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A Fully Resolved Phylogeny of the Social Amoebas (Dictyostelia) Based on Combined SSU and ITS rDNA Sequences

Maria Romeralo[a,1], **Fred W. Spiegel**[b], **and Sandra L. Baldauf**[a]

[a]Department of Systematic Botany, Evolutionary Biology Centre, Norbyvägen 18D, University of Uppsala, SE-75420 Uppsala, Sweden
[b]Department of Biological Sciences, SCEN 601, 1 University of Arkansas, Fayetteville, AR 72701, USA

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The dictyostelids possess a complex life cycle including aggregative and multicellular stages. They also include one of the most widely studied protistan model organisms, *Dictyostelium discoideum*. The current molecular phylogeny of dictyostelids is based largely on SSU (18S) rDNA sequences and shows a deep taxon consisting of four major groups, none of which correspond to the three traditional morphologically-defined genera. However, due to the generally slowly evolving nature of SSU rDNA, these data fail to resolve the majority of branches within the four groups. Given the highly morphologically mixed nature of the dictyostelid groups, it is important to resolve relationships within them. We have determined sequences for the internal transcribed spacers (ITS) of rDNA for nearly all species in the original dictyostelid global phylogeny. Phylogenetic analyses of these data, in combination with the previously determined SSU rDNA sequences, confidently resolve nearly all branches in the tree. This now fully resolved phylogeny confirms the utility of ITS for dictyostelid systematics and lays the ground work for further evolutionary study of the group.

Key words: Amoebozoa; development; *Dictyostelium*; evolution; 18S rDNA.

Introduction

The dictyostelids or “social amoebae” are a major group of soil microbes with characteristics of true multicellularity. While they spend most of their life cycle as solitary amoebae, upon starvation they can aggregate by the 100s to 100s of thousands to form sorocarps with differentiated cell types (Kessin 2001; Raper 1984). Whereas this life cycle is best characterized in the model organism *Dictyostelium discoideum*, over 100 species of dictyostelids have been described (Bonner 2009; Romeralo et al. in press). These species vary widely in various aspects, most notably in sorocarp morphology (Schaap 2007). Although the traditional dictyostelid taxonomy has been challenged on both morphological (Swanson et al. 2002) and molecular grounds (Schaap et al. 2006), their classification has yet to be formally revised. Thus, small delicate species with acellular stalks have been traditionally classified as taxon *Acytostelium* (Raper 1956), whereas species with complex multi-headed sorocarps with regular whorls of side branches constitute the traditional taxon *Polysphondylium* (Brefeld 1884). Meanwhile, the majority of described species and morphotypes

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[1]Corresponding author; fax +46 18 4716457
e-mail maria.romeralo@gmail.com (M. Romeralo).

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constitute the traditional taxon *Dictyostelium* (Brefeld 1869). The latter includes everything from the model organism *D. discoideum* to the cannibalistic *D. caveatum* and many other distinct morphologies and behaviour types. The 100+ described species of dictyostelids also differ in various other aspects of their life cycle including the mode of cellular aggregation, the behaviour of the multicellular aggregates, the branching pattern of the sorocarp and its pigmentation, the shape and size of the spores and the signaling molecule (acrasin) involved in aggregation (Raper 1984; Schaap et al. 2006; Schaap 2007).

Several species of dictyostelids have been studied in molecular, evolutionary and developmental terms (Alvarez-Curto et al. 2005; Bonner and Cox 1995; Schaap et al. 1985). However, most of this work has concentrated on a single species, *Dictyostelium discoideum*, which is now one of the most widely studied eukaryotic model organisms (Eichinger et al. 2005). However, the Dictyostelia as a whole was not subjected to systematic molecular phylogenetic study until recently (Romeralo et al. 2007; Schaap et al. 2006). These studies indicated the need for major taxonomic revision of the group at the deepest levels. Most notably, it is now clear that *Dictyostelium* is a widely paraphyletic taxon with a number of apparently distinct morphologies arising multiple times independently. Likewise, the most morphologically striking taxon, *Polysphondylium*, has been shown to be polyphyletic. Thus, it is now clear, as first suggested by Swanson et al. (2002), that traditional morphological characters are not reliable markers for major evolutionary relationships within Dictyostelia.

The first molecular phylogeny of Dictyostelia was published in 2006 and utilized the ~95% of the SSU rDNA that is universally alignable across the group (Schaap et al. 2006). These data resolved the overall phylogeny of Dictyostelia with high confidence, separating nearly all species into four major clades (referred to here as Groups 1–4). However, the majority of branches within these clades were very poorly resolved, even when the more rapidly evolving rDNA expansion segments were included for individual groups (Schaap et al. 2006; SLB unpublished).

Due to the slowly evolving nature of the SSU rDNA, species level phylogenies often utilize more rapidly evolving loci, most commonly the first internal transcribed spacer of rDNA (ITS1) (Köhsler et al. 2006; Coleman 2007). Previous work has also shown ITS rDNA to be a good marker to differentiate relationships at different levels in Dictyostelia (Romeralo et al. 2007). Therefore, in order to resolve species level relationships across the group, we sequenced this entire region (ITS1-5.8S-ITS2) for almost all of the species in the original dictyostelid molecular phylogeny (Schaap et al. 2006).

Results

ITS sequences were determined for 71 out of the 93 isolates utilized in the original global phylogeny of Dictyostelia (Schaap et al. 2006). The remaining 22 isolates were excluded, most either because their ITS sequences could not be amplified or because they were too divergent to align with other sequences (see Table 1).

The ITS sequences from the remaining 71 dictyostelid isolates were still extremely variable, both in terms of length and sequence. In fact, these sequences proved to be very difficult to align even within each of the four major groups, and no meaningful alignment of this region could be obtained across the groups. This included sequence variation, expansion/contraction of simple sequence repeats and moderate to large insertions/deletions (indels). Overall, the dictyostelid ITS sequences show length variation of 917–1250 nucleotides (NTs) in Group 4; 863–1248 NTs in Group 3; 717–1072 NTs in Group 2 and 1055–1345 NTs in Group 1. Thus, for all groups except Group 4, less than 50% of the full ITS sequence could be confidently aligned across the group. The total numbers of confidently aligned positions utilized for phylogenetic analysis were as follows: Group 4 – 685 (55%); Group 3 – 388 (31%); Group 2 – 452 (42%) and in Group 1 – 596 (44%).

Sequences were analyzed primarily using Bayesian inference. Separate analyses were run using ITS sequences alone, SSU sequences alone (using additional expansion segment positions alignable only within groups), and ITS+SSU sequences combined. The results of all four sets of analyses were consistent with each other and with the original SSU rDNA phylogeny of Schaap et al. (2006) for all four groups (data not shown). However, the number of resolved clades was highest with the combined ITS+SSU data sets.

Combined ITS+SSU rDNA Phylogeny of Group 1

This group is now almost completely resolved with nearly all branches receiving strong maximum likelihood bootstrap (mlBP) support (>70%, Hillis