Molecular Phylogenetics and Evolution 92 (2015) 53–62

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Root of Dictyostelia based on 213 universal proteins ☆

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ARTICLE INFO

Article history:
Received 18 July 2014
Revised 18 May 2015
Accepted 19 May 2015
Available online 3 June 2015

Keywords:
Aggregative multi-cellularity
Universal genes
Multigene phylogeny
Evolution of complexity

ABSTRACT

Dictyostelia are common soil microbes that can aggregate when starved to form multicellular fruiting bodies, a characteristic that has also led to their long history of study and widespread use as model systems. Ribosomal RNA phylogeny of Dictyostelia identified four major divisions (Groups 1–4), none of which correspond to traditional genera. Group 1 was also tentatively identified as sister lineage to the other three Groups, although not consistently or with strong support. We tested the dictyostelid root using universal protein-coding genes identified by exhaustive comparison of six completely sequenced dictyostelid genomes, which include representatives of all four major molecular Groups. A set of 213 genes are low-copy number in all genomes, present in at least one amoebozoan outgroup taxon (*Acanthamoeba castellanii* or *Physarum polycephalum*), and phylogenetically congruent. Phylogenetic analysis of a concatenation of the deduced protein sequences produces a single topology dividing Dictyostelia into two major divisions: Groups 1 + 2 and Groups 3 + 4. All clades in the tree are fully supported by maximum likelihood and Bayesian inference, and all alternative roots are unambiguously rejected by the approximately unbiased (AU) test. The 1 + 2, 3 + 4 root is also fully supported even after deleting clusters with strong individual support for this root, or concatenating all clusters with low support for alternative roots. The 213 putatively ancestral amoebozoan proteins encode a wide variety of functions including 21 KOG categories out of a total of 25. These comprehensive analyses and consistent results indicate that it is time for full taxonomic revision of Dictyostelia, which will also enable more effective exploitation of its unique potential as an evolutionary model system.

1. Introduction

Dictyostelia are a group of eukaryotic soil microbes found worldwide, most commonly in forest soils. They are best known for their response to starvation, where the cells aggregate by the hundreds to hundreds of thousands to form simple multicellular fruiting bodies. Because the cells in the aggregate do not fuse, dictyostelids have often been referred to as cellular slime molds or social amoebae, although non-fusing cell aggregation to form sporophores has arisen multiple times independently including at least once in bacteria (Brown and Silberman, 2013). Dictyostelids are nonetheless by far the most diverse, widespread and well-studied group of aggregating amoebae, as well as most likely the oldest (Fiz-Palacios et al., 2013).

Dictyostelia are best known for their developmental cycle, which has been most extensively studied in the model organism, *Dictyostelium discoideum. D. discoideum* cell aggregates include ~10^6 cells, of which ~20% are sacrificed to form the inert stalk of the fruiting body. This fate is largely assigned to individual cells early in the aggregation cycle (Jang and Gomer, 2011; Williams, 2010). Thus the vast majority of known dictyostelids exhibit important hallmarks of multicellularity including cellular specialization and differential survival (Raper, 1984; Schaap et al., 2006). This striking developmental cycle plus the experimental tractability of many species has resulted in dictyostelids serving as models for a wide variety of systems. These include the study of simple processes in development (Eichinger et al., 2005), cellular differentiation (Bonner, 2003, 1952), cooperation and cheating (Strassmann and Queller, 2011), cell–cell communication (Abedin and King, 2010; Coates and Harwood, 2001) and cell movement (Insall, 2013).

Roughly 150 species of Dictyostelia have been described, and these show considerable variation in aggregative behavior and

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☆ This paper was edited by the Associate Editor N. Blackstone.

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http://dx.doi.org/10.1016/j.ympev.2015.05.017
1055-7903/

sporophore morphology. However, the species are still roughly classified according to traditional taxonomy, which recognizes three genera corresponding to the three basic morphotypes: acytostelid, dictyostelid and polysphondylid (Raper, 1984; Hagiwara and Kawakami, 2008). Acytostelids (*Acytostelium* spp.) are characterized by small delicate fruiting bodies (sporophores) with acellular stalks, and thus all amoebae in the aggregate survive to form spores. Dictyostelid-type (*Dictyostelium* spp.) sporophores possess inert cellular stalks supporting one to many spore heads in various irregular arrangements. Polysphondylids (*Polysphondylium* spp.) also form multi-headed sporophores with cellular stalks, but these are more highly organized than in dictyostelids with regularly spaced whorls of side branches (Romeralo et al., 2010).

This long-standing traditional classification system was challenged first by cladistic analysis of morphological traits (Swanson et al., 2002) and then by molecular phylogeny (Schaap et al., 2006). The latter analyses further divided the taxon into four major groups, informally referred to as Groups 1–4, plus three smaller "complexes" whose relationships to the four major groups remain uncertain (Schaap et al., 2006; Romeralo et al., 2010, 2011) (Fig. 1). None of these major groups or complexes correspond to any of the three traditional dictyostelid genera. Thus both molecular and morphological analyses indicate that none of the formally recognized dictyostelid genera are valid, and the phylogeny of the group is considerably more complex than expected. The preponderance of cryptic species in Dictyostelia also suggests that much of the diversity of the group is still unknown (Flowers et al., 2010; Perrigo et al., 2012; Romeralo et al., 2011; Schaap et al., 2006). This is further supported by the predicted antiquity of the group, with molecular dating placing the last common ancestor of modern Dictyostelia at 0.6–0.7 billion years ago (Fiz-Palacios et al., 2013).

A critical question in dictyostelid phylogeny has been the position of the root, which has important implications for the interpretation of larger evolutionary trends in the group. Traditional scenarios hypothesized that the small delicate acytostelids with their simple acellular stalks should be the sister group to the "more complex" dictyostelids and polysphondylids. However SSU rDNA trees place acytostelids in a derived position, in the highly heterogeneous Group 2 together with a mixture of polysphondylid and dictyostelid morphotypes. Initially, SSU rDNA phylogeny also tentatively identified Group 1 as sister taxon to the other three major Groups, albeit with low confidence (Schaap et al., 2006). This still seemed roughly consistent with stepwise evolution of complexity, from the small usually delicate sporophores of Group 1 to the generally large robust fruiting bodies of Group 4 (Heidel et al., 2011). However, subsequent analyses using 32 universal eukaryotic proteins placed the root of Dictyostelia between Groups 1 + 2 and Groups 3 + 4 (1 + 2, 3 + 4 root), suggesting quite different interpretations of dictyostelid evolution (Romeralo et al., 2013).

The main challenge in rooting the dictyostelid tree has been the lack of data from close outgroup taxa with relatively slow evolutionary rates. This is needed in order to avoid random rooting and artifacts due to long branch attraction (LBA) (Gribaldo and Philippe, 2002; Huelsenbeck et al., 2002). However, there is still very little molecular data from other members of the dictyostelid parent taxon, supergroup Amoebozoa, other than rDNA sequences, which show markedly wide variation in mode and tempo of evolution (Pawlowski and Burki, 2009). Currently, substantial outgroup data exist for only one member of the dictyostelid sister taxon Myxogastria (Pawlowski and Burki, 2009), that of *Physarum polycephalum* (Barrantes et al., 2012). Otherwise the only other free living amoebozoan with substantial sequence data is the solitary amoeba, *Acanthamoeba castellanii* (Clarke et al., 2013). Full genome sequence data are also available for *Entamoeba histolytica*, a member of the putative sister taxon to the clade of Dictyostelia + Myxogastria (Macromycetozoa), the Archamoebae (Adl et al., 2012; Bapteste et al., 2002; Fiore-Donno et al., 2009). However, this obligate parasite has extremely fast evolutionary rates, even for generally conservative housekeeping genes (Bapteste et al., 2002; Loftus et al., 2005; Silberman et al., 1999) making it problematic for rooting a deep tree.

We sought to test the root of Dictyostelia with a comprehensive data set including all suitable protein-coding genes universal to Dictyostelia and their non-parasitic amoebozoan relatives. The data set was restricted to genes for which reliable individual phylogenies could be constructed to test for potential sources of

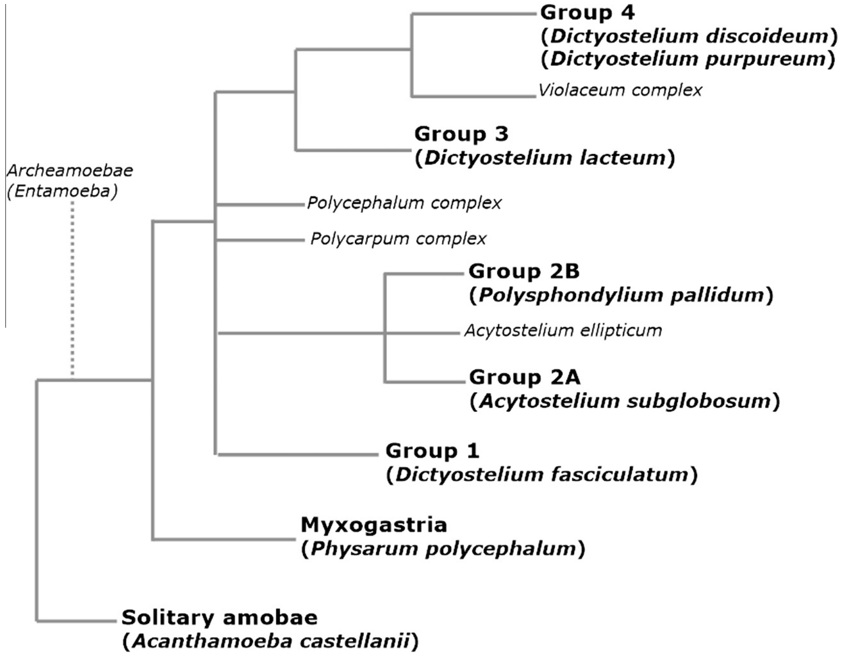

Fig. 1. Schematic phylogeny of Dictyostelia. The tree shown is a consensus phylogeny of all major groups of Dictyostelia based on SSU rDNA and α-tubulin phylogeny (Schaap et al., 2006). Species names in bold and parenthesis indicate taxa used here. A dotted line indicates the position of the Archamoeba *Entamoeba histolytica*, which was not used in these analyses due to the highly divergent nature of its sequences.