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NOTE

Molecular Systematics of Dictyostelids: 5.8S Ribosomal DNA and Internal Transcribed Spacer Region Analyses[▽]

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The variability and adaptability of the amoebae from the class *Dictyosteliomycetes* greatly complicate their systematics. The nucleotide sequences of the ribosomal internal transcribed spacers and the 5.8S ribosomal DNA gene have been determined for 28 isolates, and their utility to discriminate between different species and genera has been shown.

Dictyostelids are amoebae, frequently found in soils (9), discovered by Oskar Brefeld in 1869 (4) and grouped with myxomycetes and protostelids in the *Eumycetozoa* (3, 8). A characteristic common to all dictyostelids is the capacity to form multicellular structures by aggregation (6). The morphogenetic process is very plastic, and variations in several characteristics of the structures have been used to classify these organisms. More than 100 species have been described and grouped in three genera: *Acytostelium*, *Dictyostelium*, and *Polysphondylium*.

Systematics in dictyostelids is difficult and, in some cases, controversial due to the relatively few morphological characteristics available for classification and the large variability and adaptability of their fruiting bodies (7, 9). As a consequence, it is often difficult to ascertain whether two samples pertain to the same species. These problems have even led to the description of species complexes, such as *Dictyostelium mucoroides* or *Polysphondylium pallidum*, which group specimens with similar (7, 9), but perhaps not identical, characteristics.

Molecular phylogenetic analyses might add significant information for the systematic classification of dictyostelids. A genomic region frequently used in phylogenetic studies, including ribosomal DNAs (rDNA) and their internal transcribed spacers (ITS) (recently reviewed in references 1 and 13), was analyzed in this study.

Dictyostelids were collected from several locations in the south of Europe (Spain and Portugal) or obtained from other collections, as shown in Table 1. Cells were processed and classified according to Raper (9) using the following morphological criteria.

(i) *Acytostelium leptosomum* Raper. Sorocarps of small size (750 to 1,500 μm in length), erect, with typical cluster growth. Numerous fructifications (20 in a cluster). Sorophore acellular, no pigmentation, with piliform and very thin tip. Spores globose with polar granules distributed irregularly around them. Aggregations radiate with streams thin and long. Migratory slugs. There is considerable difference between the sorocarps of the first isolates in HI agar and those of pure cultures (smaller size in the latter).

(ii) *Acytostelium* sp. Sorocarps of very small size (300 to 600 μm in length), solitary and very delicate. Sorophore acellular and slender without pigmentation. Spores globose with polar granules. Aggregation is mound type. Long and narrow slugs that do not migrate. Grows more slowly than genus *Dictyostelium*. Need charcoal to get good fructifications. Presence of microcysts in these isolates.

(iii) *Dictyostelium giganteum* B. N. Singh. Sorocarps of big size (4 to 10 mm in length), usually prostrate. Sorophores very long and wavy with tip capitata, no branches and no pigmentation. Sori elipsoid. Spores elipsoid, 5 to 7 by 3 to 4 μm, without polar granule. Aggregation radiate. Slugs that migrate long distances producing stalk.

(iv) *Dictyostelium minutum* Raper. Sorocarps erect, of small size (0.5 to 0.85 mm in length), with cluster growth. Sorophores colorless. Spores ellipsoid and small, 5 to 6 by 3 to 3.5 μm, without polar granules. Typical aggregation of the species, mound like. Slugs do not migrate.

(v) *Dictyostelium mucoroides* Bref. Sorocarps of medium size (2 to 5 mm in length), erect or semiprostrate with solitary growth. Sorophore with no pigmentation and round base. Spores ellipsoid, 4.5 to 6 by 2.5 to 3.5 μm, without polar granules. Aggregations radiate and small, characteristic of this species.

(vi) *Dictyostelium sphaerocephalum* (Oud.) Sacc. et March. Sorocarps of medium size (2 to 4 mm in length) in relation with others species of cellular slime molds, erect or semierect with solitary growth and no pigmentation. Sorophore hyaline, sometimes irregular and sparse branching, with round base and acuminate tip with many tiers of cells. Presence of collar near the tip. Sori white, big in relation with the whole sorocarp. Spores ellipsoid and wide, 5.5 to 7 by 3 to 3.5 μm, with vacuolated cells without polar granules. Aggregations radiate and small. Some slugs migrate but not long distances.

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TABLE 1. Geographic origins of dictyostelid isolates and GenBank accession numbers of the nucleotide sequences

Species	Origin	Isolate	GenBank accession no.
Acytostelium leptosomum	Algarve, Portugal	212	AM282588
Acytostelium sp.	Tennessee	GCE9	AM282589
	North Carolina	DCB6B	AM282590
Dictyostelium giganteum	North Carolina	1880	AM282591
	North Carolina	1889	AM282592
Dictyostelium minutum	Sevilla, Spain	11C	AM282593
	Texas	L4B	AM282594
Dictyostelium mucoroides	Ciudad Real, Spain	1C	AM282595
	Huelva, Spain	89C	AM282596
	Madrid, Spain	115A	AM282597
	Soria, Spain	133B	AM282598
	Arkansas	AR4B	AM282599
Dictyostelium sphaerocephalum	Sevilla, Spain	14A	AM282600
	Huelva, Spain	20B	AM282601
	Huesca, Spain	88A	AM282602
	Huelva, Spain	89B	AM282603
	Huelva, Spain	89G	AM282604
	Madrid, Spain	118B	AM282605
Polysphondylium candidum	Ciudad Real, Spain	3A	AM282606
	Ciudad Real, Spain	7A	AM282607
	Sevilla, Spain	11F	AM282608
	Sevilla, Spain	15	AM282609
	Huelva, Spain	89A	AM282610
	Huelva, Spain	89C2	AM282611
	Huelva, Spain	89D	AM282612
	Tennessee	PH8B	AM282613
Polysphondylium pallidum	Huelva, Spain	23D	AM282614
	Ohio	PP1	AM282615

(vii) *Polysphondylium candidum*. H. Hagiw. Sorocarps of small size and solitary growth. Sorophores delicate and long with one tier of cells. Presence of supporters. Sorophores with no pigmentation and 2 to 8 whorls of 2 to 7 branches. These branches are symmetric but with different sizes. Sometimes seems to be irregular branching more than whorls. Also there are some sorocarps with no branches and no whorls. Some sorophores finish in terminal sori but the majority finish in terminal elongation. Spores ellipsoid and wide, 7 to 8 by 4 to 5 μm, with unconsolidated, but clearly evident, polar granules. Aggregates very big, radiate. Slugs that migrate large distances.

(viii) *Polysphondylium pallidum* Olive. Sorocarps solitary with 1 to 11 whorls of 2 to 6 branches, not phototropic and colorless. Sorophores without lengthened terminal segments. Spores 5 to 7 by 2.5 to 3 μm, with unconsolidated polar granules. Aggregation radiate smaller than that of *P. candidum*. Slugs that migrate.

Amoebae were grown in association with *Klebsiella aerogenes*, and their DNA was obtained using MasterAmp DNA extraction solution (Epicentre Technologies, Madison, WI). The rDNA locus coding for the 5.8S rRNA and the two flanking internal transcribed spacers, ITS1 and ITS2, was amplified by PCR using the oligonucleotides 5′-GAGGAAGGAGAAGTCGTAACAAGGTATC-3′ and 5′-GCTTACTGATATGCTTAAGTTCAGCGGG-3′. Amplified DNAs were sequenced in both strands by use of flanking and internal primers.

The sizes of the 5.8S rDNA varied between 161 and 166 nucleotides (nt) (data not shown). Multiple alignment of the sequences, made using the ClustalW program (12), allowed us to determine the percentage of nucleotide identity between samples (Table 2; summarized in Table 3). Samples classified in the same species ranged between 97 and 100% identity, indicating a low level of intraspecific variability. Identities between species from the same genera varied between 92 and 98%, the exception being *Dictyostelium minutum*, which showed an identity of around 70% (69 to 73%) with other *Dictyostelium* species, very similar to the identities of *D. minutum* with *Polysphondylium* and *Acytostelium* species (62 to 71%). The identity between *Acytostelium* species (82%) was lower than that for *Dictyostelium* and *Polysphondylium* species. The identities between samples from different genera ranged from 69.3 to 88.3%.

The 5.8S rDNA multiple alignment, excluding nonaligned positions, was used to construct a phylogenetic tree, shown in Fig. 1. Statistical significance, indicated by bootstrap values, is shown for the more consistent branches of the tree. As expected for the homology data, most of the samples for the same species branched closely together, except for the five

TABLE 2. Percentage of identity of the 5.8S rDNA regions between the different isolates

Genus	Species	Isolate	% Identity with:																										
			212	GCE9	DCB6B	1880	1889	11C	L4B	AR4B	1C	133B	115A	89C	20B	88A	89B	89G	14A	118B	PH8B	3A	7A	11F	15	89A	89C2	89D	23D	Pp1
Acytostelium	*leptosomum*	212	100	82.0	82.0	73.2	74.3	61.8	62.2	73.2	73.2	73.2	73.8	73.8	73.0	73.8	73.8	72.6	73.8	73.8	79.5	79.5	77.5	79.5	79.5	79.5	79.5	78.8	78.8
	sp.	GCE9	82.0	100	100	81.9	83.1	66.9	67.3	83.1	83.1	83.1	82.5	82.5	83.3	84.4	84.4	82.8	84.4	84.4	88.3	88.3	86.0	88.3	88.3	88.3	88.3	88.8	88.8
	sp.	DCB6B	82.0	100	100	81.9	83.1	66.9	67.3	83.1	83.1	83.1	82.5	82.5	83.3	84.4	84.4	82.8	84.4	84.4	88.3	88.3	86.0	88.3	88.3	88.3	88.3	88.8	88.8
Dictyostelium	*giganteum*	1880	73.2	81.9	81.9	100	98.7	69.9	70.4	97.5	97.5	97.5	98.1	98.1	95.7	96.9	96.9	95.1	96.9	96.9	85.8	85.8	83.5	85.8	85.8	85.8	85.8	84.5	84.5
	giganteum	1889	74.3	83.1	83.1	98.7	100	71.2	71.6	98.7	98.7	98.7	99.4	99.4	96.9	98.1	98.1	96.3	98.1	98.1	87.0	87.0	84.8	87.0	87.0	87.0	87.0	85.7	85.7
	minutum	11C	61.8	66.9	66.9	69.9	71.2	100	98.1	72.4	72.4	72.4	71.8	71.8	70.1	70.6	70.6	69.3	70.6	70.6	69.9	69.9	68.5	69.9	69.9	69.9	69.9	70.6	70.6
	minutum	L4B	62.2	67.3	67.3	70.4	71.6	98.1	100	72.8	72.8	72.8	72.2	72.2	70.6	71.0	71.0	69.7	71.0	71.0	70.4	70.4	68.9	70.4	70.4	70.4	70.4	71.0	71.0
	mucoroides	AR4B	73.2	83.1	83.1	97.5	98.7	72.4	72.8	100	100	100	99.4	99.4	96.9	98.1	98.1	96.3	98.1	98.1	85.8	85.8	83.5	85.8	85.8	85.8	85.8	85.7	85.7
	mucoroides	1C	73.2	83.1	83.1	97.5	98.7	72.4	72.8	100	100	100	99.4	99.4	96.9	98.1	98.1	96.3	98.1	98.1	85.8	85.8	83.5	85.8	85.8	85.8	85.8	85.7	85.7
	mucoroides	133B	73.2	83.1	83.1	97.5	98.7	72.4	72.8	100	100	100	99.4	99.4	96.9	98.1	98.1	96.3	98.1	98.1	85.8	85.8	83.5	85.8	85.8	85.8	85.8	85.7	85.7
	mucoroides	115A	73.8	82.5	82.5	98.1	99.4	71.8	72.2	99.4	99.4	99.4	100	100	96.3	97.5	97.5	95.7	97.5	97.5	86.4	86.4	84.1	86.4	86.4	86.4	86.4	85.1	85.1
	mucoroides	89C	73.8	82.5	82.5	98.1	99.4	71.8	72.2	99.4	99.4	99.4	100	100	96.3	97.5	97.5	95.7	97.5	97.5	86.4	86.4	84.1	86.4	86.4	86.4	86.4	85.1	85.1
	sphaerocephalum	20B	73.0	83.3	83.3	95.7	96.9	70.1	70.6	96.9	96.9	96.9	96.3	96.3	100	98.8	98.8	97.0	98.8	98.8	87.1	87.1	84.8	87.1	87.1	87.1	87.1	87.0	87.0
	sphaerocephalum	88A	73.8	84.4	84.4	96.9	98.1	70.6	71.0	98.1	98.1	98.1	97.5	97.5	98.8	100	100	98.1	100	100	87.7	87.7	84.8	87.7	87.7	87.7	87.7	87.6	87.6
	sphaerocephalum	89B	73.8	84.4	84.4	96.9	98.1	70.6	71.0	98.1	98.1	98.1	97.5	97.5	98.8	100	100	98.1	100	100	87.7	87.7	85.4	87.7	87.7	87.7	87.7	87.6	87.6
	sphaerocephalum	89G	72.6	82.8	82.8	95.1	96.3	69.3	69.7	96.3	96.3	96.3	95.7	95.7	97.0	98.1	98.1	100	98.1	98.1	86.1	86.1	83.8	86.1	86.1	86.1	86.1	86.0	86.0
	sphaerocephalum	14A	73.8	84.4	84.4	96.9	98.1	70.6	71.0	98.1	98.1	98.1	97.5	97.5	98.8	100	100	98.1	100	100	87.7	87.7	85.4	87.7	87.7	87.7	87.7	87.6	87.6
	sphaerocephalum	118B	73.8	84.4	84.4	96.9	98.1	70.6	71.0	98.1	98.1	98.1	97.5	97.5	98.8	100	100	98.1	100	100	87.7	87.7	85.4	87.7	87.7	87.7	87.7	87.6	87.6
Polysphondylium	*candidum*	PH8B	79.5	88.3	88.3	85.8	87.0	69.9	70.4	85.8	85.8	85.8	86.4	86.4	87.1	87.7	87.7	86.1	87.7	87.7	100	100	97.6	100	100	100	100	92.6	92.6
	candidum	3A	79.5	88.3	88.3	85.8	87.0	69.9	70.4	85.8	85.8	85.8	86.4	86.4	87.1	87.7	87.7	86.1	87.7	87.7	100	100	97.6	100	100	100	100	92.6	92.6
	candidum	7A	77.5	86.0	86.0	83.5	84.8	68.5	68.9	83.5	83.5	83.5	84.1	84.1	84.8	85.4	85.4	83.8	85.4	85.4	97.6	97.6	100	97.6	97.6	97.6	97.6	90.2	90.2
	candidum	11F	79.5	88.3	88.3	85.8	87.0	69.9	70.4	85.8	85.8	85.8	86.4	86.4	87.1	87.7	87.7	86.1	87.7	87.7	100	100	97.6	100	100	100	100	92.6	92.6
	candidum	15	79.5	88.3	88.3	85.8	87.0	69.9	70.4	85.8	85.8	85.8	86.4	86.4	87.1	87.7	87.7	86.1	87.7	87.7	100	100	97.6	100	100	100	100	92.6	92.6
	candidum	89A	79.5	88.3	88.3	85.8	87.0	69.9	70.4	85.5	85.5	85.5	86.4	86.4	87.1	87.7	87.7	86.1	87.7	87.7	100	100	97.6	100	100	100	100	92.6	92.6
	candidum	89C2	79.5	88.3	88.3	85.8	87.0	69.9	70.4	85.8	85.8	85.8	86.4	86.4	87.1	87.7	87.7	86.1	87.7	87.7	100	100	97.6	100	100	100	100	92.6	92.6
	candidum	89D	79.5	88.3	88.3	85.8	87.0	69.9	70.4	85.8	85.8	85.8	86.4	86.4	87.1	87.7	87.7	86.1	87.7	87.7	100	100	97.6	100	100	100	100	92.6	92.6
	pallidum	23D	78.8	88.8	88.8	84.5	85.7	70.6	71.0	85.7	85.7	85.7	85.1	85.1	87.0	87.6	87.6	86.0	87.6	87.6	92.6	92.6	90.2	92.6	92.6	92.6	92.6	100	100
	pallidum	Pp1	78.8	88.8	88.8	84.5	85.7	70.6	71.0	85.7	85.7	85.7	85.1	85.1	87.0	87.6	87.6	86.0	87.6	87.6	92.6	92.6	90.2	92.6	92.6	92.6	92.6	100	100

TABLE 3. Summary of identities[a]

Type of comparison	Species or genus (genera)[b]	% Identity (mean ± SD)		
		Complete region	5.8S rDNA	ITS1 region
Species	*D. mucoroides*	79.07 ± 12.34	99.64 ± 0.29	77.91 ± 12.26
	D. sphaerocephalum	99.07 ± 0.89	98.97 ± 0.94	99.67 ± 0.47
	P. candidum	97.57 ± 2.64	99.40 ± 1.04	96.64 ± 3.97
Same genus	*Dictyostelium*	56.78 ± 11.72	88.73 ± 12.39	49.61 ± 10.91
	Polysphondylium	50.97 ± 0.99	92.3 ± 0.79	52.39 ± 0.62
Different genera	D/A	39.79 ± 4.81	78.09 ± 6.67	39.32 ± 4.65
	P/D	33.84 ± 3.21	84.20 ± 5.61	22.74 ± 4.43
	P/A	34.64 ± 5.71	85.17 ± 4.31	24.59 ± 4.56

[a] Identities were observed between different isolates from the same species and different species, from the same genus or different genera, and for the complete region (including the rDNA and ITS1 and ITS2 regions), the 5.8S rDNA, and the ITS1 region.
[b] D, *Dictyostelium*; A, *Acytostelium*; P, *Polysphondylium*.

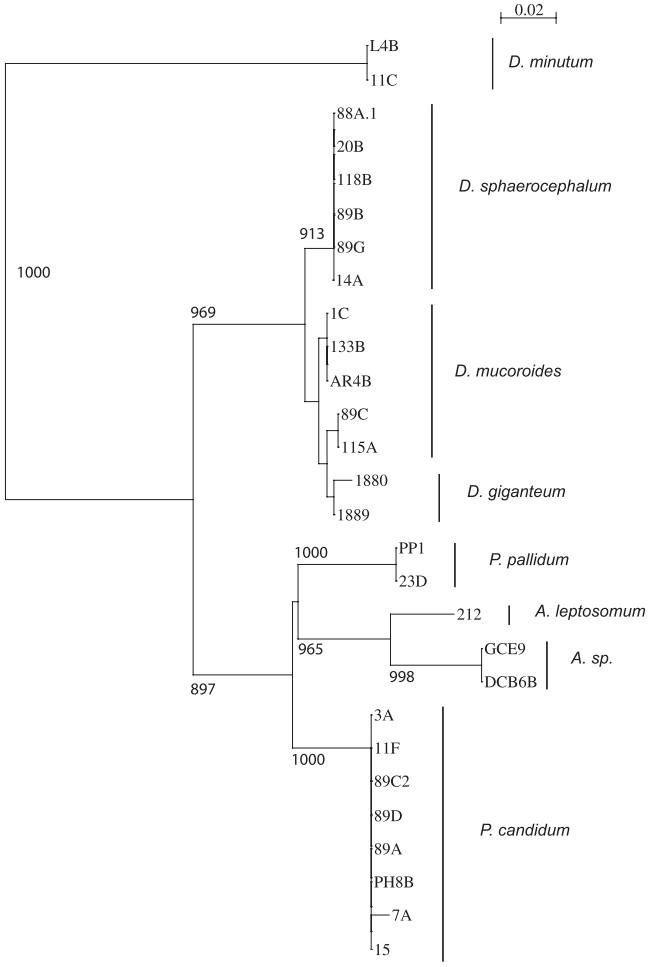

FIG. 1. Phylogenetic tree of dictyostelid species grouped according to their 5.8S rDNA sequences. The nucleotide sequences of the 5.8S rDNA from field and collection samples of different dictyostelid species, corresponding to the three genera described, *Dictyostelium*, *Acytostelium*, and *Polysphondylium*, were compared using the programs ClustalW, at the online Biology WorkBench facilities from the San Diego Supercomputer Center (http://workbench.sdsc.edu), and ClustalX (11). The 5.8S rDNA alignment was also visually optimized, and nonaligned positions were omitted. Nucleotide divergences between sequences were determined using the MVIEW program (5) at the Biology WorkBench facilities. Phylogenetic trees were determined using the neighbor-joining method (10) and the ClustalX program. A random generator seed of 111 and 1,000 bootstrap trials were calculated, and the number of times that each branch was obtained is indicated at left for values above 850. Trees were drawn using the njplot program. The evolutionary-distance scale, calculated as the fraction of nucleotide changes, is indicated in the upper right corner of the figure. The species in which the different isolates were classified are indicated at right.

samples of *D. mucoroides*, which associate in two groups: samples 1C, 133B, and AR4B and samples 89C and 115A. Samples from different species grouped in their specific genera, except for *D. minutum*, which segregated from the rest of the *Dictyostelium* samples. The closer association of *Acytostelium* species with *Polysphondylium* species than with *Dictyostelium* species is also noticeable and statistically significant.

Analyses of these data indicate that the comparison of 5.8S rDNA sequences can be very informative to discriminate between samples from different genera. Interspecific comparisons within the same genera indicate that the 5.8S rDNA sequence is highly conserved, so that the divergence between 5.8S rDNA sequences of some species (for example, *D. mucoroides* and and *D. giganteum*) was similar to intraspecific variability. This similarity makes the 5.8S rDNA sequence comparison of restricted utility to discriminate between isolates of species from the same genera.

ITS1 regions are 278 to 312 nt long in *Dictyostelium* spp. and *Acytostelium* spp. and 192 to 253 nt long in *Polysphondylium* spp., with the exception of that of *Acytostelium leptosomum*, which is 245 nt long (data not shown). The differences are larger in the ITS2 region, where sizes vary between 311 and 383 nt in *Acytostelium* spp., 417 and 486 nt in *Dictyostelium* spp., and 667 and 856 nt in *Polysphondylium* spp. (data not shown).

Most species showed high similarity between samples in their ITS1 regions (Table 4; summarized in Table 3) (similar results were obtained for ITS2), which indicates low intraspecific variability. For example, eight samples from *Dictyostelium sphaerocephalum* and six from *Polysphondylium candidum* showed 98 to 100% identity between them. *Acytostelium* spp., *D. minutum*, and *P. pallidum* also showed over 95% (95 to 100%) identity between the two samples analyzed for each species. For some of these species, one of the samples was collected in the United States and the other in the south of

TABLE 4. Percentage of identity of the ITS1 regions between different isolates

Genus	Species	Isolate	% Identity with:																										
			212	GCE9	DCB6B	1880	1889	11C	L4B	AR4B	1C	133B	115A	89C	20B	88A	89B	89G	14A	118B	PH8B	3A	7A	11F	15	89A	89C2	89D	23D	Pp1
Acytostelium	*leptosomum*	212	100	36.0	36.0	38.1	26.7	35.2	35.2	29.4	36.7	36.7	33.9	33.9	35.2	33.2	35.2	35.2	35.2	35.2	28.7	31.5	32.2	31.8	31.8	31.8	31.8	31.8	27.7	27.7
	sp.	GCE9	36.0	100	100	43.9	40.5	35.1	36.5	38.2	42.2	42.3	41.3	41.3	45.1	40.4	45.1	45.1	45.1	45.1	18.8	21.0	21.6	21.3	21.3	21.3	21.3	21.3	23.8	23.8
	sp.	DCB6B	36.0	100	100	43.9	40.5	35.1	36.5	38.2	42.2	42.3	41.3	41.3	45.1	40.4	45.1	45.1	45.1	45.1	18.8	21.0	21.6	21.3	21.3	21.3	21.3	21.3	23.8	23.8
Dictyostelium	*giganteum*	1880	38.1	43.9	43.9	100	74.1	36.1	37.0	49.7	52.2	52.4	52.0	52.0	57.2	53.4	57.2	57.2	57.2	57.2	20.5	21.7	22.0	22.0	22.0	21.7	21.7	21.7	24.7	24.7
	giganteum	1889	26.7	40.5	40.5	74.1	100	30.5	31.0	49.3	49.6	49.6	51.8	51.8	54.6	49.6	54.6	54.6	54.6	54.6	14.5	16.7	16.9	16.9	16.9	16.7	16.7	16.7	19.7	19.7
	minutum	11C	35.2	35.1	35.1	36.1	30.5	100	95.3	35.5	37.7	37.7	36.6	36.6	34.4	31.4	34.4	34.4	34.4	34.4	29.9	32.5	33.3	32.8	32.8	33.0	33.0	33.0	35.2	35.1
	minutum	L4B	35.2	36.5	36.5	37.0	31.0	95.3	100	36.3	38.5	38.5	36.5	36.5	35.2	32.3	35.2	35.2	35.2	35.2	30.6	32.8	33.3	33.0	33.0	33.0	33.0	33.0	34.2	34.2
	mucoroides	AR4B	29.4	38.2	38.2	49.7	49.3	35.5	36.3	100	82.3	82.5	65.7	65.7	58.0	54.3	58.0	58.0	58.0	58.0	20.8	22.7	23.2	22.9	22.9	22.9	22.9	22.9	22.8	22.8
	mucoroides	1C	36.7	42.2	42.2	52.2	49.6	37.7	38.5	82.3	100	99.7	70.7	70.7	59.2	55.2	59.2	59.2	59.2	59.2	20.6	22.6	23.2	22.9	22.9	22.9	22.9	22.9	24.6	24.6
	mucoroides	133B	36.7	42.3	42.3	52.4	49.6	37.7	38.5	82.5	99.7	100	70.9	70.9	59.4	55.4	59.4	59.4	59.4	59.4	20.6	22.7	23.2	23.0	23.0	23.0	23.0	23.0	24.6	24.6
	mucoroides	115A	33.9	41.3	41.3	52.0	51.8	36.6	36.5	65.7	70.7	70.9	100	100	63.0	57.9	63.0	63.0	63.0	63.0	20.0	21.6	21.6	21.9	21.9	21.3	21.3	21.3	25.1	25.4
	mucoroides	89C	33.9	41.3	41.3	52.0	51.8	36.6	36.5	65.7	70.7	70.9	100	100	63.0	57.9	63.0	63.0	63.0	63.0	20.0	21.6	21.6	21.9	21.9	21.3	21.3	21.3	25.1	25.4
	sphaerocephalum	20B	35.2	45.1	45.1	57.2	54.6	34.4	35.2	58.0	59.2	59.4	63.0	63.0	100	99	100	100	100	100	18.7	20.0	20.9	20.6	20.6	20.6	20.6	20.6	21.0	21.3
	sphaerocephalum	88A	33.2	40.4	40.4	53.4	49.6	31.4	32.3	54.3	55.2	55.4	57.9	57.9	99.0	100	99.0	99.0	99.0	99.0	19.3	20.4	21.3	21.0	21.0	21.0	21.0	21.0	17.7	18.0
	sphaerocephalum	89B	35.2	45.1	45.1	57.2	54.6	34.4	35.2	58.0	59.2	59.4	63.0	63.0	100	99.0	100	100	100	100	18.7	20.0	20.9	20.6	20.6	20.6	20.6	20.6	21.0	21.3
	sphaerocephalum	89G	35.2	45.1	45.1	57.2	54.6	34.4	35.2	58.0	59.2	59.4	63.0	63.0	100	99.0	100	100	100	100	18.7	20.0	20.9	20.6	20.6	20.6	20.6	20.6	21.0	21.3
	sphaerocephalum	14A	35.2	45.1	45.1	57.2	54.6	34.4	35.2	58.0	59.2	59.4	63.0	63.0	100	99.0	100	100	100	100	18.7	20.0	20.9	20.6	20.6	20.6	20.6	20.6	21.0	21.3
	sphaerocephalum	118B	35.2	45.1	45.1	57.2	54.6	34.4	35.2	58.0	59.2	59.4	63.0	63.0	100	99.0	100	100	100	100	18.7	20.0	20.9	20.6	20.6	20.6	20.6	20.6	21.0	21.3
Polysphondylium	*candidum*	PH8B	28.7	18.8	18.8	20.5	14.5	29.9	30.6	20.8	20.6	20.6	20.0	20.0	18.7	19.3	18.7	18.7	18.7	18.7	100	89.4	89.8	90.2	90.2	89.8	89.8	89.8	51.0	51.4
	candidum	3A	31.5	21.0	21.0	21.7	16.7	32.5	32.8	22.7	22.6	22.7	21.6	21.6	20.0	20.4	20.0	20.0	20.0	20.0	89.4	100	97.4	99.2	99.2	98.1	98.1	98.1	51.9	52.3
	candidum	7A	32.2	21.6	21.6	22.0	16.9	33.3	33.3	23.2	23.2	23.2	21.6	21.6	20.9	21.3	20.9	20.9	20.9	20.9	89.8	97.4	100	98.0	98.1	99.2	99.2	99.2	52.7	53.1
	candidum	11F	31.8	21.3	21.3	22.0	16.9	32.8	33.0	22.9	22.9	23.0	21.9	21.9	20.6	21.0	20.6	20.6	20.6	20.6	90.2	99.2	98.0	100	100	98.8	98.8	98.8	51.9	52.3
	candidum	15	31.8	21.3	21.3	22.0	16.9	32.8	33.0	22.9	22.9	23.0	21.9	21.9	20.6	21.0	20.6	20.6	20.6	20.6	90.2	99.2	98.1	100	100	98.9	98.9	98.9	51.9	52.3
	candidum	89A	31.8	21.3	21.3	21.7	16.7	33.0	33.0	22.9	22.9	23.0	21.3	21.3	20.6	21.0	20.6	20.6	20.6	20.6	89.8	98.1	99.2	98.8	98.9	100	100	100	52.7	53.1
	candidum	89C2	31.8	21.3	21.3	21.7	16.7	33.0	33.0	22.9	22.9	23.0	21.3	21.3	20.6	21.0	20.6	20.6	20.6	20.6	89.8	98.1	99.2	98.8	98.9	100	100	100	52.7	53.1
	candidum	89D	31.8	21.3	21.3	21.7	16.7	33.0	33.0	22.9	22.9	23.0	21.3	21.3	20.6	21.0	20.6	20.6	20.6	20.6	89.8	98.1	99.2	98.8	98.9	100	100	100	52.7	53.1
	pallidum	23D	27.7	23.8	23.8	24.7	19.7	35.2	34.2	22.8	24.6	24.6	25.1	25.1	21.0	17.7	21.0	21.0	21.0	21.0	51.0	51.9	52.7	51.9	51.9	52.7	52.7	52.7	100	99.6
	pallidum	Pp1	27.7	23.8	23.8	24.7	19.7	35.1	34.2	22.8	24.6	24.6	25.4	25.4	21.3	18.0	21.3	21.3	21.3	21.3	51.4	52.3	53.1	52.3	52.3	53.1	53.1	53.1	99.6	100

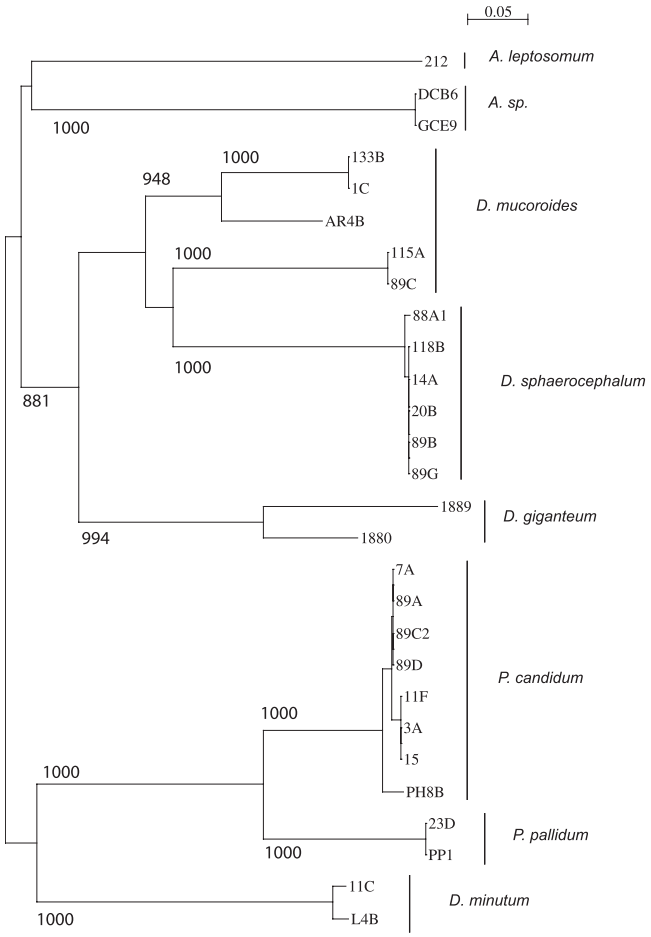

FIG. 2. Phylogenetic tree of dictyostelid species grouped according to their ITS1 nucleotide sequences. The nucleotide sequences of the ITS1 region were determined from field and collection samples of eight different species from the three dictyostelid genera: *Dictyostelium*, *Acytostelium*, and *Polysphondylium*. Sequences were aligned using the ClustalW and ClustalX programs, and the multiple alignment was used to construct a phylogenetic tree using the neighbor-joining method, as described in the legend for Fig. 1. The evolutionary-distance scale, calculated as the fraction of nucleotide changes, is shown in the upper right corner of the figure. One thousand bootstrap trials were calculated, and the values higher than 850 are shown at left for the corresponding branches. The species in which the different isolates were classified are shown at right.

Europe, two distant and different regions of the world. Only two species showed a larger divergence between samples (65 to 82%), *Dictyostelium giganteum* and *D. mucoroides*. The data on *D. mucoroides* are in concordance with those previously described for the 5.8S rDNA.

Identities between species from the same genera were on the order of 50 to 60% (Tables 3 and 4). The exception is *D. minutum*, which showed an identity of 30 to 38% with other *Dictyostelium* species, and *A. leptosomum*, which was 36% identical to other *Acytostelium* spp. Samples of different genera are less than 45% identical, which is too low for phylogenetic analyses.

The data on ITS1 alignments were used to generate the phylogenetic tree shown in Fig. 2. All of the samples from *D. sphaerocephalum* and *P. candidum* grouped closely together between them. However, for *D. mucoroides*, samples 133/1C and 115A/89C grouped together but segregated significantly between them and with sample AR4B. The two samples of *D. giganteum* also differed significantly. These data were confirmed by analyses of the 5.8S rDNA and ITS2 sequences. The larger differences found in these species could indicate higher intraspecific variability. Alternatively, these samples could have been grouped erroneously as belonging to the same species due to their similar morphological characteristics. Nucleotide sequence data would be in agreement with the division of *D. mucoroides* into, at least, two species, one represented by samples 1C, 133B, and AR4B and the other represented by samples 115A and 89C. Some morphological data also support this proposal; in particular, spores had a size of 7 by 4 μm in samples 115A and 89C and 6 by 3 μm in samples 1C, 133B, and AR4B.

These data indicate that, despite the large differences between the ITS regions of dictyostelid genera, the smaller differences between isolates from the same genera and species make these regions of utility to study their phylogenetic relationships. Actually, comparison between ITS1 sequences can be very useful to discriminate between samples from close species whose 5.8S rDNA sequences are almost identical.

The phylogenetic analyses of the 5.8S rDNA and ITS sequences have shown a good general agreement with the current systematics of the phyla, with some exceptions. The more remarkable is *D. minutum*, which showed low similarity with other *Dictyostelium* species, in either the 5.8S rDNA (70%) or the ITS1 (35%) region, compared to the average similarity that exists between *Dictyostelium* species (88.73 or 49.61%, respectively). Analyses of the small subunit rRNA sequences also indicated that *D. minutum* diverged from *Dictyostelium rosarium* and *Dictyostelium discoideum*, which were closely related between them (2). In the same previous study, *D. minutum* was closer to these two *Dictyostelium* species than to *P. pallidum*, although it was even more distant from *Dictyostelium fasciculatum*. The data presented in the present study showed significant bootstrap values for the divergence between *D. minutum* and the other *Dictyostelium* and *Polysphondylium* species but did not allow us to establish their evolutionary relationship.

The larger divergence of *D. minutum* with respect to the other *Dictyostelium* species and also to *Polysphondylium* and *Acytostelium* species could be due to a faster molecular evolution in this species. Alternatively, this divergence could indicate that *D. minutum* is distant from the three existing genera and might be the founder species of a new genus yet to be defined. Recent data obtained from the analysis of protein-coding genes support this hypothesis (10a).

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