is the nature of types in Amoebozoan systematics. Types are at the very core of Linnean systematics, as they serve as references for species. As (sexual) eukaryotes (Lahr et al., 2011; Hofstatter and Lahr, 2019), the concept of species in amoebae does not change fundamentally from the larger and better-known plants and animals; a species being the most exclusive phylogenetic unit where individuals share a common evolutionary history. It is therefore logical that typification follows the same rules. The description of any new species must be accompanied by a type that must be accessible to the other researchers; botanists for instance have access to herbaria containing dry material, from which DNA can be extracted after decades. For most amoebae, however, there is no straightforward solution. The ideal method would certainly be preserving living strains in a culture collection. Yet, many amoeboid protists cannot be kept in stable, monoprotistan cultures. Moreover, according to the provisions of the Codes of nomenclature, the type material must have a potential to be kept forever. This is often not the case for the cultures that can get degraded or lost not only due to negligence, but also for intrinsic reasons that are not entirely understood. Fixed material can alternatively be made available to researchers online, as with Eugène Penard's own collection at the Natural History Museum of Geneva (https://commons.wikimedia.org/wiki/Commons:Penard_project). These specimens are kept in Canada balsam on microscope slides, and cannot be observed with electron microscopy, a prerequisite to the identification of many amoeboid species (Kudryavtsev and Smirnov, 2006; Lara et al., 2007). For these reasons, we recommend the use of micrographs as name-bearing types as suggested for protists by (Duszynski, 1999), accompanied if needed with good line drawings. Because of the extensive cryptic diversity found in protists in general, we also strongly recommend barcoding newly described species using an appropriate marker as detailed in Pawlowski et al. (2012). A precise description of the *terra typica* of the new species and the original biotope (vegetation, climate, local parameters like pH) should be provided with all new descriptions.

Based on the communications that have been given during the symposium "Systematics of amoeboid protists" during the VIIIth ECOP/ISOP meeting in Rome, August 2nd 2019, we are giving here an overview of the latest advances and difficulties in the systematics of four chosen groups: Arcellinida, Myxomycetes, Vannellida and Himatismenida (Amoebozoa; Fig. 1) and shell-bearing Cercozoa (Rhizaria; Fig. 2). Our aim was not to provide an updated version of the classification of amoeboid protists, as it has been recently reviewed in (Adl et al., 2019) and other specialized literature. Instead, we

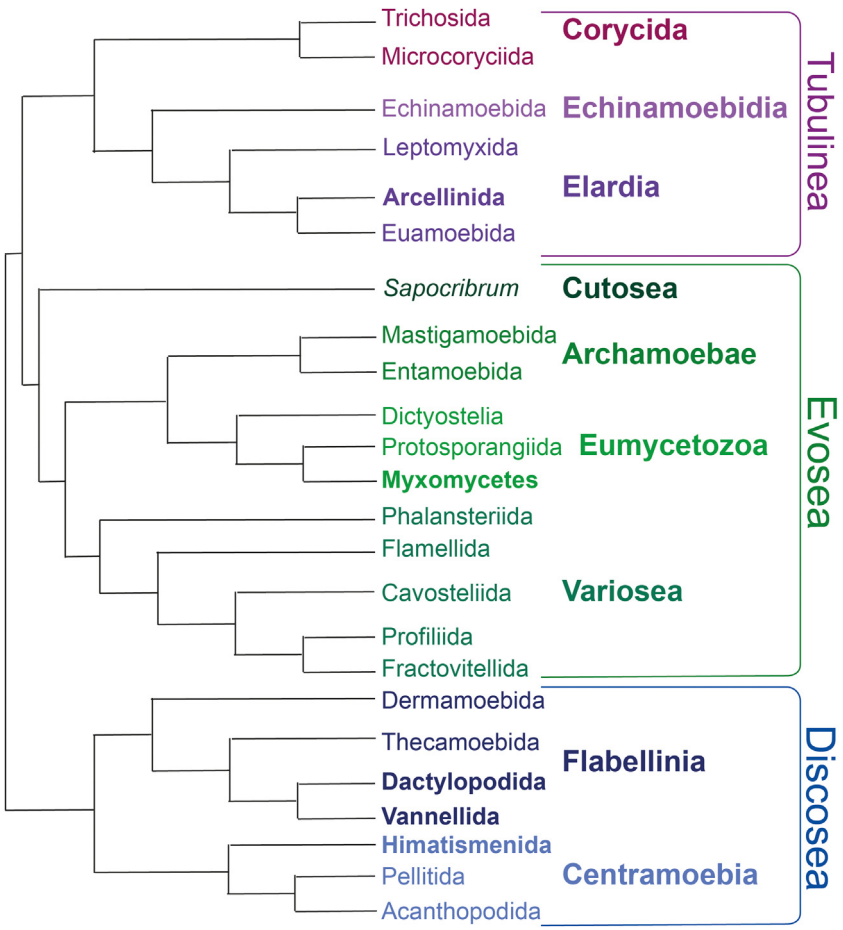

Fig. 1. Schematic tree of the Amoebozoa showing the main subdivisions. The taxa documented in this study are shown in bold.

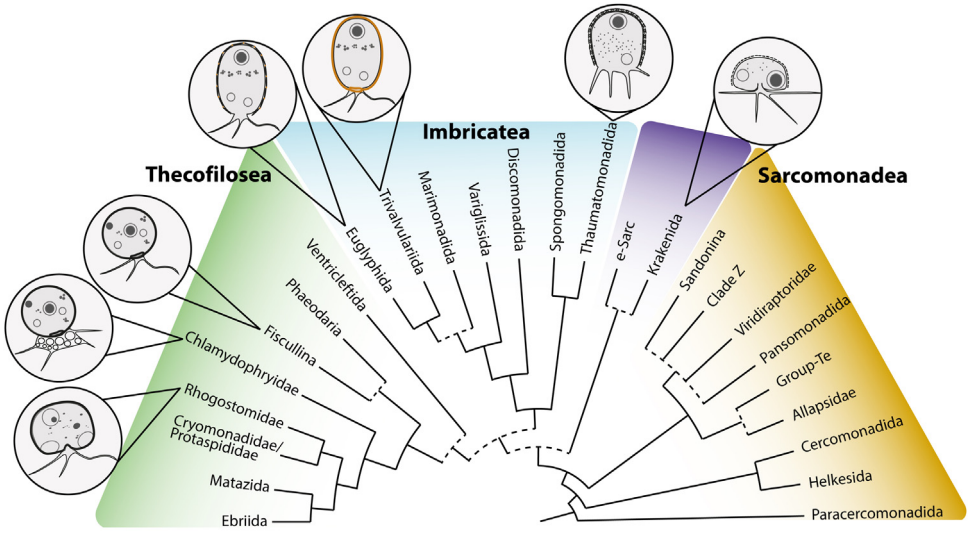

Fig. 2. Overview of cercozoan (Rhizaria; Cercozoa; Filosa) diversity. Illustrations show shell-bearing amoeboid taxa. The branching of the tree follows a consensus of current cercozoan phylogenies. Drawings are not in scale.

wished to highlight common issues in (amoeboid) protist systematics based on selected examples, and propose solutions to overcome these difficulties.

Arcellinida, a mega diverse group that emerged before the animals

Arcellinida, the lobose testate amoebae, are a group of terrestrial and freshwater protists that build a test made of protein that is in most taxa reinforced with mineral elements that can be either self-secreted or taken up from the environment. Contrary to the previous opinion that there is a major basal dichotomy of lobose amoebae between naked amoebae (Gymnamoebia) and testate amoebae (Testacealobosia, including Arcellinida) (Page, 1987), the first molecular studies have shown that Arcellinida were nested within naked lobose amoebae (Nikolaev et al., 2005). Arcellinida pre-molecular deep systematics has been rapidly challenged by the first molecular phylogenetic studies based on SSU rRNA gene, as both most species-rich genera *Nebela* (Lara et al., 2008) and *Difflugia* (Gomaa et al., 2012) were shown to be paraphyletic. In general, overall test shape proved to be a more reliable trait for broad phylogeny than test composition, in disagreement with the traditional taxonomy (Meisterfeld, 2002). In contrast, pseudopodia shape (blunt vs. conical) was retained as a major criterion, as it permitted the separation of Phryganellina from all other Arcellinida (Dumack et al., 2019). However, although the enrichment of the molecular database with new sequences improved the topology of the arcellinid tree, the long branches formed by certain taxa (Gomaa et al., 2017; Kudryavtsev et al., 2009) prevented the construction of a robust phylogenetic tree based solely on SSU rRNA gene sequences. A conserved mitochondrial marker that has been recently proposed to resolve deep nodes seems perform well, but still needs to be applied to a broader taxonomic sampling (Blandenier et al., 2017; Macumber et al., 2020). The application of single cell transcriptomics performed optimally in resolving the deepest nodes of Arcellinida (Lahr et al., 2019). Interestingly, the morphological reconstruction of the ancestral shell shapes based on knowledge from modern Arcellinida clades corresponded exactly with vase-shaped microfossils dated back from the Neoproterozoic (750 MYA), before the first animals appeared (Lahr et al., 2019). The diversity of shapes encountered in these fossils suggested that many functional types of Arcellinida were already present back then and, as almost all arcellinids are voracious microbial eukaryote predators, indicates that their protistan preys were already diversified before the first animals had appeared. Still, many genera have not been characterized molecularly, and enriching current dataset, especially with genomic data will be needed to set the backbone of the Arcellinida tree.

While deep phylogenetics aim at unravelling the first events in eukaryotic life, "shallow taxonomy" concerns species-level diversity, which is the basic unit for measuring diversity. While an approach to species delimitation based on the mayrean biological species concept (Mayr, 1942) cannot be applied easily to unculturable protists, a "three headed" approach has been proposed to discriminate individuals when morphological discontinuities correspond to genetic divergence and differing ecological preferences. Practically, this requires the use of a genetic marker that can discriminate at the species level; the first subunit of the mitochondrial cytochrome oxidase, a classical barcoding marker widely used for animals (Hebert et al., 2003) has been proven useful for certain Amoebozoa (Nassonova et al., 2010). The application of this marker to Hyalospheniformes (arguably the best known clade of Arcellinida) revealed a considerable genetic diversity within practically every morphospecies investigated (Kosakyan et al., 2012). After careful investigation, it appeared that genetically divergent forms could be separated based on morphology, at least in genus *Nebela* (Kosakyan et al., 2013). The species originally described as *Nebela collaris* (Ehrenberg), *N. flabellulum* Leidy, 1876 and *N. tincta* (Leidy, 1879) were found to fit the original descriptions, including the typical environments and the *terra typica*. Based on its position in the phylogenetic tree, *Nebela tincta rotunda* was given species rank; other forms needed to be described as new species (Kosakyan et al., 2013). A survey of the distribution of these organisms across the microniches of two peatbogs showed that each form had diverging ecological optima (Singer et al., 2018), thus fulfilling the "three headed" approach for species delimitation (Fig. 3). However, in other genera like *Hyalosphenia*, extensive phenotypic plasticity seems to hide any possible traits that could be used to discriminate species; test size and pore numbers are more correlated to local hydro-regime and climate than to genetics (Mulot et al., 2017). Nevertheless, the 13 genetic lineages that constitute the morphospecies *Hyalosphenia papilio* have different geographic distribution areas (Heger et al., 2013), a situation that can be explained by historical reasons (Singer et al., 2019). This *cryptic* diversity confirms the need for a multi-focus approach to species delimitation. Furthermore, the illustrative examples of genera *Nebela* and *Hyalosphenia* show that a considerable specific diversity still awaits to be described within Arcellinida, notably in less-known groups and in undersampled areas such as Eastern Asia (Qin et al., 2016) or the Mesoamerican corridor (Perez-Juarez et al., 2017).

Type material in Arcellinida consists in tests preserved in Canada balsam or on metallized stubs for observation with scanning electron microscope.

Molecular systematics revolutionize Myxomycete taxonomy

Myxomycetes, also known as plasmodial slime molds, are a diverse assemblage of amoeboid protists characterized by

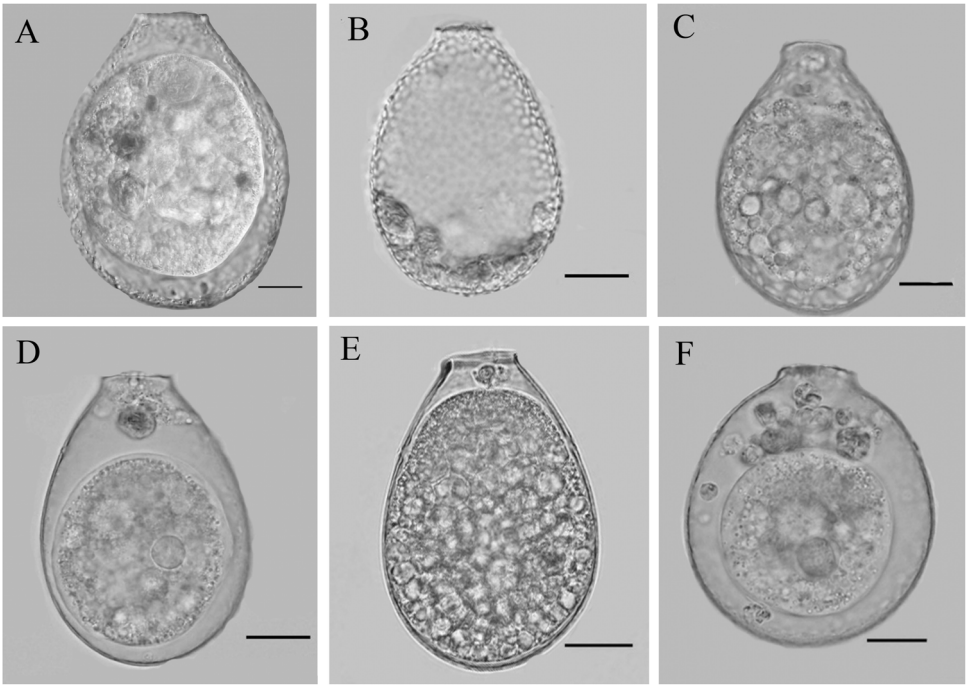

Fig. 3. Illustration of members of the genus *Nebela* inhabiting European peatlands. These species differ only slightly in morphology, are genetically distinct, (A) *N. collaris*, a generalist species; (B) *N. gimlii*, found in forested peatlands under relatively dry conditions; (C) *N. guttata* found on nutrient-poor hummocks; (D) *N. pechorensis* from forests with relatively high nitrogen content; (E) *N. tincta*, also from hummocks; (F) *N. rotunda*, also a typical forest species. Scale bar = 10 μm. Credit: photos by Anush Kosakyan.

a complex lifecycle that includes, among other stages, free living amoebae and flagellate cells feeding on soil microorganisms, and macroscopic spore-bearing structures, called sporophores. Since the nomenclature of Myxomycetes Link 1833 is specifically covered by the International Code of Nomenclature for algae, fungi, and plants ICN, and most species have been also described under that code, we advocate for following it to preserve name stability (Lahr et al., 2012), although most Amoebozoa fall within the International Code of Zoological Nomenclature ICZN (Adl et al., 2019).

Given that neither amoeboid nor flagellated life stages present obvious distinguishing characters, Myxomycete taxonomy largely relies on sporophore morphology, but it remains unclear how far the traits commonly used to separate groups are real synapomorphies that can be used among natural clades. Indeed, intergeneric morphological differences can be very subtle and some species present features that bridge the gaps among different genera or even higher ranked clades, thus casting doubt on the validity of the whole taxonomy (Eliasson, 2017; Walker and Stephenson, 2016).

In the last two decades, there have been multiple attempts to shed light on the phylogeny of Myxomycetes using SSU rRNA gene sequences, or these combined to EF-1α gene data (Cainelli et al., 2020; Erastova et al., 2013.; Fiore-Donno et al., 2012, 2013, 2010, 2019b; Kretzschmar et al., 2016; Strelow et al., 2020). According to their results, three of the five orders traditionally recognized (Martin and Alexopoulos, 1969; Nannenga-Bremekamp, 1991) are artificial, and several genera are either para- or polyphyletic (Fig. 4). Nevertheless, and despite that Myxomycetes stand among the first protists molecularly characterized (Baldauf and Doolittle, 1997; Spiegel et al., 1995), a highly robust and comprehensive phylogeny for this group, and in particular for Physarales, does not exist due to the insufficient gene and species sampling used so far (Fiore-Donno et al., 2013; García-Martín et al., 2018).

To address this issue, the evolutionary relationships of Myxomycetes have been investigated by using the first and only representative collection of transcriptome data generated for these organisms to date (García-Martín et al., unpublished data). The preliminary results of this ongoing study are promising and undoubtedly prove that the class Myxomycetes, the subgroups Amaurosporales and Lamprosporales (Lister, 1925), and both orders Physarales and Trichiales are natural groups, confirming the validity of their respective synapomorphies to define high-level relationshipsGarcía-Martín et al., 2019).

Furthermore, the internal relationships of Physarales, a group that has previously received little research attention despite its tremendous morphological and ecological diversity, have been analyzed using a four-gene dataset from an expanded taxon sampling (García-Martín et al., unpublished data). It includes the type species of most genera ascribed to Physarales, a *sine qua non* for proposing taxonomic changes,

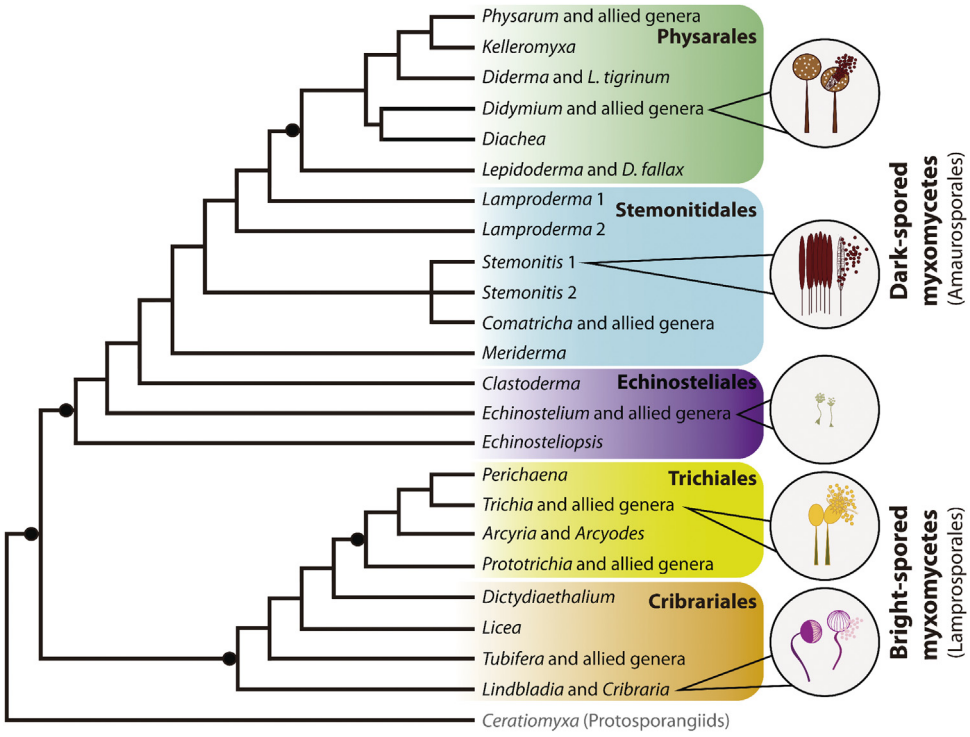

Fig. 4. Schematic representation of the Myxomycete phylogenetic tree, summarizing previous phylogenies and illustrating the non-monophyly of several genera. Black dots represent fully supported nodes.

Fig. 5. Sporophores of the species *Diachea leucopodia* (Bull.) Rostaf., the type species of genus *Diachea*. Scale bar = 0.5 mm. Credit: photo by Carlos de Mier.

and provides evidence for pervasive non-monophyly at familiar and generic levels, with only the multispecies genus *Diachea* Fr. (Fig. 5) being valid as currently circumscribed (García-Martín et al., 2019). It has also been evidenced that morphological homoplasy is exceedingly common so, on their own, most phenotypic traits traditionally used to define the genera, such as the type of sporophore and capillitium, the presence of clustered spores, the degree of calcification of some structures, etc. (Keller et al., 2017), are of limited use as taxonomic characters.

Taxonomic issues are not dealt with here, but it is important to note that para- and polyphyly are particularly rampant in Physaraceae. To solve this problem, two approaches could be used: (1) merging all genera together into a single genus or (2) splitting Physaraceae into well-supported monophyletic entities, recognizable based on a unique combination of phenotypic characters. The first strategy does not seem to be advisable as the resulting genus would be morphologically highly diverse, thus hardly diagnosable, and much nomenclatural changes would be required. Therefore, following the second approach, several species have been transferred to other genera, the resurrection of generic names for those clades comprising the type of a synonymized genus has been considered and molecular signatures have been proposed for each clade as diagnostic traits, given the scarcity of non-homoplastic morphological synapomorphies, as in (Sheikh et al., 2018).

Both unpublished studies presented in Rome provide new insights into the phylogeny, taxonomy and systematics of Myxomycetes, and introduce considerable changes to the genus-level taxonomy of Physarales. They also stress the importance of data acquisition and implementation of new methodologies, such as transcriptomics, while encourage further molecular research on these topics. However, phylogenetic relationships among and within Myxomycetes are far from being fully resolved. Given the size of this group and that sequence data are not available for many type species, generic boundaries are highly uncertain and some taxonomic problems still remain.

Only by combining additional multigene phylogenies, that must include types, with thorough morphological, ecological and ultrastructure studies we will improve our understanding of the systematics of these elusive protists.

Type material in Myxomycetes consists in dried fructifications that are preserved as herbarium material. Spores can be obtained from this material and revived after decades.

Hidden diversity in the tiny species of naked lobose amoebae

While the two groups detailed before include large sized organisms with conspicuous morphologies, naked (non-testate) amoebae always posed one of the most difficult challenges for protist taxonomists, especially those taxa that include the smallest representatives. At the very beginning of the foundation of lobose amoebae taxonomy based on reproducible morphological traits (Schaeffer, 1926), an opinion was dominating that about 75% of all known naked amoebae species could be easily classified based on morphology, while the remaining 25% were too small and too poorly studied for this (Schaeffer, 1926). After the basis of the classification was set by Schaeffer, the taxonomy has been developing through the 20th century by descriptions of more different taxa and a broad application of electron microscopy in hope to reveal more permanent characters that might prompt for phylogenetic relationships (Bovee and Sawyer, 1979; Page, 1987). However, the first molecular phylogenetic studies of amoebae showed that many structural characters had lost traces of phylogenetic signal, therefore, classifications created before wide application of molecular phylogeny turned out to be artificial in many taxonomic levels (Smirnov et al., 2011). With the introduction of molecular phylogenetics and phylogenomics, the classification has been significantly reshaped (Cavalier-Smith et al., 2015; Kang et al., 2017) showing unprecedented levels of diversity which contrast with the low numbers of species described. However, currently available research tools provide significant opportunities for correlating the observed molecular diversity with morphological innovations that exist even in the smallest species of amoebae. Given the decreasing costs of the phylogenomics data collection, the tendency emerges, to significantly rely on molecular data, and neglect the morphological evidence as the latter requires much more time-consuming investigations than sequencing.

The small amoebae from the order Vannellida are a typical example of a group where diversity has been overlooked (Kudryavtsev, 2014). Our molecular analysis based on the SSU rRNA gene showed the existence of at least six genus-level clades of marine and freshwater small vannellids (Kudryavtsev, unpublished). At the first glance, these amoebae are extremely poor in morphological characters, and may look indistinguishable (Fig. 6). However, some of these forms differ deeply in their ecological requirements (Kudryavtsev, unpublished), which justifies entirely their description as separate taxonomical entities. Careful light microscopic observation of their locomotive forms shows significant differences between these clades. To observe these, one needs to collect statistical data of the frequency of different shapes the cell takes during locomotion under standard observation conditions (Fig. 7). This requires the analysis of large numbers of active cells, a time-consuming task. However, the application of this approach highlights morphological differences between different taxa clades otherwise poor in morphological characters.

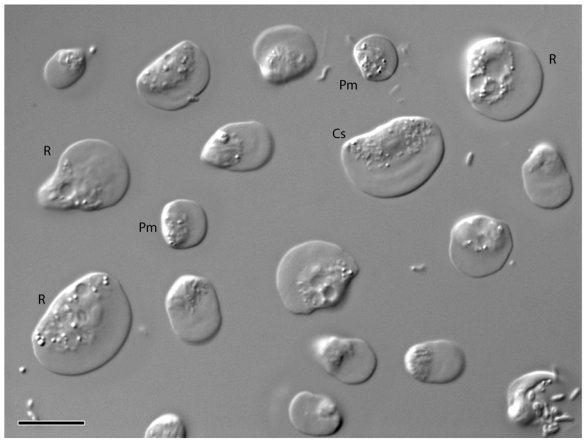

Fig. 6. Light micrographs (DIC) of the locomotive forms of various small members of Vannellida, including *Paravannella minima* (Pm) Kudryavtsev, 2014, *Ripella* spp. (R), *Clydonella sawyeri* (Cs) Kudryavtsev and Volkova, 2018, and several still unnamed genera. All to scale; scale bar = 10 μm. Credit: photos by Alexander Kudryavtsev.

Morphological and molecular diversity are related in a similar way in the order Himatismenida. Here, negligence to the electron microscopic characters may lead to a significant underestimation of the taxonomic diversity (Geisen et al., 2014b). On the other hand, there are some truly cryptic species that do not show any morphological differences and can only be distinguished by sequence data and ecological preferences, are known among naked amoebae. For example, the dactylopodid genus *Cunea* (Kudryavtsev and Pawlowski, 2015) appears to consist of at least three cryptic species differing in their temperature and salinity optima (Kudryavtsev and Volkova, 2020). In summary, classifying the smallest amoeboid protists requires, in all cases, a combination of molecular, observational and morphological data to correctly describe a species. For these reasons, **type material** from naked lobose amoebae ideally consists in cultures that are deposited in culture collections and several types of permanently stored preparations for the case if the culture is lost. Ideally, slides preserved in Canada balsam, an epoxy resin embedding for the transmission electron microscopy, and a purified DNA sample should be included in a type series whenever possible. In

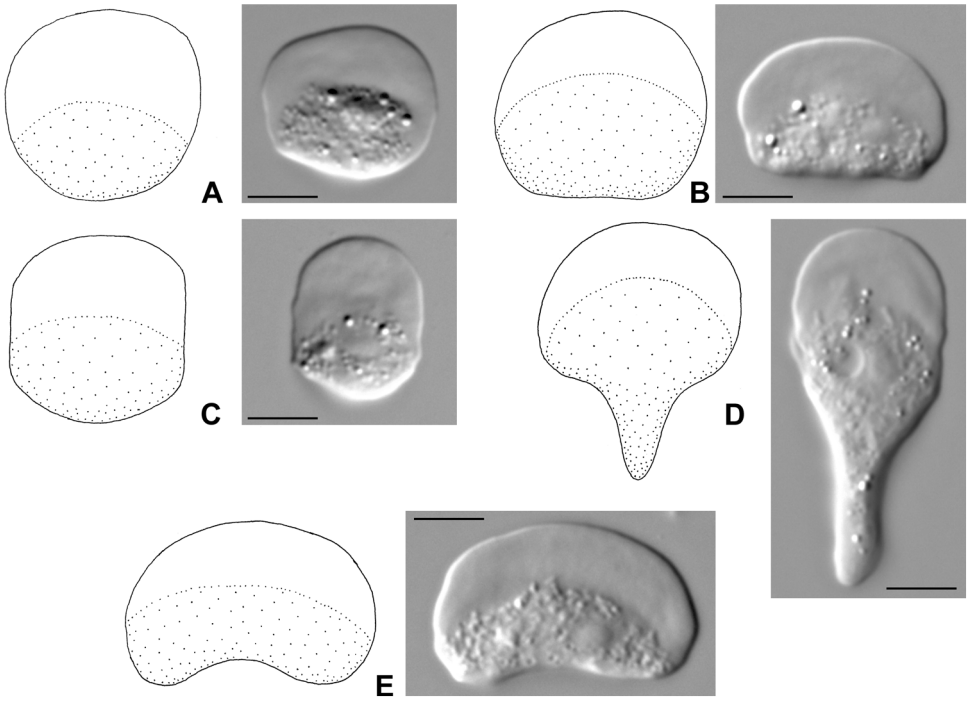

Fig. 7. Several examples of the generalized shapes of the locomotive forms in a single species of vannellid amoeba (A–E). A generalized scheme to the left, and a corresponding micrograph of a living cell (*Clydonella sawyeri* Kudryavtsev and Volkova, 2018 is taken as an example) to the right. Scale bar = 5 μm in all micrographs. Credit: photos by Alexander Kudryavtsev.

order to document at best the different locomotive forms, a video record can be made available publicly (for the example see https://www.youtube.com/channel/UCRK3mXipI41tytxPpIC1qXQ). The practice of preserving specimens only in Canada balsam on microscope slides, as was often the case with some earlier described species should be considered suboptimal.

Phylogeny and evolution of Cercozoan testate amoebae

Cercozoa belong to the eukaryotic supergroup Rhizaria together with the large and conspicuous foraminiferans and radiolarians. While the latter groups exhibit a characteristic morphology, Cercozoa display a wide diversity of shapes and have only been shown to be closely related by molecular analyses. While most diversity is composed of naked flagellates and amoeboflagellates, several taxa bear a shell. The phylogenetic position of the testate taxa with respect to other groups is shown on Figure 2.

The best known group of cercozoan testate amoebae are the Euglyphida, whose tests bear ornamented scales. While the phylogeny of large species is now relatively well-known (Chatelain et al., 2013), there is a rich diversity of forms that are known only through environmental sequencing (Lara et al., 2016). The characteristic shape of the shells is taxonomically diagnostic (Couteaux et al., 1979), which has helped considerably in developing the systematics of this groups. However, the systematics of other groups is still relatively unclear; "filose thecate amoebae are still (almost) uncharted territory" (Kosakyan et al., 2016). These organisms may play nevertheless a fundamental role in soil ecology: *Rhogostoma*, a genus of previously barely investigated testate amoebae, have been shown to exhibit tremendous abundance. Their abundance correlates with the abundance of algae (Seppey et al., 2017) on which members of this genus feed (Dumack et al., 2017a) accordingly species of *Rhogostoma* can possibly be considered as probably the most abundant algivorous organisms in soil (Seppey et al., 2017).

Shells in Cercozoa exhibit a tremendous diversity, starting from organic layers covering the cell membrane to highly elaborate shells reinforced either with xenosomes (i.e. foreign materials taken from the environment) or self-built silica scales (Dumack and Siemensma, 2020; Dumack et al., 2018a, 2018b; Scoble and Cavalier-Smith, 2014). Most shelled taxa in Cercozoa belong to Imbricatea and Thecofilosea, but the relationships between these taxa could not be assessed firmly yet. However, there is evidence that suggests a sister group relationship between these two groups which may have evolved from a scale-bearing ancestor (Cavalier-Smith et al., 2018; Dumack et al., 2017b). However, based on molecular data from both described and novel taxa (Siemensma and Dumack, 2020) it appeared that imbricatean and thecofilosean shells evolved independently. Their respective positions within Cercozoa with respect to other clades remain to be confirmed, both by expanding taxon and gene sampling.

Type material in Cercozoan testate amoebae consists in cultures, metallized specimens for scanning electron microscopy and fixed specimens on microscope slides.

Conclusions

The groups that have been illustrated here represent only a fraction of the immense diversity of amoeboid organisms that can be found in different environments. They include organisms that diverge deeply in their sizes, lifestyles, ecologies and behaviors, and have been studied by researchers stemming from totally different research traditions. In all groups, molecular phylogeny has completely revolutionized systematics. In particular, the use of multigene/genomic data has been instrumental in these changes but, perhaps even more importantly, the expansion of the number of taxa surveyed has been essential in the construction of robust phylogenetic trees. At fine taxonomic levels, single cell barcoding has considerably contributed to the knowledge of species diversity. Yet, integrating this knowledge to build new systematics needs an adaptation of nomenclatural codes to integrate the newly revealed groups; nomenclatural stability should be sought as much as possible. The process of description should be straightforward enough to make possible the treatment of the thousands of unknown species, but should be rigorous enough so that species can be found back by future researchers. It is crucial that all taxonomically relevant features (which vary among groups) are documented, but also the *terra typica* and the type of environment where the organisms have been found. For this reasons, traditional "protistological" skills and knowledge are still timely for the researchers working with amoeboid protists, and need to be taught to the next generation of researchers.

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References

Adl, S., Leander, B., Archibald, J., Anderson, O.R., 2007. Diversity, nomenclature, and taxonomy of protists. Syst. Biol. 56, 684–689.

Adl, S.M., Bass, D., Lane, C.E., Lukes, J., Schoch, C.L., Smirnov, A., Agatha, S., Berney, C., Brown, M.W., Burki, F., Cardenas, P., Cepicka, I., Chistyakova, L., Del Campo, J., Dunthorn, M., Edvardsen, B., Eglit, Y., Guillou, L., Hampl, V., Heiss, A.A., Hoppenrath, M., James, T.Y., Karnkowska, A., Karpov, S., Kim, E., Kolisko, M., Kudryavtsev, A., Lahr, D.J.G., Lara, E., Le Gall, L., Lynn, D.H., Mann, D.G., Massana, R., Mitchell, E.A.D., Morrow, C., Park, J.S., Pawlowski, J.W., Powell, M.J., Richter, D.J., Rueckert, S., Shadwick, L., Shimano, S., Spiegel, F.W., Torruella, G., Youssef, N., Zlatogursky, V., Zhang, Q., 2019. Revisions to the classification, nomenclature, and diversity of eukaryotes. J. Eukaryot. Microbiol. 66, 4–119.

Adl, S.M., Gupta, V.V.S.R., 2006. Review: protists in soil ecology and forest nutrient cycling. Can. J. For. Res. 36, 1805–1817.

Adl, S.M., Simpson, A.G.B., Farmer, M.A., Andersen, R.A., Anderson, O.R., Barta, J.R., Bowser, S.S., Brugerolle, G., Fensome, R.A., Fredericq, S., James, T.Y., Karpov, S., Kugrens, P., Krug, J., Lane, C.E., Lewis, L.A., Lodge, J., Lynn, D.H., Mann, D.G., McCourt, R.M., Mendoza, L., Moestrup, O., Mozley-Standridge, S.E., Nerad, T.A., Shearer, C.A., Smirnov, A.V., Spiegel, F.W., Taylor, M., 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. J. Eukaryot. Microbiol. 52, 399–451.

Adl, S.M., Simpson, A.G.B., Lane, C.E., Lukes, J., Bass, D., Bowser, S.S., Brown, M.W., Burki, F., Dunthorn, M., Hampl, V., Heiss, A., Hoppenrath, M., Lara, E., le Gall, L., Lynn, D.H., McManus, H., Mitchell, E.A.D., Mozley-Stanridge, S.E., Parfrey, L.W., Pawlowski, J., Rueckert, S., Shadwick, L., Schoch, C.L., Smirnov, A., Spiegel, F.W., 2012. The revised classification of eukaryotes. J. Eukaryot. Microbiol. 59, 429–514.

Baldauf, S.L., Doolittle, W.F., 1997. Origin and evolution of the slime molds (Mycetozoa). Proc. Natl. Acad. Sci. U. S. A. 94, 12007.

Blandenier, Q., Seppey, C.V.W., Singer, D., Vlimant, M., Simon, A., Duckert, C., Lara, E., 2017. *Mycamoeba gemmipara* nov gen., nov sp., the first cultured member of the environmental dermamoebidae clade LKM74 and its unusual life cycle. J. Eukaryot. Microbiol. 64, 257–265.

Bovee, E.C., Sawyer, T.K., 1979. Marine Flora and Fauna of the Northeastern United States. Protozoa: Sarcodina: Amoebae. In: NOAA Technical Report. National Marine Fisheries Service, US Department of Commerce, Washington.

Burki, Fabien, Roger, Andrew J., Brown, Matthew W., Simpson, Alastair G.B., et al., 2020. The New Tree of Eukaryotes. Trends Ecol. Evol. 35, 43–55.

Cainelli, R., de Haan, M., Meyer, M., Bonkowski, M., Fiore-Donno, A.M., 2020. Phylogeny of Physarida (Amoebozoa, Myxogastria) based on the small-subunit ribosomal RNA gene, redefinition of *Physarum pusillum* s. str. and reinstatement of *P. gravidum* Morgan. J. Eukaryot. Microbiol. 67, 327–336.

Cantino, P.D., de Queiroz, Kevin, 2020. In: Press, C. (Ed.), International Code of Phylogenetic Nomenclature (PhyloCode), Version 6. CRC Press, Boca Raton, FL, USA.

Cavalier-Smith, T., Chao, E.E., Lewis, R., 2018. Multigene phylogeny and cell evolution of chromist infrakingdom Rhizaria: contrasting cell organisation of sister phyla Cercozoa and Retaria. Protoplasma 255, 1517–1574.

Cavalier-Smith, T., Fiore-Donno, A.M., Chao, E., Kudryavtsev, A., Berney, C., Snell, E.A., Lewis, R., 2015. Multigene phylogeny resolves deep branching of Amoebozoa. Mol. Phylogenet. Evol. 83, 293–304.

Chatelain, A.P., Meisterfeld, R., Roussel-Delif, L., Lara, E., 2013. Sphenoderiidae (fam. nov.), a new clade of euglyphid testate amoebae characterized by small, round scales surrounding the aperture. Protist 164, 782–792.

Couteaux, M.M., Munsch, A., Ponge, J.F., 1979. Genus *Euglypha* — essay of numeric taxonomy. Protistologica 15, 565–579.

de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahe, F., Logares, R., Lara, E., Berney, C., Le Bescot, N., Probert, I., Carmichael, M., Poulain, J., Romac, S., Colin, S., Aury, J.M., Bittner, L., Chaffron, S., Dunthorn, M., Engelen, S., Flegontova, O., Guidi, L., Horak, A., Jaillon, O., Lima-Mendez, G., Lukes, J., Malviya, S., Morard, R., Mulot, M., Scalco, E., Siano, R., Vincent, F., Zingone, A., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Tara Oceans, C., Acinas, S.G., Bork, P., Bowler, C., Gorsky, G., Grimsley, N., Hingamp, P., Iudicone, D., Not, F., Ogata, H., Pesant, S., Raes, J., Sieracki, M.E., Speich, S., Stemmann, L., Sunagawa, S., Weissenbach, J., Wincker, P., Karsenti, E., 2015. Ocean plankton. Eukaryotic plankton diversity in the sunlit ocean. Science 348, 1261605.

Dumack, K., Bonkowski, M., Clauss, S., Volcker, E., 2018a. Phylogeny and redescription of the testate amoeba *Diaphoropodon archeri* (Chlamydophryidae, Thecofilosea, Cercozoa), De Saedeleer 1934, and annotations on the polyphyly of testate amoebae with agglutinated tests in the Cercozoa. J. Eukaryot. Microbiol. 65, 308–314.

Dumack, K., Flues, S., Hermanns, K., Bonkowski, M., 2017a. Rhogostomidae (Cercozoa) from soils, roots and plant leaves (*Arabidopsis thaliana*): description of *Rhogostoma epiphylla* sp. nov. and *R. cylindrica* sp. nov. Eur. J. Protistol. 60, 76–86.

Dumack, K., Kahlich, C., Lahr, D.J.G., Bonkowski, M., 2019. Reinvestigation of *Phryganella paradoxa* (Arcellinida, Amoebozoa) Penard 1902. J. Eukaryot. Microbiol. 66, 232–243.

Dumack, K., Mylnikov, A.P., Bonkowski, M., 2017b. Evolutionary relationship of the scale-bearing *Kraken* (incertae sedis, Monadofilosa, Cercozoa, Rhizaria): combining ultrastructure data and a two-gene phylogeny. Protist 168, 362–373.

Dumack, Kenneth, Siemensma, Ferry, 2020. Shell Colour in Cercozoa; a Simple Trait to Distinguish Thecofilosea from Imbricatea? Protist 171 (2), http://dx.doi.org/10.1016/j.protis.2020.125718.

Dumack, K., Siemensma, F., Bonkowski, M., 2018b. Rediscovery of the testate amoeba genus *Penardeugenia* (Thaumatomonadida, Imbricatea). Protist 169, 29–42.

Duszynski, D.W., 1999. Revisiting the code: clarifying name-bearing types for photomicrographs of protozoa. J. Parasitol. 85, 769–770.

Eliasson, U.H., 2017. Review and remarks on current generic delimitations in the myxomycetes, with special emphasis on *Licea*, *Listerella* and *Perichaena*. Nova Hedwigia 104, 343–350.

Erastova, D.A., Okun, M.V., Novozhilov, Y.K., Schnittler, M., 2013. Phylogenetic position of the enigmatic myxomycete genus *Kelleromyxa* revealed by SSU rDNA sequences. Mycol. Prog. 12, 599–608.

Fiore-Donno, A.M., Clissmann, F., Meyer, M., Schnittler, M., Cavalier-Smith, T., 2013. Two-gene phylogeny of bright-spored Myxomycetes (slime moulds, superorder Lucisporidia). PLoS One 8, e62586.

Fiore-Donno, A.M., Kamono, A., Meyer, M., Schnittler, M., Fukui, M., Cavalier-Smith, T., 2012. 18S rDNA phylogeny of *Lamproderma* and allied genera (Stemonitales, Myxomycetes, Amoebozoa). PLoS One 7, e35359.

Fiore-Donno, A.M., Nikolaev, S.I., Nelson, M., Pawlowski, J., Cavalier-Smith, T., Baldauf, S.L., 2010. Deep phylogeny and evolution of slime moulds (Mycetozoa). Protist 161, 55–70.

Fiore-Donno, A.M., Richter-Heitmann, T., Degrune, F., Dumack, K., Regan, K.M., Marhan, S., Boeddinghaus, R.S., Rillig, M.C., Friedrich, M.W., Kandeler, E., Bonkowski, M., 2019a. Functional traits and spatio-temporal structure of a major group of soil protists (Rhizaria: Cercozoa) in a temperate grassland. Front. Microbiol. 10.

Fiore-Donno, A.M., Tice, A.K., Brown, M.W., 2019b. A non-flagellated member of the Myxogastria and expansion of the Echinosteliida. J. Eukaryot. Microbiol. 66, 538–544.

Foissner, W., 1999. Protist diversity: estimates of the near-imponderable. Protist 150, 363–368.

García-Martín, J.M., Mosquera, J., Lado, C., 2018. Morphological and molecular characterization of a new succulenticolous *Physarum* (Myxomycetes, Amoebozoa) with unique polygonal spores linked in chains. Eur. J. Protistol. 63, 13–25.

García-Martín, Joaquina M., Zamora, Juan Carlos, Lado, Carlos, 2019. Disentangling the Systematics of the Order Physarales (Myxomycetes, Amoebozoa): an Integrated Approach Using Morphology and Multiple Gene Data. VIIIth ECOP/ISOP meeting, Rome.

Geisen, S., Fiore-Donno, A.M., Walochnik, J., Bonkowski, M., 2014a. *Acanthamoeba* everywhere: high diversity of *Acanthamoeba* in soils. Parasitol. Res. 113, 3151–3158.

Geisen, S., Kudryavtsev, A., Bonkowski, M., Smirnov, A., 2014b. Discrepancy between species borders at morphological and molecular levels in the genus *Cochliopodium* (Amoebozoa, Himatismenida), with the description of *Cochliopodium pluri-inucleolum* n. sp. Protist 165, 364–383.

Geisen, S., Mitchell, E.A.D., Adl, S., Bonkowski, M., Dunthorn, M., Ekelund, F., Fernández, L.D., Jousset, A., Krashevska, V., Singer, D., Spiegel, F.W., Walochnik, J., Lara, E., 2018. Soil protists: a fertile frontier in soil biology research. FEMS Microbiol. Rev. 42, 293–323.

George, P.B.L., Lallias, D., Creer, S., Seaton, F.M., Kenny, J.G., Eccles, R.M., Griffiths, R.I., Lebron, I., Emmett, B.A., Robinson, D.A., Jones, D.L., 2019. Divergent national-scale trends of microbial and animal biodiversity revealed across diverse temperate soil ecosystems. Nat. Commun. 10, 1107.

Gomaa, F., Lahr, D.J.G., Todorov, M., Li, J., Lara, E., 2017. A contribution to the phylogeny of agglutinating Arcellinida (Amoebozoa) based on SSU rRNA gene sequences. Eur. J. Protistol. 59, 99–107.

Gomaa, F., Todorov, M., Heger, T.J., Mitchell, E.A.D., Lara, E., 2012. SSU rRNA Phylogeny of Arcellinida (Amoebozoa) reveals that the largest arcellinid genus, *Difflugia* Leclerc 1815, is not monophyletic. Protist 163, 389–399.

Harder, C.B., Ronn, R., Brejnrod, A., Bass, D., Al-Soud, W.A., Ekelund, F., 2016. Local diversity of heathland Cercozoa explored by in-depth sequencing. ISME J. 10, 2488–2497.

Hebert, P.D.N., Ratnasingham, S., de Waard, J., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. Biol. Sci. 270 (Suppl 1), S96–99.

Heger, T.J., Mitchell, E.A.D., Leander, B.S., 2013. Holarctic phylogeography of the testate amoeba *Hyalosphenia papilio* (Amoebozoa: Arcellinida) reveals extensive genetic diversity explained more by environment than dispersal limitation. Mol. Ecol. 22, 5172–5184.

Hofstatter, P.G., Lahr, D.J.G., 2019. All eukaryotes are sexual, unless proven otherwise: many so-called asexuals present meiotic machinery and might be able to have sex. Bioessays 41, 9.

Kang, S., Tice, A.K., Spiegel, F.W., Silberman, J.D., Panek, T., Cepicka, I., Kostka, M., Kosakyan, A., Alcantara, D.M.C., Roger, A.J., Shadwick, L.L., Smirnov, A., Kudryavtsev, A., Lahr, D.J.G., Brown, M.W., 2017. Between a pod and a hard test: the deep evolution of amoebae. Mol. Biol. Evol. 34, 2258–2270.

Keller, H.W., Everhart, S.E., Kilgore, C.M., 2017. The Myxomycetes: introduction, basic biology, life cycles, genetics, and reproduction. In: Stephenson, S.L., Rojas, C. (Eds.), Myxomycetes. Biology, Systematics, Biogeography, and Ecology. Academic Press, pp. 1–40.

Kosakyan, A., Gomaa, F., Lara, E., Lahr, D.J., 2016. Current and future perspectives on the systematics, taxonomy and nomenclature of testate amoebae. Eur. J. Protistol. 55, 105–117.

Kosakyan, A., Gomaa, F., Mitchell, E.A.D., Heger, T.J., Lara, E., 2013. Using DNA-barcoding for sorting out protist species complexes: a case study of the *Nebela tincta-collaris-bohemica* group (Amoebozoa; Arcellinida, Hyalospheniidae). Eur. J. Protistol. 49, 222–237.

Kosakyan, A., Heger, T.J., Leander, B.S., Todorov, M., Mitchell, E.A.D., Lara, E., 2012. COI Barcoding of nebelid testate amoebae (Amoebozoa: Arcellinida): extensive cryptic diversity and redefinition of the Hyalospheniidae Schultze. Protist 163, 415–434.

Kretzschmar, M., Kuhnt, A., Bonkowski, M., Fiore-Donno, A.M., 2016. Phylogeny of the highly divergent Echinosteliales (Amoebozoa). J. Eukaryot. Microbiol. 63, 453–459.

Kudryavtsev, A., 2014. *Paravannella minima* n. g. n. sp (Discosea, Vannellidae) and distinction of the genera in the vannellid amoebae. Eur. J. Protistol. 50, 258–269.

Kudryavtsev, A., Pawlowski, J., 2015. *Cunea* n. g. (Amoebozoa, Dactylopodida) with two cryptic species isolated from different areas of the ocean. Eur. J. Protistol. 51, 197–209.

Kudryavtsev, A., Pawlowski, J., Hausmann, K., 2009. Description and phylogenetic relationships of *Spumochlamys perforata* n. sp. and *Spumochlamys bryora* n. sp. (Amoebozoa, Arcellinida). J. Eukaryot. Microbiol. 56, 495–503.

Kudryavtsev, A., Smirnov, A., 2006. *Cochliopodium gallicum* n. sp. (Himatismenida), an amoeba bearing unique scales, from cyanobacterial mats in the Camargue (France). Eur. J. Protistol. 42, 3–7.

Kudryavtsev, A., Volkova, E., 2020. *Cunea russae* n. sp. (Amoebozoa, Dactylopodida), another cryptic species of *Cunea* Kudryavtsev and Pawlowski, 2015, inhabits a continental brackish-water biotope. Eur. J. Protistol. 73, 125685.

Lahr, D.J.G., Kosakyan, A., Lara, E., Mitchell, E.A.D., Morais, L., Porfirio-Sousa, A.L., Ribeiro, G.M., Tice, A.K., Pánek, T., Kang, S., Brown, M.W., 2019. Phylogenomics and morphological reconstruction of Arcellinida testate amoebae highlight diversity of microbial eukaryotes in the Neoproterozoic. Curr. Biol. 29, 991–1001.e1003.

Lahr, D.J.G., Lara, E., Mitchell, E.A.D., 2012. Time to regulate microbial eukaryote nomenclature. Biol. J. Linn. Soc. 107, 469–476.

Lahr, D.J.G., Parfrey, L.W., Mitchell, E.A.D., Katz, L.A., Lara, E., 2011. The chastity of amoebae: re-evaluating evidence for sex in amoeboid organisms. Proc. R. Soc. B: Biol. Sci. 278, 2081–2090.

Lara, E., Heger, T.J., Ekelund, F., Lamentowicz, M., Mitchell, E.A.D., 2008. Ribosomal RNA genes challenge the monophyly of the Hyalospheniidae (Amoebozoa: Arcellinida). Protist 159, 165–176.

Lara, E., Heger, T.J., Mitchell, E.A.D., Meisterfeld, R., Ekelund, F., 2007. SSU rRNA reveals a sequential increase in shell complexity among the Euglyphid testate amoebae (Rhizaria: Euglyphida). Protist 158, 229–237.

Lara, E., Roussel-Delif, L., Fournier, B., Wilkinson, D.M., Mitchell, E.A.D., 2016. Soil microorganisms behave like macroscopic organisms: patterns in the global distribution of soil euglyphid testate amoebae. J. Biogeogr. 43, 520–532.

Lecroq, B., Lejzerowicz, F., Christen, R., Esling, P., Baerlocher, L., Osteras, M., Farinelli, L., Pawlowski, J., 2011. Ultra-deep sequencing of foraminiferal microbarcodes unveils hidden richness of early monothalamous lineages in deep-sea sediments. Proc. Natl. Acad. Sci. U. S. A. 108, 13177–13182.

Lejzerowicz, F., Pawlowski, J., Fraissinet-Tachet, L., Marmeisse, R., 2010. Molecular evidence for widespread occurrence of Foraminifera in soils. Environ. Microbiol. 12, 2518–2525.

Levine, N.D., Corliss, J.O., Cox, F.E., Deroux, G., Grain, J., Honigberg, B.M., Leedale, G.F., Loeblich 3rd, A.R., Lom, J., Lynn, D., Merinfeld, E.G., Page, F.C., Poljansky, G., Sprague, V., Vavra, J., Wallace, F.G., 1980. A newly revised classification of the protozoa. J. Protozool. 27, 37–58.

Lister, A., 1925. A monograph of the Mycetozoa: A descriptive catalogue sof the Species in the Herbarium of the British Museum, third edition. order of the trustees, London.

Macumber, A.L., Blandenier, Q., Todorov, M., Duckert, C., Lara, E., Lahr, D.J.G., Mitchell, E.A.D., Roe, H.M., 2020. Phylogenetic divergence within the Arcellinida (Amoebozoa) is congruent with test size and metabolism type. Eur. J. Protistol., 72.

Mahé, F., de Vargas, C., Bass, D., Czech, L., Stamatakis, A., Lara, E., Singer, D., Mayor, J., Bunge, J., Sernaker, S., Siemensmeyer, T., Trautmann, I., Romac, S., Berney, C., Kozlov, A., Mitchell, E.A.D., Seppey, C.V.W., Egge, E., Lentendu, G., Wirth, R., Trueba, G., Dunthorn, M., 2017. Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. Nat. Ecol. Evol. 1, 0091.

Martin, G.W., Alexopoulos, C.J., 1969. The Myxomycetes. University of Iowa Press, Iowa City (United States of America).

Mayr, E., 1942. Systematics and the Origin of Species from the Viewpoint of a Zoologist. Columbia University Press, New York.

Meisterfeld, R., 2002. Order Arcellinida Kent, 1880. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.), The Illustrated Guide to the Protozoa. , second edition. Society of Protozoologists, Lawrence, Kansas, USA, pp. 827–860.

Mulot, M., Marcisz, K., Grandgirard, L., Lara, E., Kosakyan, A., Robroek, B.J.M., Lamentowicz, M., Payne, R.J., Mitchell, E.A.D., 2017. Genetic determinism vs. phenotypic plasticity in protist morphology. J. Eukaryot. Microbiol. 64, 729–739.

Nannenga-Bremekamp, N.E., 1991. A Guide to Temperate Myxomycetes. Biopress, Bristol (United Kingdom).

Nassonova, E., Smirnov, A., Fahrni, J., Pawlowski, J., 2010. Barcoding amoebae: comparison of SSU, ITS and COI genes as tools for molecular identification of naked lobose amoebae. Protist 161, 102–115.

Nikolaev, S.I., Mitchell, E.A.D., Petrov, N.B., Berney, C., Fahrni, J., Pawlowski, J., 2005. The testate lobose amoebae (order Arcellinida Kent, 1880) finally find their home within Amoebozoa. Protist 156, 191–202.

Page, F.C., 1987. The classification of 'naked' amoebae (phylum Rhizopoda). Archiv für Protistenkunde 133, 199–217.

Pawlowski, J., Audic, S., Adl, S., Bass, D., Belbahri, L., Berney, C., Bowser, S.S., Cepicka, I., Decelle, J., Dunthorn, M., Fiore-Donno, A.M., Gile, G.H., Holzmann, M., Jahn, R., Jirku, M., Keeling, P.J., Kostka, M., Kudryavtsev, A., Lara, E., Lukes, J., Mann, D.G., Mitchell, E.A.D., Nitsche, F., Romeralo, M., Saunders, G.W., Simpson, A.G.B., Smirnov, A.V., Spouge, J.L., Stern, R.F., Stoeck, T., Zimmermann, J., Schindel, D., de Vargas, C., 2012. CBOL Protist Working Group: barcoding eukaryotic richness beyond the Animal, Plant, and Fungal Kingdoms. PLoS Biol. 10.

Pawlowski, J., Burki, F., 2009. Untangling the phylogeny of amoeboid protists. J. Eukaryot. Microbiol. 56, 16–25.

Perez-Juarez, H., Serrano-Vazquez, A., Kosakyan, A., Mitchell, E.A.D., Rivera Aguilar, V.M., Lahr, D.J.G., Hernandez Moreno, M.M., Cuellar, H.M., Eguiarte, L.E., Lara, E., 2017. *Quadrulella texcalense* sp. nov. from a Mexican desert: an unexpected new environment for hyalospheniid testate amoebae. Eur. J. Protistol. 61, 253–264.

Qin, Y.M., Man, B.Y., Kosakyan, A., Lara, E., Gu, Y.S., Wang, H.M., Mitchell, E.A.D., 2016. *Nebela jiuhuensis* nov. sp. (Amoebozoa; Arcellinida; Hyalospheniidae): a new member of the *Nebela saccifera - equicalceus - ansata* group described from *Sphagnum* peatlands in South-Central China. J. Eukaryot. Microbiol. 63, 558–566.

Schaeffer, A.A., 1926. Taxonomy of the Amoebas: With Descriptions of Thirty-nine New Marine and Freshwater Species. Carnegie Inst. Washington, Washington.

Scoble, J.M., Cavalier-Smith, T., 2014. Scale evolution, sequence phylogeny, and taxonomy of thaumatomonad Cercozoa: 11 new species and new genera *Scutellomonas*, *Cowlomonas*, *Thaumatospina* and *Ovaloplaca*. Eur. J. Protistol. 50, 270–313.

Seppey, C.V.W., Singer, D., Dumack, K., Fournier, B., Belbahri, L., Mitchell, E.A.D., Lara, E., 2017. Distribution patterns of soil microbial eukaryotes suggests widespread algivory by phagotrophic protists as an alternative pathway for nutrient cycling. Soil Biol. Biochem. 112, 68–76.

Sheikh, S., Thulin, M., Cavender, J.C., Escalante, R., Kawakami, S.I., Lado, C., Landolt, J.C., Nanjundiah, V., Queller, D.C., Strassmann, J.E., Spiegel, F.W., Stephenson, S.L., Vadell, E.M., Baldauf, S.L., 2018. A new classification of the dictyostelids. Protist 169, 1–28.

Siemensma, F., Dumack, K., 2020. SSU rDNA Phylogeny indicates the scale-lacking Trivalvulariida ord. nov. as a sister group to the Euglyphida (Cercozoa, Rhizaria). Protist 171, 125701.

Singer, D., Kosakyan, A., Seppey, C.V.W., Pillonel, A., Fernandez, L.D., Fontaneto, D., Mitchell, E.A.D., Lara, E., 2018. Environmental filtering and phylogenetic clustering correlate with the distribution patterns of cryptic protist species. Ecology 99, 904–914.

Singer, D., Mitchell, E.A.D., Payne, R.J., Blandenier, Q., Duckert, C., Fernandez, L.D., Fournier, B., Hernandez, C.E., Granath, G., Rydin, H., Bragazza, L., Koronatova, N.G., Goia, I., Harris, L.I., Kajukalo, K., Kosakyan, A., Lamentowicz, M., Kosykh, N.P., Vellak, K., Lara, E., 2019. Dispersal limitations and historical factors determine the biogeography of specialized terrestrial protists. Mol. Ecol. 28, 3089–3100.

Smirnov, A.V., Chao, E., Nassonova, E.S., Cavalier-Smith, T., 2011. A revised classification of naked lobose amoebae (Amoebozoa: Lobosa). Protist 162, 545–570.

Spiegel, F.W., Lee, S.B., Rusk, S.A., 1995. Eumycetozoans and molecular systematics. Can. J. Bot. Rev. Can. Bot. 73, S738–S746.

Strelow, D., de Haan, M., Bonkowski, M., Fiore-Donno, A.M., 2020. New insights into the phylogeny of the dark-spored Myxomycetes (Amoebozoa: Conosa: Myxogastria: Fuscisporidia) and polyphyly of the genus *Stemonitis*. System. Biodivers. 18, 228–236.

Walker, L.M., Stephenson, S.L., 2016. The species problem in Myxomycetes revisited. Protist 167, 319–338.