Evolution of Dictyostelid Social Amoebas Inferred from the Use of Molecular Tools

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Abstract Dictyostelid social amoebas are eukaryotic microbes distributed all around the globe. As with many other protist groups, one fundamental and revolutionary event in the study of dictyostelid (Amoebozoa) systematics has been the use of molecular tools. This has radically changed our understanding of evolution across the group and has greatly expanded the potential use of dictyostelids as model organisms for a wide range of areas including biomedicine, development, evolutionary biology, and molecular ecology. This is further supported by genome sequencing that has been carried out for at least one species in each of the major groups. Phylogenomic data are also essential to pinpointing the origin of diversification of dictyostelids in terrestrial ecosystems, which is basic for understanding the evolutionary history across eukaryotic amoeboid lineages.

Keywords Taxonomy · Protists · Amoebozoa · Soils · Molecular sequences · Molecular clocks

1 Introduction

Protist amoeboid species are difficult to differentiate based only on the morphology and to do this requires a high degree of taxonomic expertise (Caron et al. 2009). Therefore the advent of molecular tools has been a fundamental and revolutionary event in the study of protist systematics as a whole. It started with sequencing

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independent nuclear and mitochondrial molecular markers and continues now with the sequencing of whole genomes. Molecular data and phylogenetic analyses have been crucial in disentangling the phylogenetic position of different amoeboid species across the eukaryotic tree of life (Baldauf et al. 2000; Brown et al. 2009).

Strong convergent evolution is visible among morphological characters of several protist groups that are now known to belong to completely different groups in the eukaryotic tree of life (reviewed in Foissner 2006; Leander 2008). Among amoeboid protists, there are several groups that form multicellular sorocarps, called sorocarpic amoebas. These are scattered among the eukaryotic tree in six major supergroups (see Table 1). Historically, these organisms have been grouped together due to the similarity of their life cycles, but recently molecular phylogenetic analyses of sorocarpic amoebae have shown that these organisms have multiple independent origins (Adl et al. 2012; Brown et al. 2009, 2011). Important changes in the understanding of sorocarpic protist systematics can be illustrated by examples shown in Table 1, which are further described in Chap. 10 of this book.

Within Mycetozoa (Myxogastrea, Dictyostelia, Protostelia), a subgroup of the eukaryotic supergroup Amoebozoa, molecular tools, and more accurate phylogenetic analyses have been recently applied. This started with the phylogeny based on 18S rDNA and EF1a in Myxogastrids (Fiore-Donno et al. 2005, 2008) and was followed by molecular phylogenies of dictyostelid social amoeba based on 18S rDNA, alpha tubulin, and Internal Transcribed Spacer (nrITS) (Schaap et al. 2006; Romeralo et al. 2010a, 2011). Subsequently, phylogenetic analyses were also applied to the study of protostelids (Shadwick et al. 2009). These molecular phylogenies radically changed our previous vision of Mycetozoa systematics. Molecular phylogenies have confirmed that Myxogastrea and Dictyostelia form a monophyletic clade in Amoebozoa (Pawlowski and Burki 2009) while protostelids are highly polyphyletic. Within Mycetozoa, Myxogastrea are the most species-rich group (920 sps), followed by Dictyostelia (150 sps) and Protostelia (35 sps) (numbers from www.eumycetozoa.com).

Myxogastrea, or plasmodial slime molds, can be found in all terrestrial ecosystems and also some resistant stages have been found in aquatic habitats where they persist, but cannot form fruiting bodies (Stephenson et al. 2011). They present a complex life cycle with two vegetative stages: unicellular amoebas alternating with myxoflagellates and multinucleate plasmodia, culminating in the formation of fruiting bodies dispersing multiple spores. Myxogastrea have three dormant stages: microcysts, sclerotia, and spores and their life cycle can be sexually or asexually completed (Schnittler et al. 2012). On the other hand, Protostelia form very simple fruiting bodies. A single amoeba differentiates to form a single, stalked, sporocarp that is 5–500 μm tall and supports one or a few spores. Molecular studies (Fiore-Donno et al. 2010a; Lahr et al. 2011) have shown that protostelids are found in nearly every major branch of Amoebozoa, except in Tubulinea (Shadwick et al. 2009; Fiore-Donno et al. 2010a; Lahr et al. 2011).

Dictyostelid social amoebas, the subject of this book, were discovered in the nineteenth century and, because of their morphological similarity with the genus *Mucor*, were considered to be fungi and named *Dictyostelium mucoroides*.

Table 1 Species complexes across Dictyostelia

Species name	Group according to morphology	Molecular markers	Eukaryotic supergroup according to molecular data	References
Acrasis rosea	Acrasids	GAPDH, SSU + Protein coding genes	Excavate	Roger et al. (1996); Keeling and Doolittle (1996); Andersson and Roger (2002); Archibald et al. (2002); Nikolaev et al. (2004); Stechmann et al. (2006)
Guttulinopsis vulgaris	Acrasids	159 Protein dataset	Rhizaria	Brown et al. (2012)
Fonticula alba	Fungi	SSU, Actin, beta-tubulin, EF1-alpha, the cytosolic isoform of heat shock protein 70	Opisthokonta	Brown et al. (2009)
Sorogena stoianovitchae	Ciliates	Nuclear and mitochondrial SSU	Alveolate	Lasek-Nesselquist and Katz (2001); Dunthorn et al. (2008, 2011)
Sorodiplophrys stercorea	Ciliates	SSU	Stramenopiles	Baldauf et al. (2000)
Dictyostelium discoideum	Mycetozoa	SSU, ITS, Alpha tubulin	Amoebozoa	Schaap et al. (2006); Romeralo et al. (2010a, b)
Copromyxa protea	Acrasids	SSU	Amoebozoa	Brown et al. (2011)

Subsequently, some studies have demonstrated that the absence of hyphae, the presence of a cellulose wall and their aggregative behavior separates them from fungi or other groups of protists such as acrasids. Finally, the use of molecular tools such as 18S rDNA has helped to confidently place them within the eukaryotic supergroup Amoebozoa (Baldauf et al. 2000).

With phylogenetic relationships among eukaryotic groups becoming more and more resolved, analyses using molecular data are now possible to be performed. This will be of benefit for future studies on biogeography, comparative analysis, diversification rates, and reconstruction of divergence times. In fact, different approaches to reconstruct molecular clocks have been carried out recently, placing the origin of diversification of dictyostelids several hundred million years ago. Molecular tools have been key in developing this kind of analysis in groups like Dictyostelids where there is a complete absence of a fossil record.

2 Morphology Versus Molecular Data

Traditionally dictyostelid species were divided into three genera based on morphological characters: *Acytostelium* (acellular stalks), *Dictyostelium* (cellular stalks), and *Polysphondylium* (cellular stalks with whorls of branches) (Raper 1984). The first molecular phylogeny of Dictyostelia, based on two independent molecular markers (18S rDNA and alpha tubulin) divided the known species into four major groups, which do not correspond to any of the three traditional genera. Moreover, it showed a tremendous molecular depth roughly equivalent to metazoa. This phylogeny radically changed previous beliefs about evolution of form and function across the group (Schaap et al. 2006).

In 2007, Romeralo et al. demonstrated the suitability of the nrITS for assessing dictyostelid phylogenetics. Three years later a joint 18S rDNA–nrITS molecular phylogeny was produced for all species from the 2006 phylogeny, which resolved relationships to a finer level and revealed the presence of species complexes within all major morphotypes (*Acytostelium*, *Dictyostelium,* and *Polysphondylium*), (see Table 2, Romeralo et al. 2010a, b).

In 2009 molecular data (18S rDNA and nrITS sequences) were applied to confirmed species based on morphological characters. Since then, it has become standard for dictyostelid species to be described based on both morphological and molecular data (Romeralo et al. 2009, 2010a, b; Vadell et al. 2011; Cavender et al. 2013; Perrigo et al. 2013). This is helping enormously in the difficult task of resolving morphological species complex (cryptic species), across the group (e.g., *D. mucoroides*, *Polysphondylium pallidum*) (see Table 2).

In just the last 10 years the number of described species of Dictyostelia has been doubled. This is mainly due to an intensive sampling effort ("The Eumycetozoa project") to sample mycetozoa from different biomes across the world. In 2011 an updated molecular phylogeny based on 18S rDNA sequences was released (Romeralo et al. 2011). The new phylogeny continues to show the four previously

Table 2 Species of sorocarpic protists distributed among five major eukaryotic groups

Species name	Dictyostelid group	References	Molecular markers	Genome sequenced
Dictyostelium aureostipes	Group 1	Romeralo et al. (2010a, b)	18S rDNA, nrITS	Not
Dictyostelium fasciculatum	Group 1	Romeralo et al. (2010a, b)	18S rDNA, nrITS	Yes
Acytostelium leptosomum	Group 2A	Romeralo et al. (2011)	18S rDNA	Not
Acytostelium amazonicum	Group 2A	Romeralo et al. (2011)	18S rDNA	Not
Polysphondylium pallidum	Group 2B	Schaap et al. (2006); Romeralo et al. (2010a, b)	18S rDNA, nrITS	Yes
Dictyostelium tenue	Group 3	Romeralo et al. (2010a, b)	18S rDNA, nrITS	Yes
Dictyostelium sphaerocephalum	Group 4	Romeralo et al. (2007)	nrITS	Not
Dictyostelium discoideum	Group 4	Schaap et al. (2006); Romeralo et al. (2010a, b)	18S rDNA, nrITS	Yes
Dictyostelium mucoroides	Group 4	Schaap et al. (2006); Romeralo et al. (2010a, b)	18S rDNA, nrITS	Not
Dictyostelium polycarpum	Polycarpum Complex Group	Romeralo et al. (2011)	18S rDNA	Not
Dictyostelium polycephalum	Polycephalum Complex Group	Romeralo et al. (2011)	18S rDNA	Not
Polysphondylium violaceum	Violaceum Complex Group	Romeralo et al. (2011)	18S rDNA	Not

identified major groups. In addition, three previously isolated branches are now seen to form major divisions in their own right. They are formed by a lower number of isolates and provisionally named after the main species. We refer to these new groups as the "polycarpum", "polycephalum", and "violaceum" complexes in order to retain the original numbering scheme until formal names can be assigned. In addition the new species further emphasize the deep split in Group 2 recognizing two separate major groups, Group 2A and Group 2B.

Many of the species described in the last 10 years contradict the few morphological patterns tentatively identified within the first molecular phylogeny (Schaap et al. 2006) particularly for Groups 1 and 4 (e.g., some of the new species in Group 4 have clustered and coremiform sorocarps, presence of branches and polar granules inside their spores). Furthermore there is a high level of species complexes found throughout the Dictyostelia, and most morphotypes have not been examined from multiple isolates. However, since morphological patterns can be identified for more limited subgroups, morphological evolution seems to be at least moderately conservative. The lack of deeper morphological patterns should perhaps not be entirely unexpected given the small numbers of characters, the essentially simple nature of many of them, and the apparent antiquity of the group. The future discovery of additional species, together with extensive genome sequencing, should lead to a better understanding of the mechanisms and evolutionary forces shaping them.

In brief, Dictyostelids Group 1 is formed only by *Dictyostelium* morphotypes. Group 2A includes all acytostelid species except *A. ellipticum*, which belongs to Group 2B. Group 2B is a much more heterogeneous group with representatives of the three morphotypes. Group 3 is exclusively made up of *Dictyostelium* morphotypes as is Group 4, which is the richest species group and includes the model organism, *Dictyostelium discoideum.* The Polycarpum Complex Group, formed by two isolates of the morphotypes *Dictyostelium polycarpum,* whose 18S sequences are as different as any other two species in Group 4. The Polycephalum Complex Group includes five different isolates of the morphotypes *Dictyostelium polycephalum* and their 18S sequences are also extremely different. Finally the "Violaceum Complex" Group includes representatives from the *Dictyostelium* and the *Polysphondylium* morphotypes (Fig. 1). The new species revealed the presence of species complexes across the group (Table 2). Because of this inconsistency between phylogenetic groupings and the systematic naming of species in the group a major taxonomic revision of Dictyostelia is urgently needed.

Despite these advances, until recently we were still missing a very important point in the phylogenetic reconstructions: the position of the root. This is the oldest point in a phylogeny and therefore essential to infer evolutionary patterns over time. This issue has been recently addressed with the use of genomic data (Romeralo et al. 2013). Orthologs for 32 genes were retrieved from the six dictyostelid (Heidel et al. 2011) and three amoebozoan outgroup genomes and consensus alignments for the 32 encoded protein sequences were performed. The concatenated alignment of about 181,80 amino acids robustly placed the root between the branches of Groups 1–2 and 3–4 (Romeralo et al. 2013). In agreement
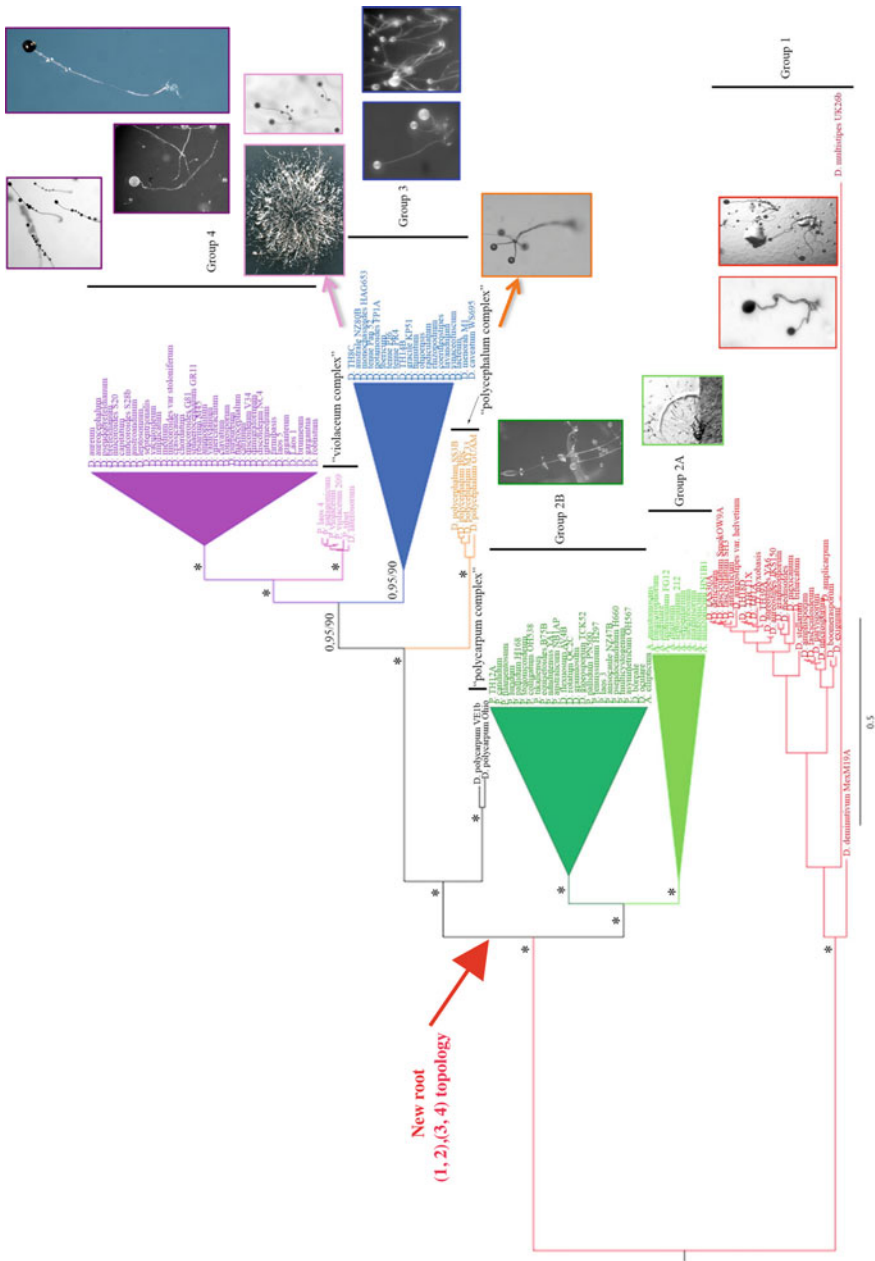
Fig. 1 Molecular phylogeny showing the major groups. (Modified from Romeralo et al. 2011)

with the previous SSU rDNA phylogenies, the inferred phylogeny largely retains the same ordering of species within the four major groups (Romeralo et al. 2011; Schaap et al. 2006), but instead of the (1), (2, 3, 4) topology shown then, now displays the (1, 2), (3, 4) topology.

Molecular tools are also becoming relevant in the study of population genetics in Dictyostelia but so far, such analyses have been carried out exclusively in Dictyostelid Group 4 species and the "Violaceum Complex" Group and show different results depending upon the species used. The species *Dictyostelium rosarium* seems to be a well-define species with all studied isolates forming a monophyletic group (Romeralo et al. 2010b) while *Dictyostelium purpureum* shows extensive genetic variation between populations and clear evidence of phylogenetic structure (Mehdiabadi et al. 2009). On the other hand, the species *Dictyostelium giganteum*, displays little genetic variation, phylogeographic structure or genetic differentiation among populations, relative to the cryptic species observed within *D. purpureum* (Mehdiabadi et al. 2010). Within the model organism *D. discoideum,* the different isolates examined form a monophyletic group, but there are several subclades and pronounced genetic differentiation among locations, suggesting the presence of geographic or other barriers between populations. These results reveal the need for further investigation into potential cryptic species (Douglas et al. 2011). Finally *Polysphondylium violaceum* phylogenetic analyses also suggest the possibility of cryptic species. The level of divergence found is comparable to the divergence between sibling species in other dictyostelids (Kalla et al. 2011). These results have important implications for our understanding of speciation and social evolution in dictyostelids in particular and eukaryotic microbes in general.

The existence of molecular tools has also allowed us to study the diversity of Dictyostelia by culture-independent techniques such as culture-independent PCR (ciPCR) (Romeralo, unpublished data). Development of ciPCR has been particularly challenging for soils, which are complex ecosystems dominated by fungi. Preliminary results show a big diversity of unknown phylotypes distributed across the molecular tree. Here again, the recent development of modern technologies of massive sequencing such as 454 or Illumina and the availability of dictyostelid-specific primers will probably change our current vision of Dictyostelia systematics and biogeography in ways that are intriguing to contemplate.

3 Diversification of Dictyostelia in the Proterozoic

Fossil calibration, the assignment of a fossil's age to its corresponding node in a phylogeny, is an essential tool used to reconstruct divergence times. Also, a fully resolve phylogeny is required for an accurate reconstruction of divergence times and this notably depends on the number of taxa and the number of genes. Unfortunately, Dictyostelia lacks a fossil record due to the absence of hard "fossilizable" structures and only two molecular markers (18S rDNA and nrITS)
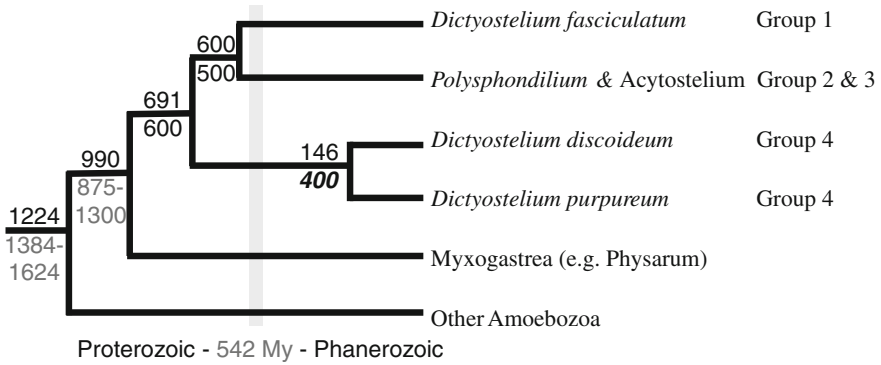
Fig. 2 Reconstructions of divergence times for Dictyostelia from different molecular studies. *Vertical gray bar* indicates the placing of the Proterozoic-Phanerozoic transition. Numbers above branches are divergence time after Fiz et al. (2013); (*bold*), below branches after Parfrey et al. (2011) (*grey*), Sucgang et al. (2011) (*kursive*) and Heidel et al. (2011). Note that branch lengths are not scaled to time

are currently sequenced for most known dictyostelids species. The nrITS marker is largely unalignable among the major groups of dictyostelids. In fact recent phylogenies use alignments of the individual groups of dictyostelids (Romeralo et al. 2010a, b). Therefore no sister group with a fossil record can be incorporated into a nrITS alignment. Since no internal calibration is possible in dictyostelids the only option is the use of a secondary calibration derived from other reconstruction, which would incorporate a huge uncertainty on reconstructed molecular ages. On the other hand, using a 18S rDNA phylogeny allows for the incorporation of other amoebozoans which do have a fossil record. However, the use of 18S rDNA for divergence time reconstruction of two closely related species may not be very robust since it may rely on few (3–4) nucleotides difference between the species. Therefore tracing a fine scale chronology within dictyostelids species becomes highly difficult with the data currently available. Alternatively, different approaches can be taken at higher taxonomic levels, such as, the use of a universal rate of substitution or the use of fossils from outside Amoebozoa.

The interest in molecular dating of protist is growing thanks to the advent of new and abundant genomic data (Groussin et al. 2011). Two recent genomic studies (Heidel et al. 2011; Sucgang et al. 2011) sought to reconstruct divergence times for major groups of dictyostelids. These two studies applied a universal rate of nucleotide substitution and then re-scaled the branch lengths for their phylogenetic tree (known as a "strict molecular clock"). In the case of Heidel et al. (2011) the tree included three dictyostelids and six representatives of fungi, animals, and plants. Heidel et al. (2011) retrieved an age for the dictyostelids' crown node of 600 My (*D. discoideum* from *Dictyostelium fasciculatum* plus *P. pallidum* split; Fig. 2) while Sucgang et al. (2011) obtained an age of 400 My for the crown age of Group 4 (*D. discoideum–D. purpureu*m split; Fig. 2). One of the risks associated with the use of strict molecular clocks (universal rate of substitution) is

the overestimation of reconstructed ages (Benton and Ayala 2003). For instance, using this method Heidel et al. (2011) estimated an age of 560 My for the crown age of land plants despite the fact that the oldest evidence of a land plant is almost 100 My younger (Rubinstein et al. 2010). On the other hand if the rate of substitution used by Sucgang et al. (2011) for Group 4 is applied in the eukaryote tree of life then the split between prokaryotes and eukaryotes would probably predate the age of any kind of eukaryote fossil including the oldest which are around 2,000 My and are very disputable (Berney and Pawlowski 2006).

Fossil calibration from outside Amoebozoa can be used for temporal reconstructions of major clades in Dictyostelids. When including only sister groups of Dictyostelids for which fossils are present (e.g., Arcellinids; Schmidt et al. 2004) the problem is the lack of resolution of the phylogenetic tree of Amoebozoa (Lahr et al. 2011). This has led to the reconstruction of divergence times using large-scale phylogenies. Different fossils from sister clades (e.g., Fungi, Metazoa) can be assigned to their corresponding nodes and the ages for major clades of Amoebozoa can be recovered. Parfrey et al. (2011) reconstructed divergence times for the eukaryote tree of life in this way using up to 15 genes. They also used multiple fossil calibration points and found ages of more than 1,000 My for the dictyostelids (Fig. 2). Unfortunately, in the calibration scheme of Parfrey et al. (2011) the oldest amoebozoan fossil (742–770 My; Porter and Knoll 2000) was assigned to the Arcellinids, which is highly debated (Cavalier-Smith 2009). A conservative approach would be to assign this fossil to Lobosa (as in Berney and Pawlowski 2006) or even more conservatively to assign it to the crown node of Amoebozoa. Interestingly, one of the alternative analyses of Parfrey et al. (2011), where Amoebozoa is sister to Opisthokonts, retrieved a crown age of these amoebas (ca. 1150 My; Fig. S5 on Parfrey et al. 2011) which is more consistent with other studies (Berney and Pawlowski 2006).

The most recent studies dating dictyostelids diversification include a wide sampling of dictyostelid species (Fiz-Palacios et al. 2013) and a high number of genes (Heidel et al. 2011). As mentioned above, Heidel et al. (2011) did not use fossil calibration but rate extrapolation while Fiz-Palacios et al. (2013) used relaxed-clock methods (that allows for branch substitution rates to be independent across the tree) together with different fossil calibration schemes from outside Amoebozoa. These two studies widely agree in placing the origin of diversification of dictyostelids (crown age). While Heidel et al. (2011) placed the origin around 600 My, Fiz-palacios et al. (2013) estimated slightly older ages within the Proterozoic (Fig. 2). Therefore both studies suggest a land colonization of dictyostelids predating the land plants' "terrestrialization" in the Ordovician. Different evolutionary perspectives can support the early colonization of land by dictyostelids. On one hand, Heidel et al. (2011) argue that dictyostelids have (1) non-aquatic fruiting bodies and (2) high resistance to DNA damage by UV due to absence of plant canopy. On the other hand, Fiz-Palacios et al. (2013), in line with other authors (Kenrick and Crane 1997 and Porter and Knoll 2000), suggest that dictyostelids diversification happens thanks to the rise of terrestrial ecosystems. A synchrony of diversification among different eukaryotes can be inferred when

considering the fossil record of land plants (470 My; Rubinstein et al. 2010), arbuscular mycorrhizal fungi (ca. 460 My; Taylor and Barbee 2006) and different soils arthropods (see Rehm et al. 2011). Then the question remains regarding the dictyostelids' sister group, Myxogastrea, which is a species-rich clade (ca. 920 species) of terrestrial organisms. Did both sister clades (Dictyostelids and My-xogastrids) colonize land independently? Or did the last common ancestor colonized land with a later specialization on coarse woody debris, ground litter, and the bark surface of living trees in Myxogastrea and forests soil/litter microhabitat in Dictyostelia? We hope that the coming genomic data of Dictyostelida and sister groups, as well as the improvement of molecular clocks techniques, will help resolving these and other evolutionary questions.

4 Ecology and Biogeography of Protists?

An important outcome of the use of molecular tools is their relevance in the fields of ecology and biogeography. Since as little as 1–10 % of all microbial organisms can be cultured (Handelsman and Smalla 2003; Pace 1997; Foissner 2006), environmental surveys based on the amplification, cloning, and sequencing of small-subunit ribosomal RNA genes (18S rDNA) directly from the environment, are a powerful tool to study the diversity of microorganisms (Lara et al. 2007; Caron et al. 2012). This is especially true with protists, where the extent of total diversity is unknown and of considerable debate (review in Foissner 2006). Environmental DNA surveys can also be used to compare several taxonomical groups within a locality (Fierer 2007; Moreira and Lopez-Garcia 2002) or to test biogeographical hypothesis such as the "everything is everywhere (EiE), but the environment selects" hypothesis (Bass et al. 2007). However, there is still no common species concept for protists as a group and it remains difficult to evaluate to which extent morphological, ecological, and/or ultrastructural variation is associated with genetic variation (Boenigk et al. 2012).

These taxonomical uncertainties together with the ancient origin of the protist groups may have led to the establishment of hypothesis such as the EiE (Fenchel and Finlay 2004). This controversy over microbial biogeography (Foissner 2006) presents two main hypotheses. On the one side of the EiE debate it is argued that free-living microbial eukaryotes can reach any geographic location due to their small size, large number of progeny, and great dispersal capabilities, and as a result they will establish at any favorable environment (Fenchel and Finlay 2004; Finlay 2002). On the other side it is argued that dispersal is restricted in some protists and this is reflected in the geographic distribution patterns of different species (i.e., not any habitat favorable for a micro-eukaryote species will be occupied due to dispersal limitations). This is commonly referred to as the moderate endemicity model (Foissner 2006). Biogeography is a quite well-developed field for animals and plants and a hot topic for protist nowadays (Fierer 2008). Protist species with restricted distributions have already been found (Foissner

2006, 2008; Smith and Wilkinson 2007). Furthermore, the use of molecular data and phylogenetic analyses is helping enormously to resolve the common problem of geographically restricted cryptic species across groups (Foissner 2006; Epstein and Lopez-Garcia 2008). Molecular clocks can further help in untangling species delimitations by providing a temporal framework: i.e., a clade of 50 myr is likely to harbor more cryptic species than a clade of 1 myr. This highlights the critical importance of molecular data in protist species delimitations and thus in differentiating cosmopolitan versus geographically restricted species.

Within the soil, Dictyostelia are major consumers of bacteria and play an important role in nutrient cycling and soil health (Raper 1984; Hagiwara 1989). However, information about the role of dictyostelid amoebas in soils remains limited, mostly because they can only be differentiated by their multicellular structures, which are microscopic and therefore only observable in laboratory-based cultures. As a consequence, we know relatively little about the behavior of dictyostelids in their natural habitats. Their ecological relationships with other taxonomic groups are also unknown, other than the fact that they are bacterivores. Some general trends have been suggested over the years, but these have been difficult to test by traditional methods. The culture-independent approach allows us to examine these questions on a broad scale for the first time and lays the necessary groundwork for future quantitative studies. These will allow us to study soil samples worldwide and get insights into the ecological role dictyostelid amoebas play in soil ecosystems. At the same time, this will allow us to have a better understanding of dictyostelid diversity and distribution and therefore be in a better position to answer questions such as: is everything really everywhere?

The genomic era has brought about a great variety of techniques for measuring biodiversity. The latest advance in the field is the single-cell genome sequencing which could be a highly beneficial approach to understand the microbial diversity and evolution of different ecosystems worldwide (Kalinsky and Quake 2011; Kalinsky et al. 2011). This is especially important in protists where the known diversity is very small and only 1–10 % of the species are cultivable. Therefore, the application of this technique is an exciting and promising approach for the field, as shown by some examples from uncultured bacteria from the human mouth (Marcy et al. 2007) and the first single-cell archaeal genome (Blainey et al. 2011).

5 Concluding Remarks

The study of dictyostelids has enormously benefited from the arrival of the genomic era. Thanks to molecular data we know that the genetic diversity of the group is enormous and equivalent to that of metazoan, however, only ca. 150 species are known at the time of writing. Multiple comparisons can be made across dictyostelid social amoeba (Amoebozoa) and Metazoa, especially considering that many genes have been conserved in this group and lost in fungi, metazoa's sister group (for more details see Chap. 1 in this book). In the near future the availability

of genomes within Amoebozoa and other protists groups will allow us to extend these comparisons to other major groups across the tree of life. Especially interesting will be the sequencing of genomes among the sorocarpic protist groups, which are distributed in five major eukaryotic groups (Amoebozoa, Alveolates, Excavata, Opisthokonta, and Rhizaria), in order to gain insights into the evolution of multicellularity across the tree of life. Finally, molecular clocks assessments will also play a key role in the genomic era, thus making it possible to date big evolutionary events such as the origin(s) of multicellularity.

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