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Dictyostelids from a Tropical Forest in Cuba

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Abstract: Six species of dictyostelid cellular slime molds are reported from ten samples of ground soil and ten samples of aerial "canopy soil" collected in November 2002 from a montane tropical forest in the Guamuahaya Mountains of central Cuba. Four species were recovered from the ground samples and five species from the aerial samples. The two most abundant species were *Dictyostelium mucoroides* and *Heterostelium pallidum*. There appear to be no previous records of dictyostelids from Cuba.

Keywords: canopy soil, cellular slime molds, ground soil, Neotropics

Introduction

During the course of a survey for myxomycetes carried out on the Alturas de Banao Ecological Reserve (21°57′ N, 79° 36′ W) in Cuba during November 2002, the second coauthor collected a small set of samples of ground soil and aerial "canopy soil" to be processed for dictyostelids. The Alturas de Banao Ecological Reserve is located in the province of Sancti Spicitus in the Guamuahaya Mountains of central Cuba. The predominant vegetation of the reserve is a montane tropical forest. The primary habitat for dictyostelids is the soil/litter interface zone of forest ecosystems, and the assemblage of species associated with tropical forests has been shown to be particularly biodiverse (e.g., Cavender and Raper 1969, Cavender et al. 2013). However, dictyostelids are also known to be associated with the mass of soil-like organic material ("canopy soil") that accumulates beneath the covering of vascular and nonvascular epiphytes that develops on the larger branches of trees or at the base of a large vascular epiphyte such as a bromeliad or orchid that grows attached to the trunk of a tree (Stephenson and Landolt 1998). Presumably, dictyostelids have the same role (as bacterivores) in both ground soils and canopy soils.

There appear to be no previous records of dictyostelids from Cuba, and the survey for myxomycetes in that country carried out in 2002 represented an opportunity to collect samples that could be processed for dictyostelids. It was anticipated that dictyostelids could be isolated from these samples, and thus confirm their presence in another area of the Neotropics. Nomenclature used herein for dictyostelids follows Shrikh et al. (2018).

Materials and methods

Five samples of ground soil and five samples of canopy soil were collected from each of two localities on the Alturas de Banao Ecological Reserve for a total of 20 samples. Both localities had similar vegetation and were located on the same general area. All samples, each approximately 30 to 50 g, were

collected and placed in sterile whirl-pack plastic bags. Samples were mailed to the laboratory at Shepherd University and processed as soon as possible, as recommended by Cavender and Raper (1965).

The isolation methods were those described by Cavender and 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 95 x 15 mm Petri dishes prepared with hay (leached and dried, mostly *Poa* sp.) infusion agar (Raper 1984). This produced a final dilution of 0.02 g of soil per plate. Approximately 0.4 ml of a heavy suspension of 12-24 hour *E. coli* was added to each culture plate, and plates were incubated under diffuse light at 20-25°C. A pH measurement was taken of each diluted soil sample. Each inoculated plate was examined at least once a day for several days following appearance of initial aggregations, and the location of each aggregate clone marked. Aggregations, pseudoplasmodia, and sorocarps appeared from 2 to 10 days following inoculation of the plates. Isolates of interest were subcultured from spores on low nutrient agar with *E. coli* and also spores were conserved in tubes of silica gel granules at 4°C as described in Raper (1984).

Results

Six species of dictyostelids were identified from the 158 isolates recovered from the twenty samples (Table 1). Five of these could be assigned to described species, while the identification of one species of *Dictyostelium* was problematic and it is listed as "*Dictyostelium* sp." in the table. Four species were recovered from the ground samples and five species from the aerial samples. *Dictyostelium mucoroides* and *Heterostelium pallidum* (listed in older treatments of the dictyostelids as *Polysphondylium pallidum*) were the most abundant species. *Dictyostelium mucoroides* alone accounted for 57% of all clones. No single species was recorded from all four sets of samples.

Values of pH recorded for the samples ranged from 4.3 to 6.7, with the lowest value recorded for an aerial sample from site 2 and the lowest value recorded from an aerial sample from site 1. As a general observation, there was not a large difference between the ground samples and aerial samples with respect to pH.

Discussion

The biodiversity of dictyostelids in the ground soils of tropical forests tends to be higher than in temperate deciduous forests (1965c), so the number of species recorded in the present study would be considered low. Aerial "canopy soil" essentially does not exist in temperate forests with the exception of temperate rain forests, and there is only limited information on the assemblages of dictyostelids found in this microhabitat (Stephenson and Landolt 2011).

The fact that *Dictyostelium mucoroides* and *Heterostelium pallidum* were the two most abundant species might have been expected, since these are two of the more abundant species worldwide. Interestingly, these were the first two species of dictyostelids formally described by science. Two of the other species recorded (*Dictyostelium citrinum* and *D. tenue*) are relatively rare, although already known from ground soil at scattered localities in the Neotropics. The former was isolated from aerial canopy soil collected from site 2, which was characterized by the lowest pH values recorded in the present study. Numbers of clones/g varied rather widely, from a low of only three for aerial canopy soil from site 2 to 380 for a sample of ground soil from site 1. When the data for all samples of each type were pooled,

ground soil samples were more productive for dictyostelids than aerial canopy soil samples. This pattern has been reported previously (Stephenson and Landolt 2011) and probably reflects the fact that ground soil, as the primary microhabitat for dictyostelids (Cavender and Raper 1965b), is simply more favorable for these organisms.

Table 1. Dictyostelids recovered from 10 samples of ground soil (G) and 10 samples of aerial canopy soil (A) in Cuba.

Species	**Number of clones**			
	Site 1A	**Site 1G**	**Site 2A**	**Site 2G**
Dictyostelium citrinum			1	
Dictyostelium mucoroides	12	78		
Dictyostelium purpureum	1	9		2
Dictyostelium tenue				2
Dictyostelium sp.	1			
Polysphondylium pallidum	15	27		10
Total clones	29	114	1	14
Clones/gram	97	380	3	47
pH	6.1–6.7	6.2–6.6	4.2–5.5	5.6–6.1

It should be noted that aerial canopy soil was rather sparse and relatively thin in the two situations (beneath mats of vascular and nonvascular epiphytes on the branches of trees and at the bases of large vascular epiphytes on the trunks of trees) from which samples were collected in the present study. As such, the occurrence of dictyostelids in these samples might seem somewhat surprising, especially the number of clones recorded from aerial canopy soil from site 1. However, very high numbers of clones have been reported for particular microsites in many other studies of dictyostelids.

In summary, the present study provided the first data on dictyostelids for the island of Cuba, but these data are quite limited. Additional sampling is warranted in order to develop a more complete understanding of the biodiversity of these organisms in this region of the Neotropics.

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