

Costa Rican dictyostelids, with notes on the taxonomy, phylogeny and ecology of *Dictyostelium discoideum*

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In 1962 and then over the period of 1993–2001, samples of soil/litter for isolation of cellular slime molds (dictyostelids) were collected throughout Costa Rica. Collecting sites included examples of all major forest types found in the country and ranged in elevation from 10 to 3000 m. More than 3300 clones of dictyostelids were recovered from these samples. The clones included representatives of at least 24 described species along a number of isolates that do not conform closely to any known species and thus may be new to science. Greatest diversity was found in the montane/cloud forests, whereas the average number of clones per gram was highest in moist/wet forests. Distribution of species on a country-wide basis is compared with that of Mexico, another tropical country. In addition, phylogenetic analyses of 18S rDNA sequences were carried out on 12 different isolates of *Dictyostelium discoideum*, a predominantly temperate species that was recorded from both Costa Rica and Mexico.

Keywords: biogeography, distribution, ecology, Neotropics, soils.

Cellular slime molds (dictyostelids) are single-celled, eukaryotic, phagotrophic bacterivores usually present and often abundant in terrestrial ecosystems (Raper 1984). These organisms represent a normal component of the biota of soils and apparently play a role in maintaining the natural balance that exists between bacteria and other microorganisms in the soil environment. While feeding on bacteria, dictyostelid myxamoebae reproduce mitotically and, if subjected to relative starvation conditions, form multicellular, sporulating structures. These dictyostelid fruiting bodies are microscopic and rarely observed except in laboratory culture. Under favorable condi-

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tions, the spores germinate to release myxamoebae, and the life cycle begins anew. Dictyostelids are most abundant in the surface humus layer of forest soils, where populations of bacteria are the highest and microenvironmental conditions appear to be the most suitable for dictyostelid growth and development (Raper 1984).

Approximately 130 species of dictyostelids have been formally described. Some species appear to be cosmopolitan, whereas others have a more restricted distribution (Swanson *et al.* 1999). Studies on the occurrence and distribution of dictyostelids in the Neotropics began with three series of collections made by James Cavender in various parts of Mexico during the early 1960s. In 1962, Cavender carried out another survey in Costa Rica (Cavender & Raper 1968). Results from this initial Mexican survey were combined with comparable data sets obtained by Landolt, Stephenson and Suthers to produce the first comprehensive treatment of the dictyostelids of that tropical country (Cavender *et al.* 2012). In the present paper, the different data sets from Costa Rica have been combined to produce the second comprehensive study of dictyostelids in the Neotropics. As such, our primary objective was to characterize the assemblage of dictyostelids associated with tropical forests of Costa Rica. A secondary objective was to compare these data with data from Mexico (Cavender *et al.* 2012)

The country of Costa Rica occupies a total area of approximately 51100 km² in Central America between latitudes 8° and 12° N and longitudes 82° and 86° W. Costa Rica borders Nicaragua to the north and Panama to the south, with the Caribbean Sea located to the east and the Pacific Ocean to the west. The general topography of the country is surprisingly mountainous, and the highest point (Cerro Chirrip) reaches an elevation of 3819 m. Although the climate of Costa Rica as a whole is considered as tropical, the varied topography and the wide range in elevation cause distinctly different conditions of rainfall and temperature to exist from place to place. In general, the Caribbean side (except for the Pacific SW) is wetter than the Pacific side, and the very wettest portions of Costa Rica receive an annual rainfall in excess of 5000 mm. Two main seasons can be recognized—a dry season that extends from December to April and a wet season that goes from May to November. Mean annual temperatures are about 27 °C in the coastal lowlands, 20 °C in the central part of the country and below 10 °C at the very highest elevations. These different conditions are reflected in differences in the predominant forest type present in a particular region of the country. Costa Rica is characterized by one of the highest levels of biodiversity of any country in the world, and about 25 % of the total land area of the country occurs in national parks and other protected areas (Janzen 1983).

The approach used in the present study was to assign the various types of forest habitats from which samples were collected to four different types that are based largely on the Holdridge *et al.* (1971) life zone system. These four forest types are (1) dry forest, (2) moist/wet forest, combined (3) cloud/montane forest, combined (above an elevation of 800 m), and (4) oak cloud

forest. These forest types are comparable to those recognized by Schnittler & Stephenson (2000) in their studies of myxomycetes in Costa Rica. The dry forest type has a pronounced dry season between December and April, with an annual precipitation of less than 2000 mm. Dominant trees are deciduous or semi-deciduous. One of the best examples is the Area de Conservación Guanacaste (10° 51' N, 85° 36' W, 300 m elevation). The moist/wet forest type is characterized by a less pronounced dry season, with annual precipitation between 2000 and 3000 mm. A variety of non-deciduous tree species make up this forest type. Examples of such forests sampled in the present study were sites near Cahuita on the southern Caribbean coast (9° 45' N, 82° 58' W, 10-30 m elevation), Selva Verde (near La Selva station of the Organization of Tropical Studies, 10° 25' N, 84° 00' W, ca. 80 m elevation) and La Marina, Alajuela (10° 23' N, 84° 23' W, 400 m elevation). Sampling sites assigned to the cloud/montane forest type included the lower slopes of Volcán Cacao (10° 56' N, 85° 28' W, ca. 1200 m elevation) and Braulio Carillo (9° 56' N, 84° 07' W, 1400-1600 m elevation). Dominant trees in this forest type are evergreen, and average annual precipitation ranges between 3000-4000 mm. The oak cloud forest type, including the example located near Cerro de la Muerte (9° 34' N, 85° 44' W, 3150 m elevation) in the Talamanca range, occurs at the very highest elevations sampled in the present study. These forests, which are evergreen, tend to be characterized by almost daily exposure to clouds, and the mean annual precipitation sometimes exceeds 4000 mm. The geographic locations for the sampling sites in this study are given in Tab. 1 and are depicted on the map Fig. 1.

Materials and methods

In 1962 and then over the period of 1993-2001, a total of 341 samples for isolation of dictyostelids were collected from study sites throughout Costa Rica. These included examples of all of the major forest types described in the previous section. Six to 12 samples (but mostly 10), each consisting of 10-20 g were collected from soil/humus layer at each site. All samples were placed in sterile plastic bags and returned to the laboratory at Shepherd University, Ohio University or the University of Wisconsin for processing. Samples were processed to isolate dictyostelids within a week of sample collection.

As in the prior Mexican study (Cavender *et al.* 2012), isolation procedures used for dictyostelids were those described by Cavender & Raper (1965). Each sample was weighed and enough sterile distilled water added to obtain an initial soil/water dilution of 1:10. This mixture was shaken to disperse the material and to suspend the cells of dictyostelids present. A 5.0 ml volume of this initial dilution was added to 7.5 ml of sterile, distilled water to create a 1:25 dilution of sample material. Aliquots (each 0.5 ml) of this suspension were added to each of two or three 95 x 15 mm culture plates prepared with hay (leached and dried, mostly *Poa* sp.) infusion agar (Raper

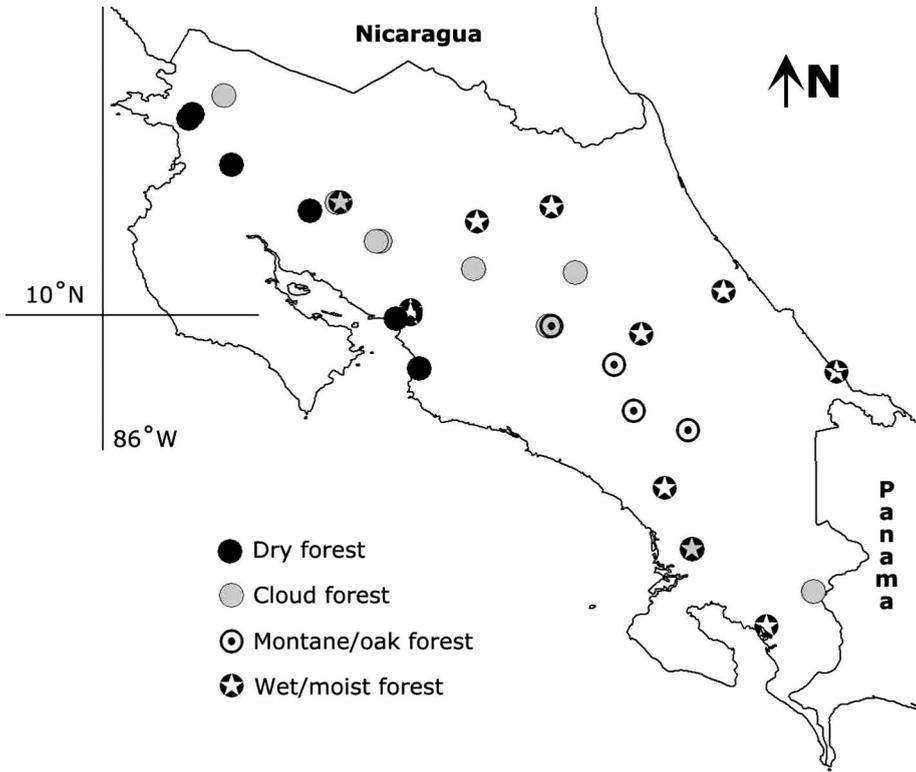


Fig. 1. Locations of collecting sites in the four different forest types in Costa Rica. Place names and geographic coordinates for all sites are provided in Tab. 1.

1984). This produced a final dilution of 0.02 g of soil per plate. Approximately 0.4 ml of a heavy suspension of *E. coli* was added to each culture plate, and plates were incubated under diffuse light at 20–25 °C. Each plate was examined at least once a day for several days following appearance of initial aggregations, and the location of each aggregate clone marked. When necessary, isolates were subcultured to facilitate identification. Spores of isolates used for further study, including DNA sequencing, were conserved on silica gel granules or by lyophilization. Nomenclature used herein essentially follows Raper (1984).

For DNA extraction, 12 different isolates from Mexico and Costa Rica, including ten new isolates that had been conserved were grown on SM plates (standard medium: 20 g/l peptone; 2 g/l yeast extract; 20 g/l glucose; 2 g/l MgSO₄; 3.8 g/l KH₂PO₄; 1.2 g/l K₂HPO₄; 2% agar). Cells from the edge of plaques growing on these plates were collected with a sterile tip, mixed with DNA extraction solution from Epicentre and heated for 30 min at 60 °C followed by 8 min at 98 °C. Cell lysates were used directly for PCR amplifica-

Tab. 1. Dictyostelid collections from Costa Rican forests. ^aDenotes an estimated no. of species

Locality	Province	Elev.	Coordinates (approx.)	No. of species	Clones/gram
Moist and wet forest sites					
Selva Verde I	Heredia	100 m	10° 27' N; 84° 04' W	7	185
Selva Verde II	Heredia	100 m	10° 27' N; 84° 04' W	6	28
Cahuita	Limón	10 m	9° 44' N; 82° 51' W	7	495
Batán	Limón	100 m	10° 05' N; 83° 20' W	8 ^a	535
Fortuna	Limón	400 m	9° 14' N; 83° 35' W	10 ^a	174
Golfito	Puntarenas	450 m	8° 38' N; 83° 09' W	5 ^a	9
La Marina	Alajuela	400 m	10° 23' N; 84° 23' W	10 ^a	542
Palmar I	Puntarenas	<100 m	8° 58' N; 83° 28' W	6 ^a	225
Palmar II	Puntarenas	<100 m	8° 58' N; 83° 28' W	6 ^a	105
Palmar III	Puntarenas	<100 m	8° 58' N; 83° 28' W	7 ^a	115
Turrialba I	Cartago	800 m	9° 54' N; 83° 41' W	6 ^a	140
Turrialba II	Cartago	800 m	9° 54' N; 83° 41' W	10 ^a	263
Tilaran I	Guanacaste	526 m	10° 28' N; 84° 58' W	4 ^a	28
Esparza	Puntarenas	200 m	9° 59' N; 84° 40' W	7 ^a	110
Farm 18	Puntarenas	<100 m	10° 00' N; 84° 40' W	8 ^a	163
Cloud and montane forest sites					
Monteverde I	Puntarenas	1300 m	10° 18' N; 84° 48' W	7	305
Monteverde II	Puntarenas	1300 m	10° 18' N; 84° 49' W	6	210
Monteverde III	Puntarenas	1300 m	10° 18' N; 84° 49' W	7	103
Volcán Cacao	Guanacaste	1250 m	10° 56' N; 85° 28' W	7	363
Braulio Carillo	Heredia	1000 m	10° 10' N; 83° 58' W	6	145
Wilson I	Puntarenas	1000 m	8° 47' N; 82° 57' W	8	100
Wilson II	Puntarenas	1000 m	8° 47' N; 82° 57' W	9	43
Palmar IV	Puntarenas	1000 m	8° 58' N; 83° 28' W	10 ^a	23
San José	San José	1000 m	9° 56' N; 84° 05' W	10 ^a	275
Zarcero	Alajuela	1000 m	10° 11' N; 84° 24' W	12 ^a	240
Tilaran II	Guanacaste	1000 m	10° 28' N; 84° 59' W	10 ^a	200
Dry forest sites					
Carara	Puntarenas	100 m	9° 45' N; 84° 38' W	5	493
Santa Rosa I	Guanacaste	300 m	10° 50' N; 85° 37' W	5	18
Santa Rosa II	Guanacaste	300 m	10° 51' N; 85° 36' W	6	43
Cañas	Guanacaste	< 100 m	10° 26' N; 85° 06' W	2	25
Barranca	Puntarenas	<100 m	9° 58' N; 84° 44' W	8 ^a	235
Liberia	Guanacaste	<100 m	10° 38' N; 85° 26' W	6 ^a	35
Oak cloud forest sites					
Villa Mills Oak	Cartago	2900 m	9° 34' N; 83° 43' W	3	15
Chirripó/Tapantí	Cartago	2500 m	9° 29' N; 83° 29' W	3	75
National Parks			9° 46' N; 83° 48' W		
El Empalme	Cartago	2000 m	9° 56' N; 84° 04' W	2	205

tion. Amplification consisted of an initial denaturing step at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 52 °C for 1 min and 72 °C for 2 min, with a final polishing step of 10 min at 72 °C. Sequences were amplified with primers 18S-FA: 5' AACCTGGTTGATCCTGCCAG 3' and 18SRB: 5' TGATCCTTCTGCAGGTTTAC 3', as described in Perrigo *et al.* 2013. Sequencing was performed at Macrogen on an ABI 3730XL3-1523-027 sequencing machine using the aforementioned primers along with internal sequencing primers D542F and D1340R (Schaap *et al.* 2006). Sequence data were edited with the program Staden (Staden 1996).

SSU rDNA sequences, including those from the ten new isolates (GenBank database accession numbers KJ394470 to KJ394480) were added by hand to an existing SSU rDNA alignment (Romeralo *et al.* 2011). Phylogenetic analyses were conducted with the general time reversible nucleotide substitution model corrected for the proportion of invariant sites and with four rate categories for varying evolutionary rates across sites (GTR + I + GAMMA model). All parameters for the model were determined by the respective programs. An optimal tree topology was sought with Bayesian inference with the MC3 search algorithm and two independent sets of four chains with MrBayes (Ronquist & Huelsenbeck 2003) and by maximum likelihood (ML) criteria with RaxML 7.0.4 (Stamatakis 2006, Stamatakis *et al.* 2008). The optimality and robustness of the tree were evaluated respectively with posterior probabilities, performed sampling, 2 000 000 generations and 20 % burning (BIPP, MrBayes) and 100 bootstrap replicates (MLBP, RaxML).

Data recorded from the plates prepared with samples collected during the course of the present study were number of clones (colonies), frequency of occurrence and number of clones per gram for each species in every one of the collecting sites. Many of the species formerly placed in the *Dictyostelium mucoroides* complex were not well defined at the time (1962) when the earliest samples were collected. As such, the latter would have included a number of forms now recognized as separate species, including *D. giganteum*, *D. firmibasis*, *D. implicatum*, *D. sphaerocephalum* and *D. aureostipes*. The probable number of clones likely to have been represented by each of these species in the earlier data was estimated from their respective proportions of each in the totals represented in the comparable data sets compiled more recently by Landolt and Stephenson (unpubl).

Results

A total of 3349 clones representing at least 27 different taxa were isolated, including one species of acrasid slime mold, *Guttulinopsis nivea*. This acrasid was first reported from Panama (Raper 1960) and also occurs in wet forests of Guatemala and Mexico, possibly associated with spider and howler monkeys. It is likely that some clones that were isolated and identified at the time as *Polysphondylium pallidum* may actually represent more recently described, white-spored members of the genus (e.g. *P. colligatum* and *P. asy-*

Tab. 2. Alphabetical list of Costa Rican dictyostelids and one acrasid with authorities

<i>Acytostelium leptosomum</i>	Raper, Mycologia 48(2): 179 (1956)
<i>Dictyostelium aureostipes</i>	Cavender, Raper & Norberg, Amer. J. Bot. 66(2): 209 (1979)
<i>D. aureum</i>	Olive, Proc. Amer. Acad. Arts & Sci. 37(12): 340 (1901)
<i>D. citrinum</i>	Vadell, M.T. Holmes & Cavender, Mycologia 87(4): 553 (1995)
<i>D. coeruleostipes</i>	Raper & Fennell, Amer. J. Bot. 54(5): 519 (1967)
<i>D. dimigraformum</i>	Cavender, J. Gen. Microbiol. 62: 115 (1970)
<i>D. discoideum</i>	Raper, J. Agric. Res. 50(2): 135 (1935)
<i>D. dumosum</i>	Cavender, Vadell, Landolt & Romeralo, Mycologia 105: 610-35 (2013)
<i>D. firmibasis</i>	H. Hagiw., Bull. Natl. Sci. Mus. Tokyo, B 14(3): 356 (1971)
<i>D. giganteum</i>	B. N. Singh., J. Gen. Microbiol. 1: 17 (1947)
<i>D. implicatum</i>	H. Hagiw., Bull. Natl. Sci. Mus. Tokyo, B 10(2): 63 (1984)
<i>D. intermedium</i>	Cavender, Amer. J. Bot. 63(1): 63 (1976)
<i>D. lavandulum</i>	Raper & Fennell, Amer. J. Bot. 54(5): 519 (1967)
<i>D. maendriiforme</i>	Cavender, Vadell, Landolt & Romeralo, Mycologia 105: 610-35 (2013)
<i>D. mexicanum</i>	Cavender, Worley & Raper, Amer. J. Bot. 68(3): 379 (1981)
<i>D. monochasoides</i>	H. Hagiw., Bull. Natl. Sci. Mus. Tokyo, B 16(3): 494 (1973)
<i>D. mucoroides</i>	Bref., Abh. Senckenb. Naturf. Ges. 7: 85 (1869)
<i>D. m. var. stoloniferum</i>	Cavender & Raper, Amer. J. Bot. 55(4): 510-511 (1968).
<i>D. polycephalum</i>	Raper, J. Gen. Microbiol. 14: 717 (1956)
<i>D. purpureum</i>	Olive, Proc. Amer. Acad. Arts, 37(12): 340 (1901)
<i>D. rhizopodium</i>	Raper & Fennell, Amer. J. Bot. 54(5): 517 (1967)
<i>D. sphaerocephalum</i>	(Oudem.) Sacc. & Marchal, Bull. Soc. Roy. Bot. Belg. 24: 74 (1885)
<i>D. tenue</i>	Cavender, Raper & Norberg, Amer. J. Bot. 66(2): 213 (1979)
<i>D. vinaceofuscum</i>	Raper & Fennell, Amer. J. Bot. 54(5): 522 (1967)
<i>Guttulinopsis nivea</i>	Raper, Worley & Kessler, Mycologia 69: 1016-1030 (1977)
<i>Polysphondylium asymmetricum</i>	Vadell & Cavender, Mycologia 90(4): 719 (1998)
<i>P. colligatum</i>	Vadell & Cavender, Mycologia 90(4): 719 (1998)
<i>P. pallidum</i>	Olive, Proc. Amer. Acad. Arts, 37(12): 341 (1901)
<i>P. violaceum</i>	Bref., Unters. Gesamtgeb. Mykol. 6: 5 (1884)

metricum) first described from Guatemala (Vadell & Cavender 1998). We also consider the likelihood that due to their close similarity, a few clones might equally represent *D. tenue* or *D. monochasoides*. Thus in the tabulation of the numbers of different dictyostelid species recovered from each collection site, some values are estimated. Dictyostelid species isolated from each of the four forest types are given in Tab. 1.

An alphabetical list of cited Costa Rican dictyostelids and the one acrasid together with authorities is provided in Tab. 2.

Tab. 3. Numbers of clones and frequencies of Costa Rican dictyostelid cellular slime molds in the four forest types. M-W = Moist-Wet; C-M = Cloud-Montane; C-O = Cloud-Oak and Dry. Cl+Freq represent combined clone numbers and frequencies of all four forest groups for each species and represents an index of importance. ^aAn acrasid slime mold. ^bNewly described species. ^cNumbers of forms include *G. nivea*, *Polysphondylium* sp. and both *D. monochasoides* and *D. tenue*. Three additional, rare species (*Acytostelium minutissimum*, *A. ellipticum* and *D. deminutivum*) were reported earlier (Stephenson & Landolt 2011) from epiphytic soils in Monteverde cloud forests.

Species	M-W clones	M-W Freq.	C-M clones	C-M Freq.	Dry clones	Dry Freq.	C-O clones	C-O Freq.	Cl + Freq.
<i>Dictyostelium purpureum</i>	218	520	175	380	105	135			1533
<i>Polysphondylium pallidum</i> /P. sp.	514	460	72	172	55	79	4	20	1376
<i>D. mucoroides</i>	107	309	262	310	1	15	91	89	1184
<i>D. sphaerocephalum</i>	387	309	5	22	144	140			1007
<i>D. giganteum</i>	4	77	110	148			67	121	527
<i>D. monochasoides/tenue</i>	114	260	9	71	13	40			507
<i>P. violaceum</i>	9	80	68	111	43	105	1	10	427
<i>D. firmibasis</i>			226	137	3	31			397
<i>D. sp.</i> (purple)	240	90							330
<i>D. sp.</i>	29	40	32	120	1	10	1	10	243
<i>D. vinaceofuscum</i>	4	20	48	123	18	50			263
<i>D. aureostipes</i>	18	155	3	30	1	14			221
<i>D. lavandulum</i>	41	100	4	40					185
<i>D. implicatum</i>			23	54					77
<i>Guttulinopsis nivea</i> ^a			18	20	12	10			60
<i>D. discoideum</i>			6	52					58
<i>D. meandriforme</i> ^b	6	50							56
<i>D. mucoroides</i> var. <i>stoloniferum</i>	10	40							50
<i>D. polycephalum</i>			5	21	2	20			48
<i>D. rhizopodium</i>	10	30							40
<i>Acytostelium leptosomum</i>	2	20	1	10					33
<i>D. mexicanum</i>	1	10	2	20					33
<i>D. aureum</i>			1	10					11
<i>D. citrinum</i>					1	10			11
<i>D. coeruleostipes</i>			1	10					11
<i>D. dumosum</i> ^b			1	10					11
Total clones	1714		1072		399		164		
Total frequency		2570		1871		659		250	
No. of samples			103				30		
Avg. clones/gram	208		182		170		98		
Number of different forms ^c	19		23		13		5		

A summary of dictyostelid species recovered from Costa Rican sampling, ranked in descending order of importance as indexed by combining clones per gram recovered with frequency of occurrence (Cl+Freq.), is provided in Tab. 3.

Phylogenetic analysis of 18S rDNA sequences from 29 isolates of *Dictyostelium discoideum* (12 isolates originating from Costa Rica and Mexico and an equal number of other strains of *D. discoideum* isolated from a wide, global distribution) plus those from four other, similar species produced a phylogenetic tree showing an optimal topology using both Bayesian inference (BI) and maximum likelihood (ML) as shown in Fig. 2.



Fig 2. Phylogenetic analyses of 18S rDNA sequences from isolates of *Dictyostelium discoideum*. In addition to the *D. discoideum* isolates assigned here to Costa Rica and Mexico, the other isolates in the tree are from: temperate USA (V12, WS582, AX2, WS10, WS691, AX4, MF2, NC4, LC3); Japan (ST3, KK2, H23); Mexico (TO9); Pakistan (V34) Honduras (HN28A); Guatemala (OH494, OH562); Trinidad (AR5b) and Indonesia (PJ11). The geographic origins for strains X00134 and 91HO9 are unknown. Strain 2a3a is the same as ZA3A. – Dic_ = *Dictyostelium*, Dic_discoideum = *D. discoideum*. The tree shown is the optimal topology found by using both Bayesian inference (BI) and maximum likelihood (ML). Nodes with PP 1.00 and BS 100 are denoted with an asterisk (*), and indicate very strong support. While other nodes in this optimal topology have less than total support using BI and ML measures, they do represent relative degrees of similarity.

Discussion

With few exceptions, the described species of dictyostelids recovered from Costa Rica were either species considered to be cosmopolitan in distribution (e.g. *Polysphondylium violaceum*, *Dictyostelium mucoroides*, and *Polysphondylium pallidum*) or forms with recognized tropical and subtropical affinities in the New World or Asia (e.g., *Dictyostelium purpureum*, *D. citrinum*, and *D. lavandulum*). Species such as *D. discoideum* that are generally considered to have a distribution centered largely in temperate regions of the world were uncommon but still represented by an appreciable number of isolates. The moist/wet forest had the highest average number of clones/g at 208, including the three highest counts—La Marina at 542, Batán at 535, and Cahuita at 495. Counts were lowest in the oak cloud forest at 98 and in the dry forest at 170 (Tab. 3). The montane cloud forests had an intermediate average count of 182 clones/g but had the greatest species diversity at 23, as compared to 19 for the moist/wet forest. These results contrast with those from Mexico (Cavender *et al.* 2012), where the greatest diversity was recorded for lowland seasonal rain forests (24 species compared to 19 in Costa Rica). The same pattern was evident for the number of clones per gram (291 compared to 208). Numbers per gram were actually highest in tropical deciduous forests in Mexico (315), a forest type that is less defined in Costa Rica since the latter country is characterized by an overall wetter climate as well as one that is more tropical. The dry forest type in both countries had lower counts than the more moist seasonal forests as well as lower values of species richness. The more tropical character of Costa Rican montane/cloud forests is reflected in the less frequent occurrence of one temperate species, *Dictyostelium discoideum*, as well as the absence of another temperate species, *D. minutum*. A change in the dominant species also was apparent, with *D. sphaerocephalum* becoming a clear dominant in the dry and moist/wet forests of Costa Rica. As already indicated, *D. mucoroides* was the major dominant in all forest types in Mexico except for thorn forests and desert, whereas *D. sphaerocephalum* was not a dominant species in any Mexican forest type. The tropical form of this species differs somewhat morphologically from its temperate counterpart but has a similar genetic profile (Romeralo *et al.* 2007). *Polysphondylium violaceum* was most prominent in dry forests as it was in Mexico, but its overall ranking in Costa Rica was much lower than in Mexico, where it was the second ranking species. The species of *Polysphondylium* with lighter spores (Vadell & Cavender 1998) and *D. purpureum* were dominants in most of the Costa Rican forests as they were in Mexico, with the exception of Costa Rican oak cloud forests. The wettest environments in Costa Rica (e.g. the oak cloud forests and the rain forest at Golfito, where annual rainfall is 3000–4000 mm) had generally lower numbers of dictyostelids. It may be that these organisms do better in somewhat drier seasonal rainfall environments, as previously suggested by Cavender & Raper (1968).

Dictyostelium discoideum has been used as a model organism since the 1960s to study basic processes in cell and developmental biology (Raper 1984, Kessin 2001). In terms of distribution, *D. discoideum* is a rather common species that has been found in temperate deciduous forests of North America and elsewhere but which has rarely been reported from the tropical lowlands. It was found at higher elevations (Zarcero and Monteverde) in Costa Rica but is less frequent than was the case for the cloud forests of Mexico. In this study we have included 29 different isolates morphologically identified as *Dictyostelium discoideum* and sequences for the 18S rDNA gene were generated for all of them. Ten of the gene sequences are new and the remaining come from isolates used in a prior phylogenetic analysis of dictyostelids (Schaap *et al.* 2006). Phylogenetic analyses of 18S rDNA sequences from these 29 isolates show that isolates of *Dictyostelium discoideum* form a monophyletic clade with a tremendous genetic difference (Fig. 2). Isolates from Mexico and Costa Rica are mixed, showing no clear pattern of geographical differentiation (Fig. 2). It is possible that this molecular tree reflects a pattern of ecological differentiation. This echoes other previous analyses of molecular characters of *D. discoideum* isolates that suggest great genetic differences within this morphospecies and perhaps the existence of cryptospecies, especially in tropical isolates (Evans *et al.* 1988, Douglas *et al.* 2011). A more detailed study, including more isolates of *D. discoideum* with a re-evaluation of morphological characters as well as an in-depth study of both biotic and abiotic data is currently ongoing (Romeralo *et al.*, unpubl). This will allow clarifying biogeographic and ecological patterns within this model species of the Dictyostelia.

Surprisingly, *Dictyostelium minutum*, another temperate species that was found to co-occur with *D. discoideum* in the cloud forest of Mexico, was not recorded in Costa Rica. However, other isolates that are members of phylogenetic Group 3 (Romeralo *et al.* 2011) were recovered from Monteverde and elsewhere. The crampon-based species of dictyostelids (*Dictyostelium lavandulum*, *D. rhizopodium*, *D. vinaceofuscum* and *D. coeruleostipes*) appear to be well represented in Costa Rica. The holotype for *D. lavandulum* (TU-5a), most prominent in wet forests, was isolated at the Turrialba site, whereas the holotype for *D. vinaceofuscum* (Li-2), prominent in dry forests, is from the Liberia site (Raper & Fennell 1967). *Dictyostelium rhizopodium* was isolated in a wet forest at Turrialba and La Marina, and *D. coeruleostipes* was recorded from a cloud forest at Monteverde. *Dictyostelium lavandulum* was not recorded from Mexico, although the other crampon species occur there (Cavender *et al.* 2012).

Other rare species occurring in both Mexico and Costa Rica were *D. mucoroides* var. *stoloniferum* (the holotype TU II-6 is from Turrialba, Costa Rica; Cavender & Raper 1968), *D. mexicanum* and *D. citrinum* (Vadell *et al.* 1995). Costa Rica is also the source of the very rare species *Acytostelium minutissimum* (Cavender & Vadell 2000). Two small species belonging to phylogenetic Group 3 (*D. maeandriforme* and *D. dumosum*) and described re-

cently from Guatemala and Belize (Cavender *et al.* 2013), were recorded from cloud forests and wet forests, respectively.

An undescribed species, purple in color but without a crampon base (VQ 3a, OH 243), was found in relatively large numbers at La Marina. Some of the isolates of *Polysphondylium* also could represent species new to science (Cavender, unpubl. data). *Dictyostelium polycephalum* was much more common in Mexico than in Costa Rica. Another difference in the two countries was the relative prominence of *D. sphaerocephalum*. This species was never abundant in any forest type in Mexico but was a dominant (a combination of frequency of occurrence and numbers of clones per gram sample) in dry and moist/wet forests in Costa Rica. In contrast, *D. mucoroides* was the single most dominant species in Mexico in all forest types except for thorn and desert (Cavender *et al.* 2012), whereas in Costa Rica it was the prominent species present only in cloud/montane and oak cloud forests. *Polysphondylium violaceum*, the second most important species in Mexico, was far less important in Costa Rica. *Dictyostelium purpureum* and members of the genus *Polysphondylium* with lighter spores, including *P. pallidum*, were equally abundant in both countries and also ranked among the dominant species present except in the oak cloud forests, where *D. mucoroides* was clearly dominant. When frequencies and numbers of clones for each species as listed in Tab. 3 are added together (Cl+ Freq.) to create an importance value, a more complete picture of the occurrence and distribution of each species is given and *D. purpureum* becomes the single most important species; however, when only numbers of clones are used—as was done for Mexico—it has the same relative position as in Mexico.

There have been relatively few intensive studies of dictyostelids in tropical regions of the world, but the data reported herein indicate that the biodiversity of these organisms is high in Costa Rica. This conforms to the earlier set of data from Mexico (Cavender *et al.* 2012) and what is known about the dictyostelids of Guatemala and the Peruvian Amazon (Swanson *et al.* 1999, Cavender 1996). In general, biodiversity of dictyostelids appears to be lower in temperate regions, although a comparable number of species was reported from the Great Smoky Mountains National Park by Cavender *et al.* (2005). However, the park is characterized by an unusually wide range of habitats for a temperate region of the world and has been subjected to some of extensive sampling over a period of several decades in the context of the All Taxa Biodiversity Inventory project (Stephenson & Landolt 2009). Although temperate and tropical regions share numerous species in common, there is a subset of species that clearly have a distribution centered in the tropics. In contrast, a few species that are common in at least the temperate forests of eastern North America are comparatively rare at lower latitudes. Phylogenetic analyses of 18S rDNA sequences carried out on 12 different strains of *D. discoideum*, which have been isolated from high-elevation forests of Costa Rica and Mexico, indicate that an unexpectedly high level of genetic variation exists in this otherwise predominantly temperate species.

These results suggest that comparable studies of a number of other morphospecies are warranted.

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