

Taxonomy, slime molds, and the questions we ask

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Abstract: Taxonomic treatments often influence the way we both ask and attempt to answer certain biological questions. The classical taxonomy of the dictyostelid cellular slime molds (Dictyosteliales) involves a convenient set of categories that were developed independent of phylogeny. In order to test whether the characters supporting the classical taxonomy hold any phylogenetic signal, we subjected 19 described taxa belonging to two families (Acytosteliaceae and Dictyosteliaceae) and three genera (*Acytostelium*, *Dictyostelium*, and *Polysphondylium*) to rooted cladistic analyses using PAUP* v 4.0b4a. Neither family nor any of the three genera were found to represent monophyletic groups. These results confirm that the classical taxonomy used to delineate families and genera within these slime molds carries very little phylogenetic signal. Taxonomic character sets should be scrutinized phylogenetically in order to determine what information they provide about the relatedness of taxa within a group. Because taxonomy often drives the nature of biological inquiry, caution should be exercised when drawing conclusions regarding the evolution of developmental systems in *Dictyostelium*.

Key Words: *Acytostelium*, *Dictyostelium*, Eumycetozoa, Phylogeny, *Polysphondylium*

INTRODUCTION

A phylogeny is a scientific hypothesis. A taxonomy by itself is not. Yet we often treat taxonomies as hypotheses, and the questions we pose regarding phylogenetic relatedness are often driven by the insinuations taken from the underlying taxonomy. The rules of both botanical and zoological nomenclature empha-

size stability (Mayr and Ashlock 1991, Greuter et al 2000). With the classical identification, naming, and subsequent cataloging of new species, evolutionary theory has historically taken a secondary role to the traditional (and intuitive) view that similarity of form (whether macroscopic, microscopic, or molecular) should indicate relatedness between taxa. Morphological, physiological, behavioral, and genetic characteristics are certainly important in the development of a taxonomic system. Once in place, however, a taxonomic system intrinsically drives the nature of biological inquiry, typically by inspiring assumptions about the evolutionary processes that contribute to the diversity of form.

The classical taxonomic grouping of Oomycota with fungi, for example, encouraged the assumption that filamentous growth and absorptive nutrition were synapomorphies unifying them with the Fungi. We now know that oomycetes are phylogenetically allied with the heterokont algae (Barr 1992), a lineage quite removed from the Opisthokonts [Fungi and Animals (Cavalier-Smith 1998)]. This phylogenetic realization verifies convergence of a key taxonomic character—filamentous hyphae—and leads to the awareness that traditional taxonomy has impeded formulation of the most fundamental and pertinent questions regarding hyphal origins in all groups of mycelial organisms (e.g., how many times have hyphae originated?).

Similarly, the aggregation of single amoebae into a multi-celled fruiting body was an important taxonomic character that unified the acrasids (sensu Olive 1975) and dictyostelid cellular slime molds into a larger Class Acrasiomycetes (Raper 1984). Olive's (1975) observations leading to studies by Page and Blanton (1985), and Roger et al (1996) have demonstrated that the acrasids are members of the Heterolobosea, a group phylogenetically distant from the Eumycetozoa. Differences in morphology have turned out to be more important than superficial similarities of aggregation, and again, convergence has been recognized in what was traditionally viewed as a unique evolutionary event.

Taxonomic treatments influence the way we form our inquiries, and we often fail to ask the right questions about the evolutionary mechanisms involved if, for instance, convergence of characters is never con-

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TABLE I. Probable synapomorphies among the Dictyosteliales

Synapomorphy	Reference
Streaming aggregation of individual myxamoebae followed by differentiation into a multicellular fruiting body composed of stalk and spore mass	Shaffer 1964; Bonner 1967; Hohl et al. 1968; Olive 1975; Bonner 1982; Raper 1984
Stalk tube synthesis & ultrastructure	Gezelius, 1959; Hohl et al., 1968; George et al., 1972
Spore ultrastructure	Gezelius, 1959; Hohl et al., 1968; Hohl & Hamamoto, 1969
Myxamoeba ultrastructure	Gezelius, 1959; Hohl et al., 1968
Nature of mitosis	Roos, 1975; Moens, 1976; Heath, 1980; Raper, 1984
Ultrastructure of microtubule centers (MTC's)	Guhl & Roos, 1994
Nucleoli peripheral in the nucleus	Gezelius, 1959; Hohl et al, 1968
Pseudoplasmodium organization	MacWilliams & Bonner, 1979

sidered. This trap is well camouflaged, owing first to modern taxonomy's presumed acceptance of an evolutionary worldview, and second to the history of nomenclature in each group of related organisms. Biologists must be mindful that our ideas about how characters evolve can be highly influenced by taxonomy.

Subclass Dictyosteliidae, Order Dictyosteliales is clearly a monophyletic assemblage (TABLE I) within the Eumycetozoa, a natural group that includes the protostelid, dictyostelid, and myxogastrid slime molds (Olive 1975, Dykstra 1977, Drouin et al 1995, Spiegel et al 1995, Keeling and Doolittle 1996, Baldauf and Doolittle 1997, Baldauf 1999). The Dictyosteliales have traditionally been divided into two families: the Acytosteliaceae (which includes *Acytostelium*), with an acellular, hollow stalk, and the Dictyosteliaceae (which includes *Dictyostelium* and *Polysphondylium*), which have a cellular stalk (Olive 1975, Raper 1984). Oskar Brefeld (1869) was the first

to isolate and describe a dictyostelid, *Dictyostelium mucoroides*, whose generic name was chosen based on the net-like appearance of the fruiting body's stalk cells (Raper 1984). Members of *Dictyostelium* possess relatively large fruiting bodies that are typically unbranched or irregularly branched (FIG. 1A). Brefeld later (1884) described a second species, *Polysphondylium violaceum*, complete with a new generic designation based on the regularly-whorled branches of the fruiting body's cellular stalk (FIG. 1B). These two genera were included in the family Dictyosteliaceae. *Acytostelium leptosomum*, described by Raper in 1956, and later characterized fully by Raper and Quinlan (1958), possessed tiny, delicate fruiting bodies with acellular hollow stalks (FIG. 1C), and was deemed unique enough to be assigned to a third genus in its own, new family Acytosteliaceae.

Much speculation has been made on the phylogenetic relationships within the dictyostelids, but none of these studies has questioned 2 basic assump-

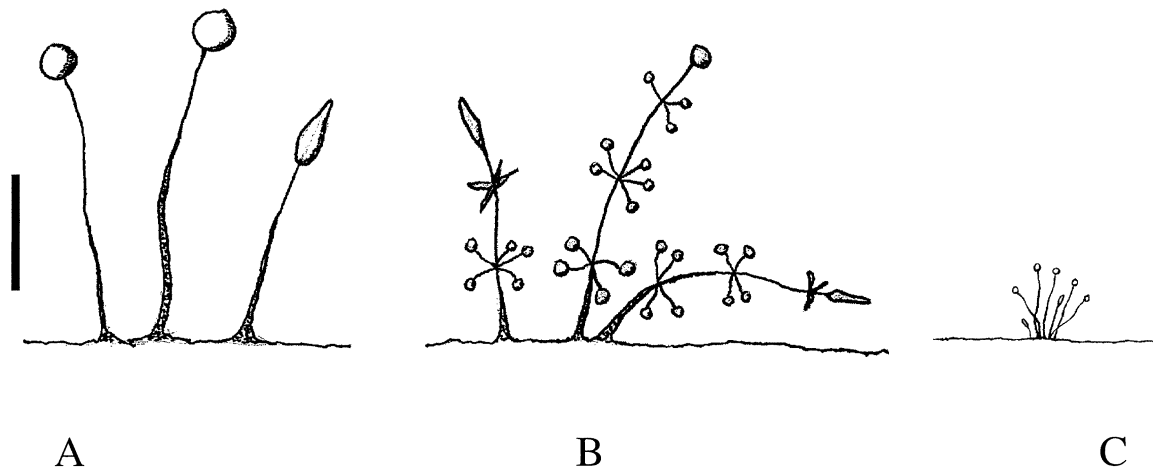


FIG. 1. a. *Dictyostelium mucoroides*, b. *Polysphondylium violaceum*, c. *Acytostelium leptosomum*. Bar = 1 mm.

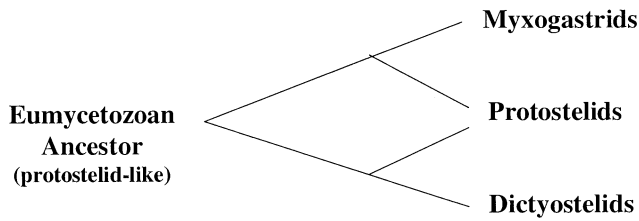


FIG. 2. Proposed evolutionary relationships among the Eumycetozoa (modified from Olive 1975).

tions implied by the taxonomy: (i) the first dictyostelid had acellular stalks, and cellular stalk evolved only once; (ii) regular, whorled branching evolved only once. Holmes (unpubl), in a preliminary phylogenetic study of 24 species of dictyostelids, placed several of the smallest species (including *D. minutum*) at early branching points, suggesting their primitive evolutionary position. Vadell and Cavender (1991) presented a phylogeny of 31 dictyostelid taxa, showing a monophyletic *Polysphondylium* emerging from within a paraphyletic *Dictyostelium*. The cladograms of both Holmes and Vadell and Cavender suggested that the genus *Dictyostelium* is paraphyletic. However, both of these analyses used *Acytostelium* as an outgroup, rather than including this dictyostelid within the analysis group.

Outgroup selection is obviously an important matter, and for examining relationships within the Dictyosteliales, the use of a Eumycetozoan sister taxon is most appropriate. Olive and Stoianovitch (1960) hypothesized a relationship between the protostelid *Protostelium mycophaga* and the dictyostelid genus *Acytostelium* based on the two groups' very similar non-flagellated amoebae and acellular fruiting body stalks. Molecular work has supported a close relationship between *Protostelium* and dictyostelids (Dutta and Mandel 1972, Spiegel et al 1995), as well as between the protostelid *Planoprotostelium* (a close relative to *Protostelium* (Spiegel 1990)) and *Dictyostelium* (Baldauf and Doolittle 1997). Spiegel et al (1979) suggested that the similarities during culmination among stalk tube-synthesizing cells of dictyostelids and protostelids indicated a shared evolutionary history as well. These studies have lent considerable support to Olive's (1975) hypothesis that a protostelid-like ancestor gave rise to Dictyostelids (FIG. 2).

In this paper, we use formal phylogenetic analysis to investigate whether the traditional taxonomic characters impart any information about the evolutionary relatedness of 19 members of the Dictyosteliales in order to determine if those characters support the current classification of two families and three genera. We test two hypotheses that are con-

sistent with the current taxonomy: (i) acellular stalk is a plesiomorphic character state, while cellular stalk is a synapomorphy that defines the family Dictyosteliaceae; (ii) evenly-spaced whorled branching is a synapomorphy that defines the genus *Polysphondylium*. We also discuss the influence our results may have on the formulation of questions about the evolution of key characters defining the two families and three genera of the group.

MATERIALS AND METHODS

A data matrix containing 18 characters was constructed for 1 protostelid outgroup (*Protostelium mycophaga*) and 19 in-group taxa (TABLE II). Characters were drawn from the taxonomic literature and chosen according to their universality among members of the Dictyosteliales. Character coding was made according to the taxonomic works of Raper (1984), Hagiwara (1989), Olive (1975), and original published species descriptions. The 19 dictyostelid taxa were chosen to cover the range of morphological and developmental diversity found in the roughly 65 described species (Swanson et al 1999). The final matrix was analyzed using PAUP* v 4.0b5 for Macintosh (Swofford 1999) applying branch and bound methods for maximum parsimony, with characters defined as unordered and with equal weights. Unrooted strict and 80% majority rule consensus trees were constructed.

RESULTS

Thirty-six equally parsimonious trees were generated, each with 58 steps. The strict and 80% majority rule consensus trees generated from these data (FIG. 3A, B) do not support the hypotheses that either family of the Dictyosteliales is monophyletic, or that any of the three genera is monophyletic.

An important feature to note is that *Dictyostelium lacteum* is always positioned basal to a clade that contains both *Acytostelium ellipticum* and all of the dictyostelids with cellular stalks.

Using strict consensus, there is no support for a single clade that contains all of *Polysphondylium*, although the 80% consensus tree lends some support for a monophyletic group containing the white-spored species of *Polysphondylium* (FIG. 3B).

DISCUSSION

The trees generated from the present character set do not support the classical arrangement of families into Acytosteliaceae and Dictyosteliaceae, nor do they support a monophyletic genus, *Polysphondylium*.

The current taxonomy of dictyostelids implies

TABLE II. Character data matrix for 19 dictyostelid taxa and 1 outgroup taxon

Taxon ^a	Characters ^b																		
	Polar granules	Spore shape	Micro-cysts	Macro-cysts	Type of acrasin	Sorocarp branching	Relative sorocarp size	Slug behavior	Growth habit	Sporangium pigment	Spore shape	Stalk base shape	Stalk tip shape	Phototropism	Aggregation type	Stalk cellularity	Nucleolus position	Stalk pigment	Whorled branching
Daur	2	1	1	?	?	1	1	2	0	0	0	0	1	1	4	1	1	2	0
Ddemi	2	1	1	?	?	0	0	2	1	0	0	0	0	?	?	1	1	0	0
Ddisc	0	1	0	1	1	0	1	3	0	0	2	1	1	1	3	1	1	0	0
Dlact	0	0	1	1	2	0	0	1	1	0	0	0	0	?	?	1	1	0	0
Dlate	2	1	0	?	?	1	2	2	0	3	1	?	?	1	?	1	1	3	0
Dmacr	0	1	?	?	?	0	0	2	0	0	2	1	1	1	3	1	1	0	0
Dmexi	2	1	1	1	?	1	1	1	1	2	2	1	?	?	?	1	1	2	0
Dminu	1	1	1	1	4	1	0	1	0	0	0	0	0	0	2	1	1	0	0
Dmuco	0	1	1	1	1	0	1	2	0	0	0	1	1	1	3	1	1	0	0
Dpoly	1	1	1	?	?	0	0	3	2	0	0	0	0	0	3	1	1	0	0
Dpurp	0	1	0	1	1	0	2	2	0	3	0	1	1	1	3	1	1	3	0
Drhiz	2	1	1	?	?	1	1	2	1	3	1	?	?	1	?	1	1	3	0
Drosa	0	0	1	1	1	2	2	2	1	0	?	?	?	?	4	1	1	0	0
Pfila	1	1	0	?	?	2	2	2	1	0	?	?	0	0	4	1	1	0	1
Ppall	1	1	1	3	3	2	1	2	0	0	0	0	0	0	4	1	1	0	1
Pviol	2	1	1	3	3	2	1	2	0	3	0	0	1	1	4	1	1	3	1
Aelli	1	1	1	?	?	0	0	1	1	0	0	0	0	0	?	0	1	0	0
Alept	0	0	1	?	?	0	0	1	1	0	0	0	0	0	?	0	1	0	0
Asubg	0	0	1	?	?	0	0	1	0	0	0	0	0	0	?	0	1	0	0
Pmyco	?	0	1	0	0	0	0	0	0	1	0	0	0	?	0	0	0	0	0

^a Taxa: Daur = *Dictyostelium aureostipes*, Ddemi = *D. deminutivum*, Ddisc = *D. discoideum*, Ddact = *D. lacteum*, Dlate = *D. laterosorum*, Dmacr = *D. macrocephalum*, Dmexi = *D. mexicanum*, Dminu = *D. minutum*, Dmuco = *D. mucoroides*, Dpoly = *D. polycephalum*, Dpurp = *D. purpureum*, Drhiz = *D. rhizopodium*, Drosa = *D. rosarium*, Pfila = *Polysphondylium filamentosum*, Ppall = *P. pallidum*, Pviol = *P. violaceum*, Aelli = *Acytostelium ellipticum*, Alept = *A. leptosomum*, Asubg = *A. subglobosum*, Pmyco = *Protostelium mycophaga*.

^b Character State Codes: ? = unknown; Polar Granules absent = 0, unconsolidated = 1, consolidated = 2; Spore Shape spherical = 0, elliptical/oblong = 1; Microcysts absent = 0, present = 1; Macrocyts absent = 0, present = 1; Acrasin absent = 0, cAMP = 1, pterin der. = 2, gforin = 3, folic acid der. = 4; Sorocarp branching absent = 0, irregular = 1, regular = 2; Sorocarp size small (0.1-2.0 mm) = 0, medium (2.1-4.5 mm) = 1, large (4.6-10 mm) = 2; Slug n/a = 0, absent = 1, migrating with stalk = 2, stalkless migrating = 3; Growth habit solitary = 0, clustered/gregarious = 1, coreiform = 2; Sorus/spore pigment absent = 0, orange = 1, yellow = 2, blue/brown/lavender = 3; Stalk base simple = 0, digitate = 1, discoid = 2; Stalk tip simple = 0, compound = 1; Phototropism absent = 0, present = 1; Aggregation absent = 0, microsporium-type = 1, minutum-type = 2, mucoroides-type = 3, violaceum-type = 4; Stalk acellular = 0, cellular = 1; Amoebal nucleolus central = 0, peripheral = 1; Stalk pigment absent = 0, orange = 1, yellow = 2, blue/brown/lavender = 3; Branches non-whorled = 0, whorled = 1.

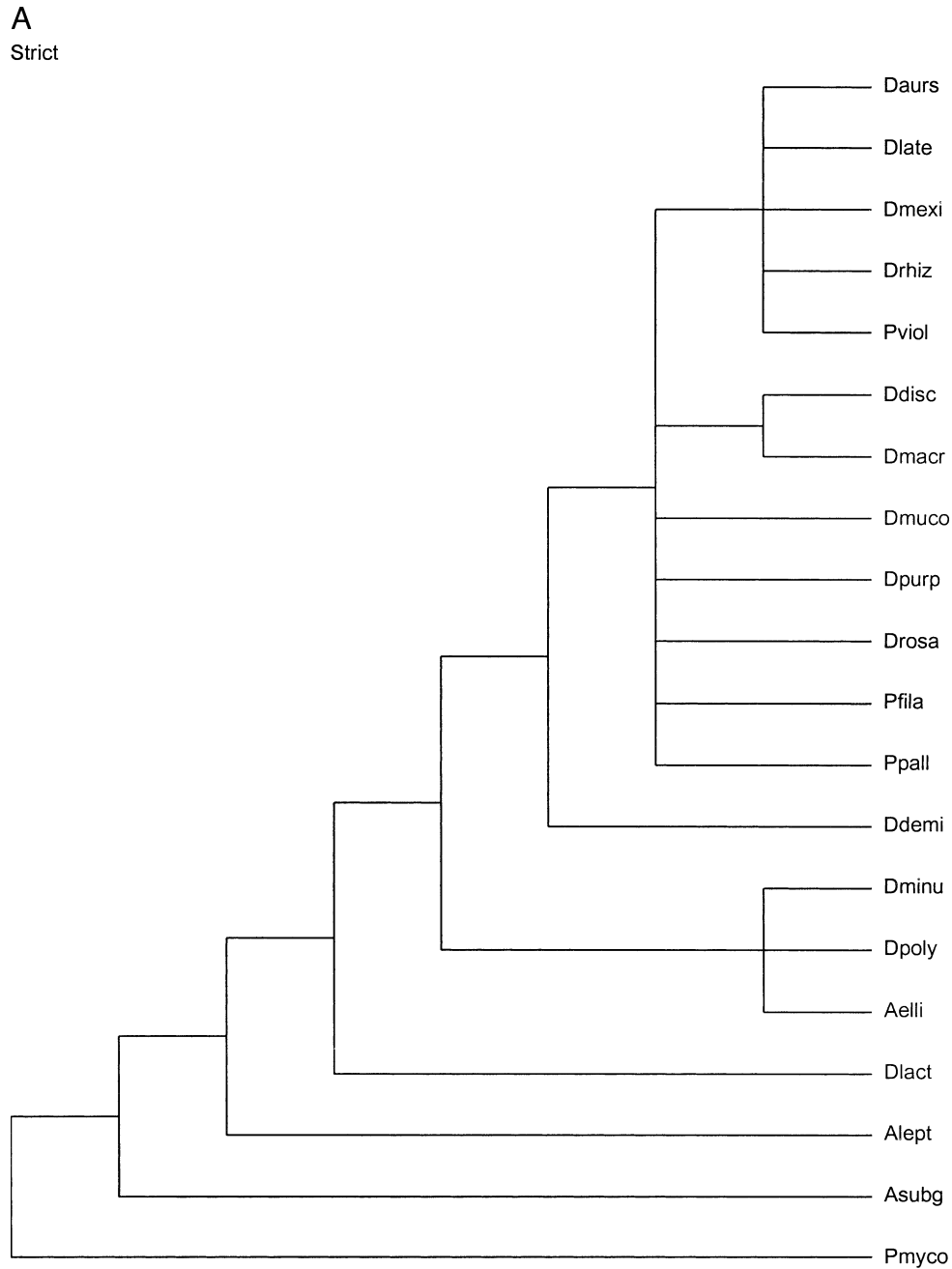


FIG. 3. A. Strict consensus of 36 most parsimonious trees. B. 80% Majority rule consensus of 36 most parsimonious trees; numbers indicate percent of trees that support the topology. (see TABLE II, footnote 'a' for species abbreviations)

that cellular stalk arose once, and is a synapomorphy of the Dictyosteliaceae, and that whorled branching arose once, and is a synapomorphy of the genus *Polysphondylium*. These have been unquestioned assumptions in all published speculation on the phylogeny of the dictyostelids. Four possible phylogenetic arrangements are consistent with the hypotheses implied by the current taxonomy (FIG. 4A–D). At one extreme (FIG. 4A), all three genera and both

families are monophyletic. At the other extreme, the only monophyletic genus is *Polysphondylium* and the only monophyletic family is the Dictyosteliaceae (FIG. 4D). In one intermediate tree, *Acytostelium* and *Polysphondylium* are monophyletic, and both families are monophyletic (FIG. 4B). In the other intermediate tree, the Acytosteliaceae and *Acytostelium* are paraphyletic, the Dictyosteliaceae is monophyletic, and within the Dictyosteliaceae, both *Dictyos-*

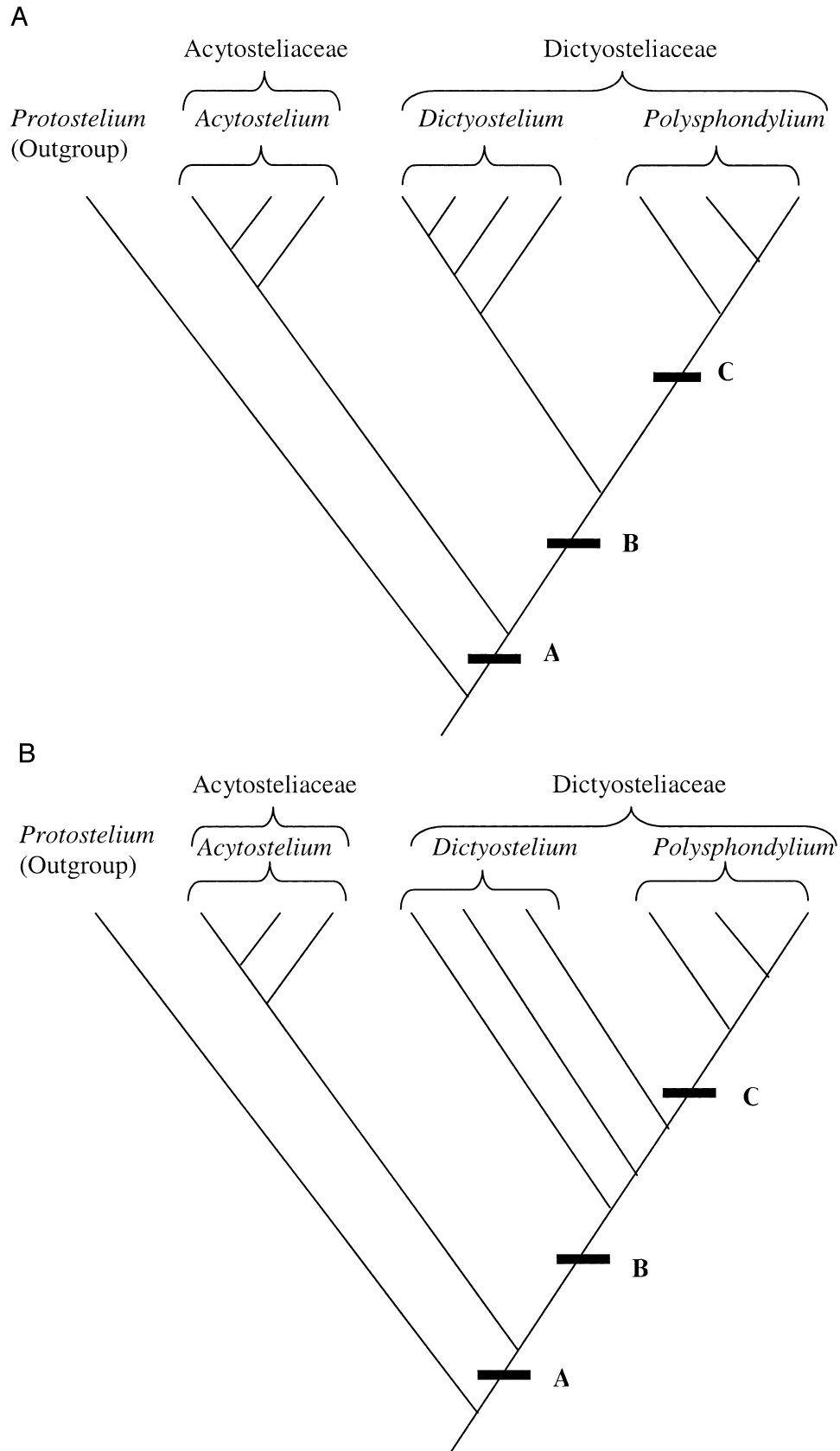


FIG. 4. A–D. Four phylogenetic arrangements consistent with the current taxonomy. A, B, and C represent the origins of streaming aggregation, cellular stalks, and whorled branching respectively.

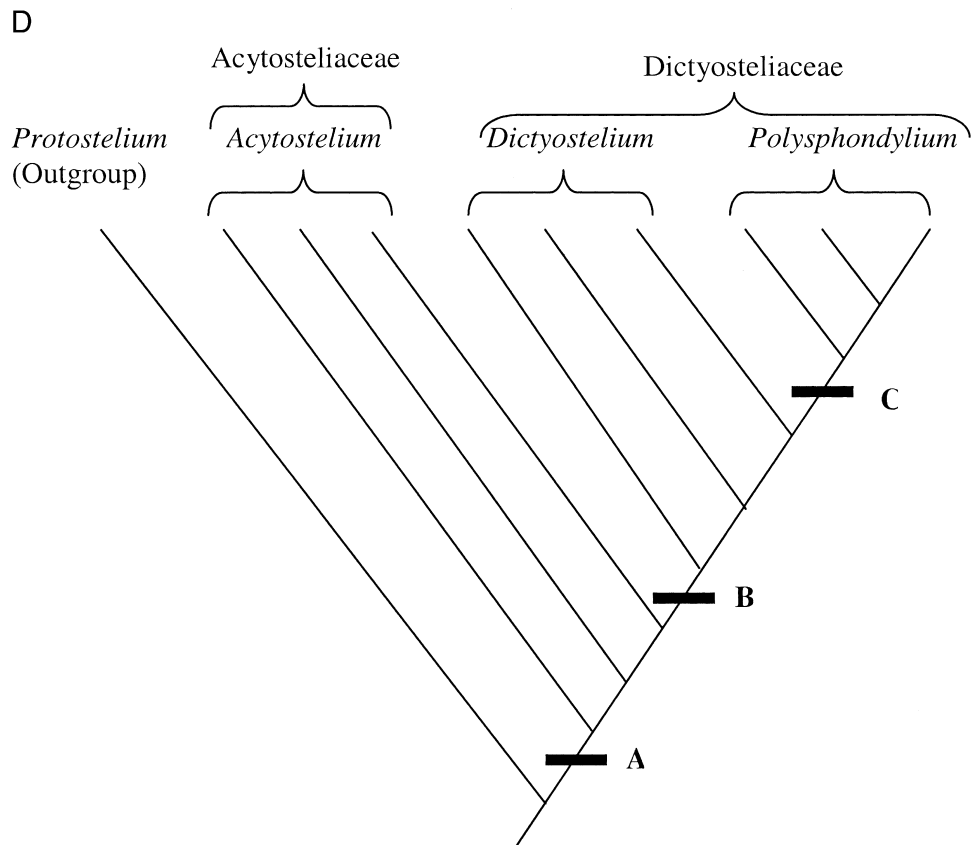
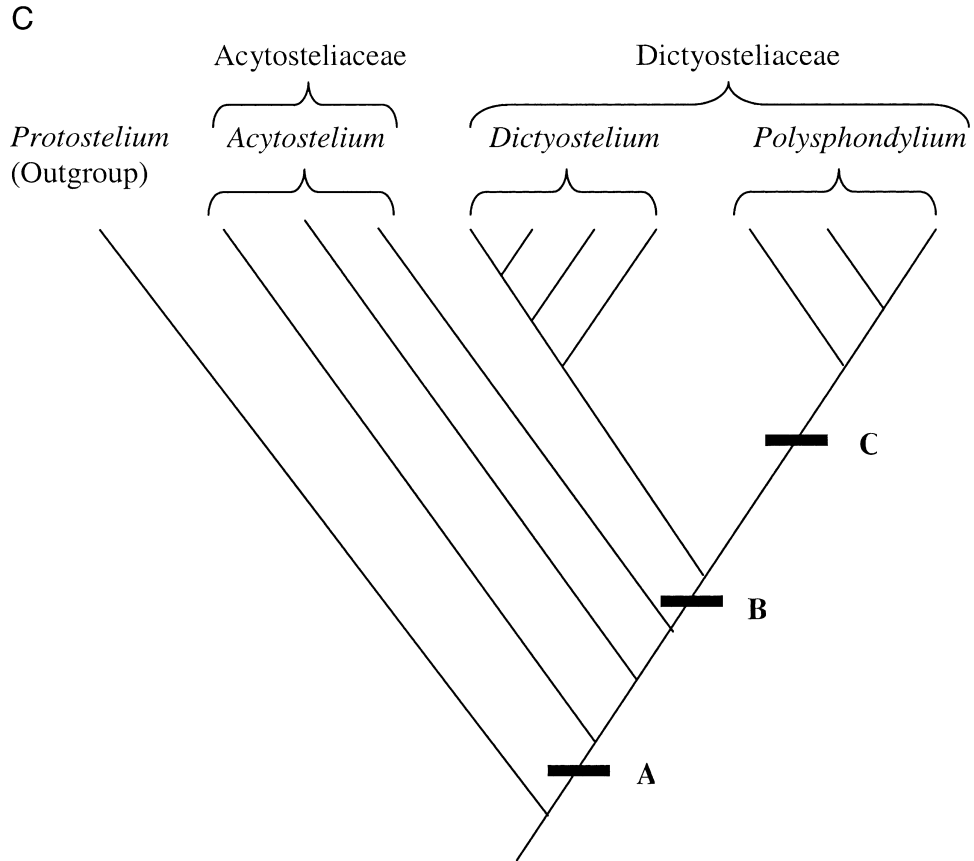


FIG. 4. Continued.

cAMP-induced aggregation, and periodic production of microcysts. By including additional morphological and physiological properties in their new concept of taxonomy, Traub and Hohl (1976) urged biologically sound groupings that reflected the biochemical aspects central to dictyostelid growth and development. We have found that when numerous taxonomically useful characters are used in a rigorous phylogenetic analysis, there is no support for the classical arrangements of families and genera within the Dictyosteliales.

When the characters drawn from the traditional taxonomy are used for phylogenetic analysis, *Acytostelium* is paraphyletic, found in several clades basal to the clade that includes the bulk of *Dictyostelium* and *Polysphondylium* (FIG. 3A). Therefore, it is likely that acellular stalk, the character state that defines *Acytostelium*, is indeed plesiomorphic for some of the *Acytostelia*. The basal position of *Dictyostelium lacteum*, however, lends support to the idea that cellular stalk, the character state that defines the Dictyosteliaceae has arisen more than once. Alternatively, the cellular stalk may have arisen only once, just basal to the clade that contains *D. lacteum*, and a character state reversal in the lineage leading to *A. ellipticum* is the basis for this species' secondarily acellular stalk. This being said, because of the implications of the classical taxonomy, the question of *how* cellular stalks evolved has not been adequately addressed. By refuting the hypothesis that cellular stalks have evolved only once, a wide range of evolutionary and developmental questions become apparent, questions that have not been posed due to restraints implicit in the taxonomy.

The position of *D. lacteum* is not entirely surprising. Robertson and Cohen (1972) speculated a "primitive" position for this species in the genus based on the relative complexity of developmental control systems and morphogenesis. Various other investigators have pointed out the close resemblance of this species to members of *Acytostelium* (Bonner 1967, Olive 1975). In fact, Bonner and Dodd (1962) reported that the stalks of *D. lacteum* contained lower cellular as well as upper acellular portions.

Using all of the taxonomic characters for phylogenetic analysis, there is no support for a monophyletic *Polysphondylium*. Members of the genus *Polysphondylium* emerge from two separate relatively apical unresolved polytomies (FIG. 3A). This analysis supports the notion that regularly spaced, whorled branching, the character state that defines *Polysphondylium*, is homoplastic, having likely arisen more than once (although it is possible that some mem-

bers of *Dictyostelium* could be secondarily whorlless). Debate about the significance of whorled branching has endured since Van Tieghem (1884) first questioned its suitability for defining a new genus (Potts 1902, Olive 1902, Rai and Tewari 1963, Raper 1984). Cox et al (1988) have suggested a relatively simple model for whorl formation that involves the interplay between chemotactic movement of cells forward, and cohesion of cells to each other. The spatial patterns of whorl formation are genetically controlled (Spiegel and Cox 1980, Cox et al 1988), but the spacing of whorls in nature may be induced or constrained by the specific micro-spatial environment that the organism occupies. Spore dissemination is the key driving force to extending the spore mass upward and/or outward, regardless of structural mechanism, and successful dispersal in nature often depends on the extension of the spore masses into appropriately large spaces in the soil. A whorling dictyostelid would therefore be able to place spores in many interstitial spaces that could be traversed by invertebrate vectors. Species with a single sorus of spores would only extend into one space. Selective pressure would likely favor "opportunistic whorling" in these instances, wherein the effectiveness of spore dispersal is maximized, without unessential cell differentiation in the migrating slug.

Polysphondylium violaceum, the type species of the genus (Brefeld 1884), emerges from within a terminal clade that includes other pigmented dictyostelids. This result is not altogether remarkable, as *P. violaceum* differs from the unpigmented members of *Polysphondylium* in several other respects, including the presence of consolidated polar granules in the spores and a marked phototropism of the migrating slug. If Brefeld had defined the genus *Polysphondylium* based on *P. violaceum*'s unique sori and stalk pigmentation, rather than on its stalk's regular whorled branching pattern, perhaps the range and scope of evolutionary questions raised about the group would be quite different. Speculation aside, based on our data set, we reject the hypothesis that *Polysphondylium* is monophyletic and that whorled branching has arisen only once.

Following the proposals of Graybeal (1998), additional taxa and characters were added to and removed from the data matrix in an attempt to support one of the hypothetical trees consistent with the classical taxonomy of the group. Only slight differences in topologies were generated, and these each remained inconsistent with the classical taxonomy. No clades were found that exclusively contained all members of either Acytosteliaceae or Dic-

tyosteliaceae. *Dictyostelium lacteum* never grouped with the cellular-stalked dictyostelids. *Polysphondylium* could be made monophyletic with the removal of several characters from the data matrix (i.e., growth habit, pigmentation, and base/tip shape), but this made the a priori assumption that whorled branching was a unifying synapomorphy. When whorled branching was removed from the data matrix, no combination of the remaining characters could hold *Polysphondylium* together in a single clade. We conclude that it is unlikely that adding more developmental and/or morphological characters will generate cladograms with topologies consistent with the classical taxonomy, although perhaps data from molecular analyses may generate trees with different topologies.

Clearly, a phylogenetic analysis using taxonomically valuable characters does not support a classification that is consistent with the current taxonomy. Further, equation of the classical taxonomy with a phylogenetic hypothesis has prevented us from asking the most appropriate evolutionary and developmental questions. For example, what evolutionary processes could lead to differences in stalk cellularity and/or regular whorled branching? Are the developmental genetics for stalk cellularity in *D. lacteum* the same for other members of *Dictyostelium*? Are the developmental genetics for whorled branching the same in *P. violaceum* and *P. pallidum*? In highlighting the poor understanding we have of dictyostelid evolution, we also emphasize our limited understanding of the evolution of many intricate processes that have made these slime molds (particularly *Dictyostelium discoideum*) such attractive and powerful model systems for the study of basic cell and developmental biology. If *D. discoideum* is a model system, then we need to know how it fits into the phylogenetic milieu of the dictyostelids in order to fully understand the implications of its biology. The evolution of cellular stalks, delays in stalk synthesis, and the "altruistic" nature of stalk formation for example, are significant events in slime mold evolution. We must be careful to consider the question of what is strictly a *D. discoideum* characteristic, what is a *Dictyostelium* characteristic, and what is a general biological characteristic. We should not rely on the prevailing taxonomy to make those decisions for us. It would benefit us to evaluate how generally applicable these characteristics are within the group before we can speculate on how generally applicable they are outside the group.

We are not presenting these phylogenetic hypotheses as a final answer, nor are we suggesting taxonomic revision. Certainly, molecular work may result

in trees with different topologies, perhaps even a tree supporting one of the hypotheses implied by the current taxonomy. Our point is, whatever taxonomic character set is adopted for a particular group of organisms should be phylogenetically tested so as not to preclude a wider range of potential hypotheses from being tested, and a more pertinent range of questions from being posed. Each time new characters are introduced, they too should be processed phylogenetically to determine what, if anything, they indicate about the evolutionary relationships within the group. In this time of integration of biological thought and synthesis of a new biological paradigm, it is important to recognize that as much as taxonomic criteria aid in the development of phylogenetic hypotheses, so obviously should phylogenetics guide accurate taxonomy.

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LITERATURE CITED

- Baldauf SL. 1999. A search for the origins of animals and fungi: comparing and combining molecular data. *Am Naturalist* 154(suppl):S178–S188.
- , Doolittle WF. 1997. Origin and evolution of the slime molds (Mycetozoa). *Proc Nat Acad Sci, USA* 94: 12007–12012.
- Barr DJS. 1992. Evolution and kingdoms of organisms from the perspective of a mycologist. *Mycologia* 84(1):1–11.
- Bonner JT. 1967. *The cellular slime molds*. Princeton, New Jersey: Princeton University Press. 205 p.
- . 1982. Evolutionary strategies and developmental constraints in the cellular slime molds. *Am Naturalist* 119:530–552.
- , Dodd MR. 1962. Aggregation territories in the cellular slime molds. *Biol Bull* 122:13–24.
- Brefeld O. 1869. *Dictyostelium mucoroides*. Ein neuer Organismus und der Verwandtschaft der Myxomyceten. *Abh Seckenberg Naturforsch Ges* 7:85–107.
- . 1884. *Polysphondylium violaceum* und *Dictyostelium mucoroides* nebst Bemerkungen zur Systematik der Schleimpilze. *Unters Gesamtgeb Mykol* 6:1–34.
- Cavalier-Smith T. 1998. Neomonada and the origin of animals and fungi. In: Coombs GH, Vickerman K, Sleight MA, Warren A, eds. *Evolutionary relationships among Protozoa*. London: Kluwer. p 375–407.
- Cox EC, Spiegel FW, Byrne G, McNally JW, Eisenbud L.

1988. Spatial patterns in the fruiting bodies of the cellular slime mold *Polysphondylium pallidum*. *Differentiation* 38:73–81.
- Drouin G, Moniz de Sa M, Zuker M. 1995. The *Giardia lamblia* actin gene and the phylogeny of eukaryotes. *J Mol Evol* 41:841–849.
- Dutta SK, Mandel M. 1972. Deoxyribonucleic acid base composition of some cellular slime molds. *J Protozool* 19:538–540.
- Dykstra MJ. 1977. The possible phylogenetic significance of mitochondrial configurations in the acrasid cellular slime molds with reference to members of the Eumycetozoa and the fungi. *Mycologia* 9:579–591.
- George RP, Hohl HR, Raper KB. 1972. Ultrastructural development of stalk-producing cells in *Dictyostelium discoideum*, a cellular slime mould. *J Gen Microbiol* 70:477–489.
- Gezelius K. 1959. The ultrastructure of cells and cellulose membranes in Acrasidae. *Exp Cell Res* 18:425–453.
- Graybeal A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? *Syst Biol* 47(1):9–17.
- Greuter W, McNeill J, Barrie FR, Burdet HM, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth DL. 2000. International code of botanical nomenclature. Koenigstein, Germany: Koeltz. 474 p.
- Guhl B, Roos U-P. 1994. Microtubule centers and the interphase microtubule cytoskeleton in amoebae of the cellular slime molds (Mycetozoa) *Acytostelium leptosomum* and *Protostelium mycophaga*. *Cell Motil Cytoskel* 28:45–58.
- Hagiwara H. 1989. The taxonomic study of Japanese Dictyostelid cellular slime molds. Tokyo, Japan: National Science Museum. 131 p.
- Heath IB. 1980. Variant mitoses in lower eukaryotes: indicators of the evolution of mitosis? *Int Rev Cytol* 64:1–80.
- Hohl HR, Hamamoto ST. 1969. Ultrastructure of spore differentiation in *Dictyostelium discoideum*: the prespore vacuole. *J Ultrastruct Res* 26:442–453.
- , ———, Hemmes DE. 1968. Ultrastructural aspects of cell elongation, cellulose synthesis, and spore differentiation in *Acytostelium leptosomum*, a cellular slime mold. *Am J Bot* 55(7):783–796.
- Keeling PJ, Doolittle WF. 1996. Alpha-Tubulin from early-diverging eukaryotic lineages and the evolution of the tubulin family. *Mol Biol Evol* 13(10):1297–1305.
- Konijn TM, Barkley DS, Chang Y-Y, Bonner JT. 1968. Cyclic AMP: a naturally occurring acrasin in the cellular slime molds. *Am Naturalist* 102:225–233.
- MacWilliams HK, Bonner JT. 1979. The prestalk-prespore pattern in cellular slime molds. *Differentiation* 14:1–22.
- Mayr E, Ashlock PD. 1991. Principles of systematic zoology. 2nd ed. New York: McGraw-Hill. 475 p.
- Moens, PB. 1976. Spindle and kinetochore morphology of *Dictyostelium discoideum*. *J Cell Biol* 68:113–122.
- Olive EW. 1902. Monograph of the Acrasidae. *Proc Boston Soc Nat Hist* 30:451–513.
- Olive LS. 1975. The Mycetozoa. New York: Academic Press. 293 p.
- . 1978. Sorocarp development by a newly discovered ciliate. *Science* 202:530–532.
- , Stoianovitch C. 1960. Two new members of the Acrasiales. *Bull Torr Bot Club* 87:1–20.
- Page FC, Blanton RL. 1985. The Heterolobosea (Sarcodina: Rhizopoda), a new class uniting the Schizopyrenida and the Acrasidae (Acrasida). *Protistologica* 11:121–132.
- Potts G. 1902. Zur Physiologie des *Dictyostelium mucoroides*. *Flora (Jena)* 91:281–347.
- Rai JN, Teari JP. 1963. Studies in cellular slime molds from Indian soils. II. On the occurrence of an aberrant strain of *Polysphondylium violaceum* Bref., with a discussion on the relevance of mode of branching of the sorocarp as a criterion for classifying members of Dictyosteliaceae. *Proc Indian Acad Sci* 58:201–206.
- Raper KB. 1984. The Dictyostelids. Princeton, New Jersey, USA: Princeton University Press. 453 p.
- Raper KB, Quinlan MS. 1958. *Acytostelium leptosomum*: a unique cellular slime mould with an acellular stalk. *J Gen Microbiol* 18:16–32.
- Robertson A, Cohen MH. 1972. Control of developing fields. *Ann Rev Biophys Bioeng* 1:409–464.
- Roger AJ, Smith MW, Doolittle RF, Doolittle WF. 1996. Evidence for the Heterolobosea from phylogenetic analysis of genes encoding glyceraldehyde-3-phosphate dehydrogenase. *J Euk Microbiol* 43(6):475–485.
- Roos U-P. 1975. Mitosis in the cellular slime mold *Polysphondylium violaceum*. *J Cell Biol* 64:480–491.
- Shaffer BM. 1964. Intracellular movement and locomotion of cellular slime-mold amoebae. In: Allen RD, Kamiya N, eds. Primitive motile systems in cell biology. New York: Academic Press, Inc. p 387–405.
- Spiegel FW. 1990. Phylum plasmodial slime molds, Class Protostelida. In: Margulis L, Corliss JO, Melkonian M, Chapman DJ, eds. Handbook of Protoctista. Boston, Massachusetts, USA: Jones & Bartlett. p 484–497.
- , Cox EC. 1980. A one-dimensional pattern in the cellular slime mould *Polysphondylium pallidum*. *Nature* 286:806–807.
- , Feldman J. 1985. Obligate amoebae of the protostelids: significance for the concept of Eumycetozoa. *BioSyst* 18:377–386.
- , Lee SB, Rusk SA. 1995. Eumycetozoa and molecular systematics. *Can J Bot* 73(suppl. 1):S738–S746.
- , Olive LS, Brown RM Jr. 1979. Roles of actin during sporocarp culmination in the simple mycetozoa *Planoprotostelium aurantium*. *Proc Nat Acad Sci, USA* 76:2335–2339.
- Swanson AR, Vadell EM, Cavender JC. 1999. Global distribution of forest soil dictyostelids. *J Biogeog* 26:133–148.

- Swofford DL. 1999. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, Massachusetts, USA: Sinauer Associates.
- Traub F, Hohl HR. 1976. A new concept for the taxonomy of the family Dictyosteliaceae (cellular slime molds). *Am J Bot* 63(5):664–672.
- Vadell EM, Cavender JC. 1991. Phylogeny of the Family Dictyosteliaceae. Abstracts: IV Int. Dicty. Conf. Univ. of British Columbia, Vancouver.
- Van Tieghem P. 1884. *Coenonia*, genre nouveau de Myxomycètes à plasmode agrégé. *Bull Soc Bot Fr* 31:303–306.