# Cellular slime molds isolated from Lubang Island, Occidental Mindoro, Philippines

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Dictyostelid species (cellular slime molds) were isolated and purified from 200 soil samples collected from coastal and lowland mountain forests of Lubang Island in Occidental Mindoro, Philippines. Five dictyostelid species, identified as *Dictyostelium discoideum*, *D. mucoroides*, *D. purpureum*, *Polysphondylium pallidum* and *P. violaceum* were previously known from Philippines. Two other species, *Dictyostelium aureo-stipes* and *D. laterosorum*, were isolated for the first time from Philippine soil. Another species, *Dictyostelium* sp. L08-09, could be identified only to the genus level and may be an undescribed species. Our study is the first report of Philippine dictyostelids in over 30 years.

**Key words** – dictyostelids, forest soil, species list, tropical rain forest, island biodiversity

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## Introduction

Dictyostelids or cellular slime molds are micropredators commonly found in forest soil. They are found in most terrestrial ecosystems around the world, particularly in the humus layer of forest soils (Cavender & Raper 1965b, 1965c, Swanson et al. 2002). Much like other terrestrial organisms, their diversity increases as one nears the equator (Cavender 1973, Watson et al. 1995) and at lower altitudes (Cavender 1980, Hagiwara 1984). Unfortunately, not much is known about the total number and global biodiversity of cellular slime molds around the world (Swanson et al. 1999). Although the diversity of these organisms has been well studied in Europe and the Americas, the species found in tropical South East Asia have received little attention.

The cellular slime molds collected in South East Asia were observed to be very diverse and even comparable to those of Central and South America (Swanson 1999). However, the region may not have been sampled enough. Aside from the cosmopolitan species complex composed of *Dictyostelium purpureum*, *D. mucoroides*, *Polysphondylium violaceum*, and *P. pallidum*, together with the equatorially restricted species *D. tenue*, Cavender (1976) collected the following species of cellular slime molds from different places in the region: *Acytostelium subglobosum*, *D. bifurcatum*, *D. coeruleo-stipes*, *D. intermedium*, *D. lacteum* var. *papilloideum*, *D. lavandulum*, *D. multi-stipes*, *D. polycephalum*, *D. rhizopodium*, *D. vinaceo-fuscum*. *D. macrocephalum* was also found in South East Asia (Swanson et al. 1999). In Papua New Guinea, *D. monochasioides*, *D.*

*purpureum*, *D. sphaerocephalum*, and *P. pallidum* were isolated by Kobayasi & Miura (1970). In the Philippines, only eleven species of dictyostelids have been identified so far by Dogma & Blancaver (1965), Dogma (1975) and Cavender (1976). Seven species from the genus *Dictyostelium*, three from *Polysphondylium*, and one from *Acytostelium* were listed. The species were isolated from soil samples collected from Corregidor Island, Mt. Makiling and Ipo Dam in Luzon, and Panay Island in the Visayas. Unfortunately, many other areas in the country remain unexplored for dictyostelids. The forests of Lubang Island in Occidental Mindoro thus offer an ideal location to check for the presence of dictyostelids and spark an interest in these organisms. Due to its geographic isolation, the island is known for its many endemic flora and fauna, and thus, could potentially harbour new species of cellular slime molds. The present paper records the species of dictyostelids isolated and collected from soil samples in coastal and lowland forests in Lubang Island during April and August 2009.

## Methods

### Study site

The Lubang Group of Islands (13$^{O}$49'15.6" N, 120$^{O}$09'54.8" E) is located northwest of Mindoro and about 150 km southwest of Manila (Fig. 1). Lubang Island is about 30 km in length and 10 km in width. To the east of the main island of Lubang lies Ambil Island, to the southeast is Golo Island and to the northwest is Cabra Island. The area has an annual temperature range of 23-31$^{O}$C and an annual rainfall range of 9 to 623 mm. The study site has two pronounced seasons: dry from November to April and wet during the rest of the year. The island is mostly covered with thick tropical virgin lowland rainforests that host large dipterocarp trees.

### Collection of soil samples

Twenty collection sites were used in this study: ten sites along coastal forests surrounding the main island of Lubang (coastal area) and ten sites within the forested area of Mount Gonting, the highest point in Lubang (lowland mountain area) (Fig. 1). Five soil samples were collected randomly from each of the ten coastal sites and from each of the ten mountain forest sites during the dry (April 2009) and wet (August 2009) seasons. This gave a total number of 200 soil samples throughout the duration of the study. Soil samples (~30g from each sampling site) were collected from the surface layer of forest soils by scraping soil, together with decomposing leaves and plant matter. It has been noted that the number of dictyostelids in soil soon decrease after collection (Cavender & Raper 1965a) so soil samples were immediately processed after collection.

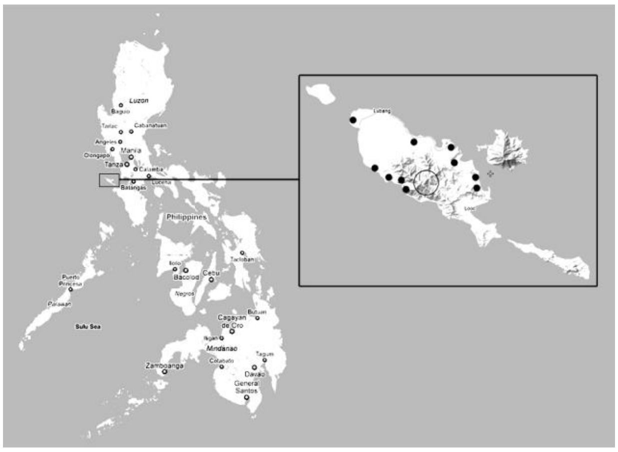


**Fig 1** – Map of the study area: Lubang Island, Occidental Mindoro. Mt. Gonting is located in the middle of the main island of Lubang and served as collection site for mountain soil samples (encircled area). Coastal soil samples were collected around the island (dots).

### Isolation and purification of dictyostelid

Cellular slime molds or dictyostelids were isolated following the protocol of Cavender & Raper (1965a). Bacterial and soil suspensions were spread over the surface of hay infusion agar plates until all areas were covered. All culture plates were incubated for a period of 7 days at room temperature under diffuse light. Three replicates of culture plates were prepared from each soil sample, giving a total of 600 isolation plates. After incubation, the culture plates were observed under a stereomicroscope for the presence of dictyostelids. Dictyostelid species were isolated and further purified by spore touch technique.

**Identification of cellular slime molds**

The isolated cellular slime molds were identified based on their distinct morphological characteristics. Initially, pure cultures of the isolated dictyostelids were grown on freshly prepared hay-infusion agar plates with *Escherichia coli* as food source. Then, the stages in their life cycle as well as the spore morphologies of each of the isolated dictyostelids were observed under a stereomicroscope and bright field compound microscope. The morphological features used for their identification were as follows: the shape and size of the dictyostelid spores, the aggregation patterns of the streaming amoeboid cells, the shape and behavior of the forming pseudoplasmodium, and the size, colour, and branching patterns of mature fruiting bodies. Identification of the isolated dictyostelids was done by comparing their morphologies with those of published literature and web-based ID guides: Cavender (1970), Cavender (1980), Cavender et al. (1979), Cavender & Vadell (2006), the Eumycetozoan project (http://slimemold.uark.edu/) and Discover Life (http://www.discoverlife.org/). Fruiting bodies of the isolated dictyostelids were also measured and photographed.

**Results**

Cellular slime molds were isolated from 88 of the 200 soil samples and comprised eight species belonging to two genera (*Polysphondylium* and *Dictyostelium*). Through morphological characterization these species were identified as: *Dictyostelium aureo-stipes*, *D. discoideum*, *D. laterosorum*, *D. mucoroides*, *D. purpureum*, *Polysphondylium pallidum*, and *P. violaceum*. One isolate could be identified only to the genus level, as *Dictyostelium* sp. L08-09. Descriptions of the different dictyostelids are given below:

***Dictyostelium mucoroides***

Fruiting bodies are commonly unbranched or very sparsely branched (Fig. 2). Sorocarps are usually erect, semi-erect, inclined or partially prostrate, phototactic and have a variable length of about 2-5 mm or more. Sori are globose, appear milk-white, and are commonly 125-200 µm in diameter. Fruiting bodies arise from characteristic clavate to expanded bases. Cell aggregations are radiate in pattern, vary in size from 1-3 mm in diameter, and may form larger convergent streams that could segment and form separate sorocarps.

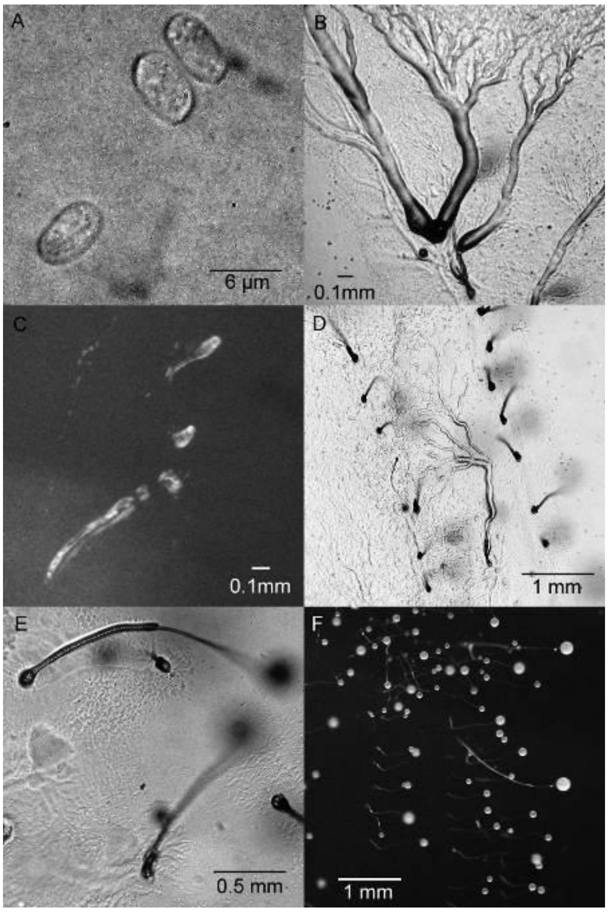


**Fig 2** – *Dictyostelium mucoroides*. A. Oblong to elliptical spores with abundant small vesicles. B. Asymmetrical aggregations. C. Early sorogens. D. Irregular clavate to conical bases. E. Lower prostrate sorophore (upper left) with erect sorophore (lower right). F. Mature fruiting bodies.

***Dictyostelium purpureum***

This was one of the first isolates to be identified primarily because of its distinctive large purple sori (Fig. 3). Sorocarps are usually solitary, erect to inclined, strongly phototactic and are variable in length. No branching was observed. Sori are globose and appear dark purple to black when mature. Spores are capsule shaped and are about 5-8 µm in length. Fruiting bodies arise from expanded bases. Cell aggregations are commonly large and may exceed 1 cm in diameter. These may start as

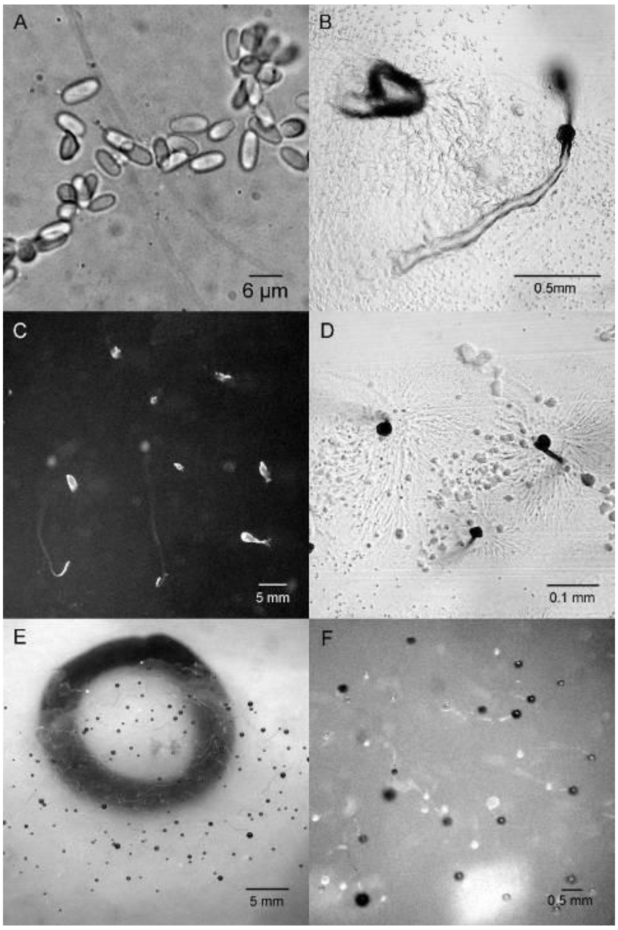


**Fig 3** – *Dictyostelium purpureum*. A. Elliptical spores. B. Irregular radial aggregation. C. Early solitary sorogens forming from a rounded center. D. Conical base. E-F. Sorocarps with distinctive dark purple sori.

broad aggregations that eventually become well developed converging streams.

***Polysphondylium violaceum***

Sorocarps usually appear solitary but may sometimes be clustered (Fig. 4). Mature fruiting bodies have a variable length that may reach 1 cm or more and produce branches and whorls. Sorocarps produce about 3-8 whorls that are evenly spaced, sometimes interrupted. Whorls were observed to typically have 2-3 branches. Sori are globose and lightly pigmented (violet). Sorocarps are highly phototactic, often orienting themselves heavily towards an available light source. Bases are slightly clavate. Aggregations are radiate and variable in size. Smaller aggregations range from 2-3 mm in diameter but may form large converging streams of up to 1cm in length (Cavender & Vadell 2006).

***Polysphondylium pallidum***

Mature fruiting bodies of *P. pallidum* are characterized by their white sori and typically colourless sorophores. Sorocarps commonly have 3-8 whorls with 3-6 branches per whorl and are seen to be solitary or weakly clustered, erect or semi-erect, richly branched and symmetrical (Fig. 5). Fruiting bodies vary in length from 3-8 mm and rise from clavate or rounded bases. Sori are globose and appear white. Terminal sori are mostly 75-150 µm in diameter while lateral sori appear smaller at 40-65 mm in diameter. Spores are elliptical and may appear slightly reniform measuring mostly 5-7 µm in length. Aggregations are radiate and compact measuring about 1-2 mm in diameter.

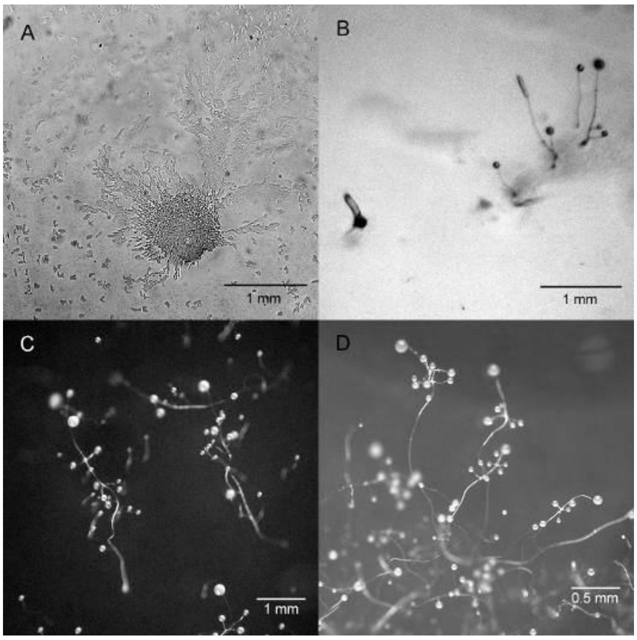


**Fig 4** – *Polysphondylium violaceum*. A. Early stages of typical "violaceum" aggregation. B. Late-stage sorogen beside cluster of asynchronous fruiting bodies. C. Solitary, erect, whorled sorocarps. D. Mature sorocarps with violaceous sori with few whorls, and purple sori.

***Dictyostelium discoideum***

This species is identified by its basal disc, yellowish sorus, and more notably, its stalkless migrating pseudoplasmodia or slugs (Fig. 6). Sorocarps are commonly unbranched, erect, rise from tapered sorophores, and relatively small at about 1.8-3.8 mm in height. Cell aggregations are radiate and usually have a diameter of 2-3 mm. Each aggregation produces a characteristic slug that is able to move or migrate.

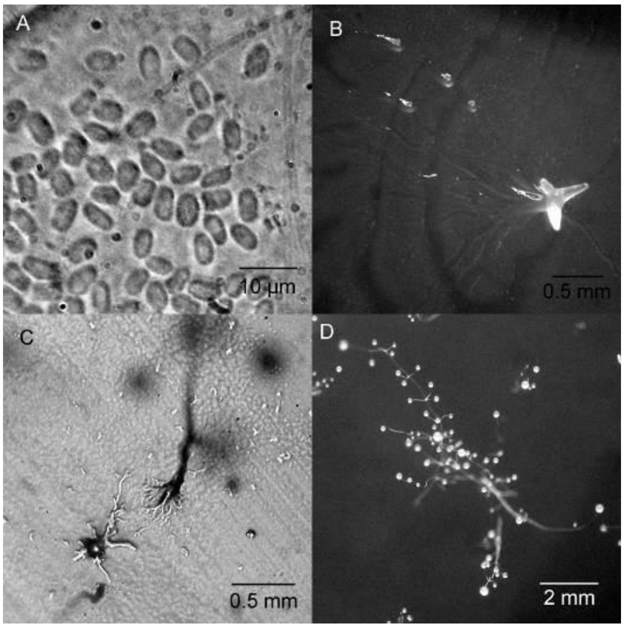


**Fig 5** – *Polysphondylium pallidum*. A. Oblong to elliptical to broadly reniform spores, with unconsolidated polar granules. B. Early clustered sorogens. C. Radial aggregation (lower left) and clavate to expanded base (right). D. Mature whorled sorocarps.

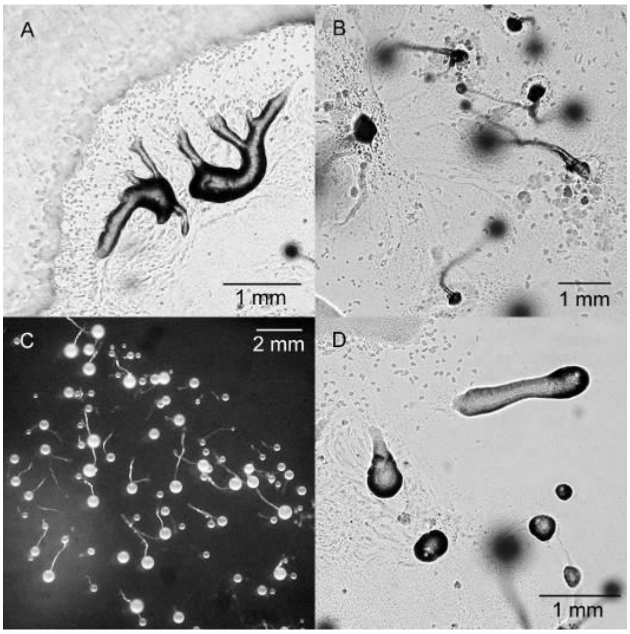


**Fig 6** – *Dictyostelium discoideum*. A. Characteristic radiate late aggregation. B. Large, cellular, broadly conical base, C. Mature sorocarps with rounded terminal sorus. D. Migrating pseudoplasmodium or "slug".

***Dictyostelium aureo-stipes***

Sorocarps may appear solitary or clustered and are often erect (Fig. 7). These are phototactic and may vary in length from 3-7 mm. Sorophores were observed to be white and bear multiple (15-22) lateral branches that are irregular in size and spacing. *D. aureo-stipes* can be easily identified by its numerous, irregular branching. Sori are globose and white. Spores are elliptical to slightly reniform, have visible polar granules, and vary in length from about 4.7-7 mm.

***Dictyostelium laterosorum***

Sorocaps of this organism are usually erect or inclined but may also appear prostrate (Fig. 8). Sorophores are rarely branched, vary in size from 5-10 mm, and are seen to rise from expanded bases. Lateral sori are notably smaller than the terminal sorus of a fruiting body.

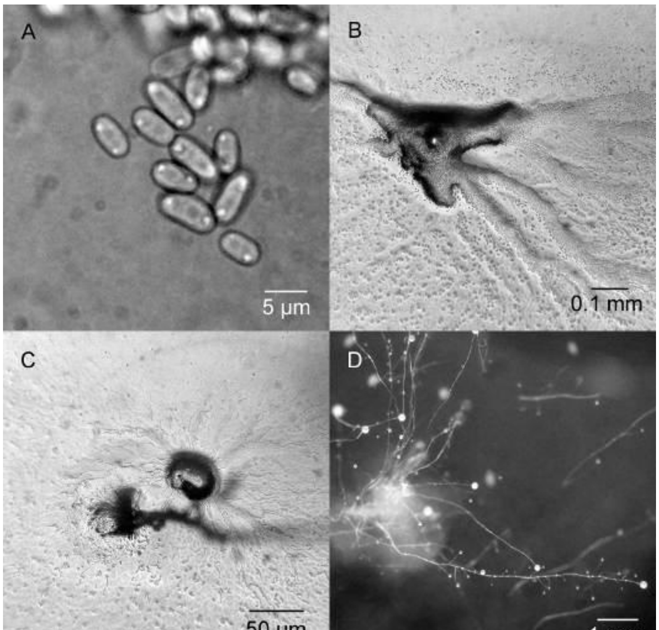


**Fig 7** – *Dictyostelium aureo-stipes*. A. Oblong to elliptical spores with consolidated polar granules. B. Lacunose violaceum type aggregation. C. Knobbed sorophore base (left) and main aggregation (right). D. Sorocarp with profusely branched habit.

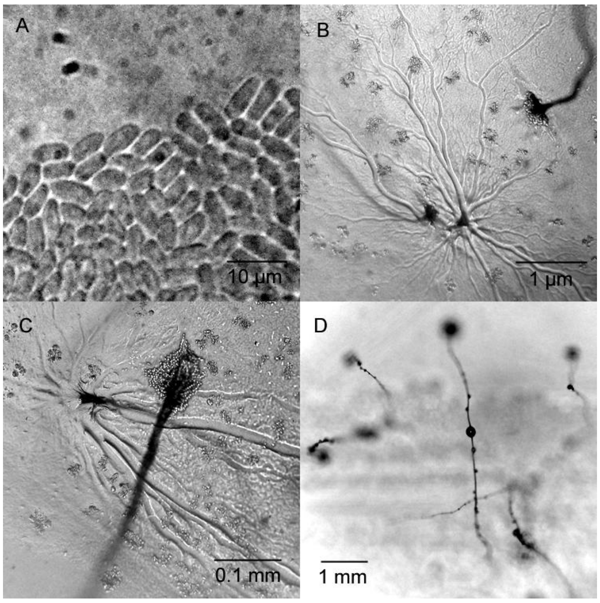


**Fig 8** – *Dictyostelium laterosorum*. A. Elliptical to reniform spores. B. Long radial aggregations. C. Weakly digitate, crampon like base. D. Light violet sorophore with distinct sori along its stalk.

***Dictyostelium*** **sp. L08-09**

Sorocarps resemble those of *D. rosarium* having a similar beaded appearance, but could also take on branched forms (Fig. 9). Branching, when present, is irregular and appear as single branches or as whorls having 2-3 branches each. Sorophores appear white, vary in length and grow beyond 8 mm under diffused light. Pseudoplasmodia were even observed to have been "crawling" on the inner surface of the plate covers. Sori are globose and appear white. Fruiting bodies rise from clavate to expanded bases. Cell aggregations are radiate, of the violaceum-type and measure about 2 mm in diameter. Each aggregation can give rise to long pseudoplasmodia that eventually develop into mature fruiting bodies.

**Discussion**

Only a handful of reports about dictyostelids from Philippine soil have been published. *D. discoideum*, *D. mucoroides*, *P. pallidum*, and *P. violaceum* were first isolated by Dogma & Blancaver from Philippine soil in 1965. Dogma (1975) reported *D. rosarium* from soil collected from Los Baños, Laguna. Cavender (1976) further reported the isolation of *A. subglosum*, *D. lacteum* var. *papilloideum*, *D. mucoroides*, *D. mucoroides* var. *stoloniferum*, *D. polycephalum*, *D. purpureum*, *Dictyostelium* sp., *P. pallidum*, and *P. violaceum* from Corregidor Island and Ipo Dam in Luzon, and from Lambuan in Panay Island, the Visayas. Since these publications, there have been no other reports on Philippine dictyostelids. This prompted us to conduct a study on the dictyostelids from soil samples collected in coastal and lowland montane forests in Lubang Island, Occidental Mindoro. This geographically isolated island was virtually in isolation from the other islands of the Philippines and is an ideal site to explore island dictyostelids.

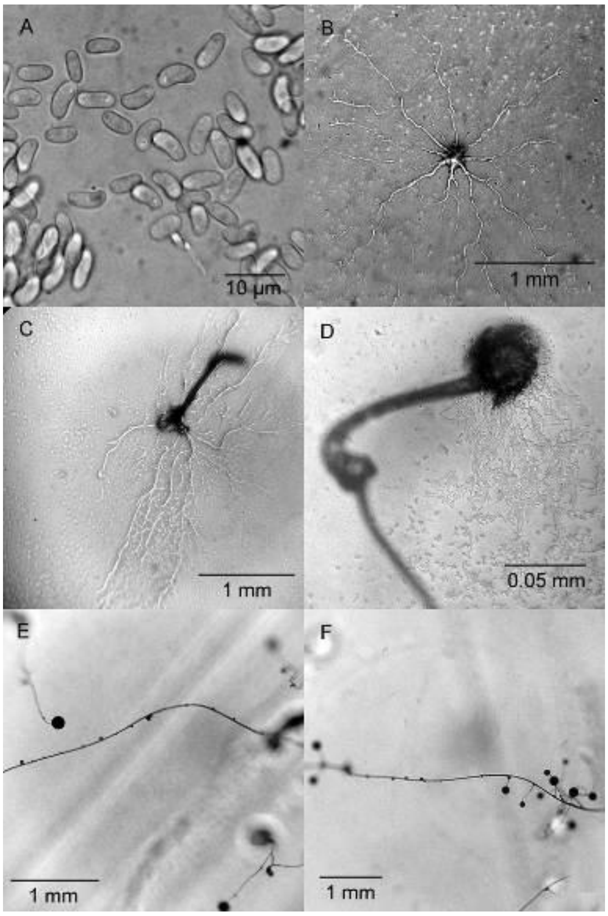


**Fig 9** – *Dictyostelium* sp. L08-09. A. Elliptical to reniform spores with prominent polar granules. B. Long radial aggregation. C. Young sorophore rising from radial aggregation. D. Expanded base with stream of amoeboid cells. E. Sori along length of unbranched sorophore. F. Branched formation with sori along the stalk.

*D. mucoroides* was the most abundant species of dictyostelid in Lubang Island (Fig. 2). The organism was isolated from 8 out of 20 collection sites throughout the island. It is notable that *D. mucoroides* is one of the most widely distributed species of dictyostelids around the world. It prefers somewhat acidic soil (Cavender & Vadell 2006), which explains why it was the most abundant dictyostelid in the mountain forests of Lubang (average soil pH = 6.55). *D. purpureum* was the second most abundant isolate (Fig. 3). It was also the most frequently occurring dictyostelid in Lubang Island, having been isolated from 9 out of 20 collection sites. This species is known to have a very wide distribution around the world (Swanson et al. 1999), but incidence of *D. purpureum* increases as one approached the tropics (Cavender 1973). It was also found to occupy lower altitude forest soils (Cavender 1965b). It was also the most abundant species in the coastal forests of Lubang.

*P. violaceum* was found to be the third most abundant species from the Island of Lubang (Fig. 4). It is considered to have a cosmopolitan distribution, meaning it can be found in forest soils around the world (Swanson et al. 1999). It was found on both the coastal and mountain forest collection sites but only during the month of August. This may be attributed to its preference for very moist sites (Cavender & Vadell 2006). The average moisture content of the forest soils in Lubang was observed to have increased by 3 percent from April to August. *P. pallidum* was the fourth most abundant cellular slime mold from Lubang Island (Fig. 5). It was also the third most frequently occurring isolate, having been seen in 8 out of 20 collection sites. It prefers acidic soils as opposed to *P. violaceum*, which thrives in more neutral habitats (Cavender & Vadell 2006). It is known to be one of the most dominant species of dictyostelids in most habitat types around the world (Swanson et al. 1999).

*D. discoideum* was found in 6 out of the 20 collection sites in Lubang (Fig. 6). Studies show that this species is mostly found in cool temperate regions such as hemlock-white pine forests (Cavender 1973) and cool temperate northern hardwood forests in North America and Japan (Cavender & Kawabe 1989). It may also be found in tropical regions, although rare (Swanson et al. 1999). *D. discoideum* is known to have a disjunct distribution, suggesting that it may be part of the few remaining populations of a previously widely distributed species (Watson et al. 1995). This widely disjunct distribution pattern could be traced to the permanent destruction of forests habitats, which it could have occupied otherwise (Swanson et al. 1999). This may explain why *D. discoideum* has not been found in temperate deciduous forests in Europe and India even though these have similar habitat characteristics (Swanson et al. 1999). *D. aureo-stipes* was seen in 3 out of 20 collection sites in Lubang (Fig. 7). This organism is known to have a preference for mesic or moist environments (Cavender & Vadell 2006) and is widespread in temperate regions but is more common in sub-tropical and tropical zones (Swanson et al. 1999). It is interesting to note that this species was only found during August in 3 coastal forest sites where the average moisture content was relatively high at 21%. *D. laterosorum* was the least abundant and least occurring dictyostelid isolate from Lubang Island (Fig. 8). Clones were isolated from 2 collection sites in the mountain forest only during August, suggesting a very limited incidence of the organism. *D. laterosorum* was first isolated in South America and is known to inhabit tropical moist forest soils (Cavender 1970). It is also classified as a pantropical species (Swanson et al. 1999). Pantropical species of cellular slime molds are found in a variety of tropical deciduous and seasonal evergreen forest soils around the world. These species deserve special mention as they are very seldom, if ever, found to occupy forest soils of the temperate zone (Swanson et al. 1999). This organism was initially identified as *D. rosarium* as it has the same beaded appearance, but was later identified as *D. laterosorum* after differences in pigmentation and spore shape were observed. *D. rosarium* is characterized by milk-white sori while the isolated organism possessed terminal sori that were light violet in appearance. Furthermore, *D. rosarium* fruiting bodies have distinct globose spores while the isolated organism clearly had elliptical to reniform spores. *Dictyostelium* sp. L08-09 had the second lowest clonal count and was found in only 3 collection sites in coastal forests during August (Fig. 9). This organism was also initially considered to be *D. rosarium* because of its combined beaded and branched appearance. Under certain conditions, stalked lateral sori reminescent of *D. rosarium* could develop (Cavender & Vadell 2006). However, the initial identification was eventually overturned when the morphological characteristics of its spores differed from those of *D. rosarium*. *Dictyostelium* sp. has elliptical to slightly reniform spores with visible polar granules, unlike those of *D. rosarium*, which are globose or spherical. No other literature about dictyostelids was found to have a similar set of morphological descriptions prompting us to tentatively label this isolate as *Dictyostelium* sp. It is possible that this is a new species of *Dictyostelium*.

Prior to the present study only 11 species of dictyostelids have been reported in the Philippines. Considering that dictyostelids in South East Asia are known to be very diverse (Swanson et al. 1999), the data gathered about cellular slime molds in the Philippines and perhaps generally in South East Asia leaves much room for discovery. The isolation of *D. aureo-stipes* and *D. laterosorum* from Lubang Island brings the number of isolated cellular slime molds from Philippine soil to 13 species. The isolation of a potentially novel species, *Dictyostelium* sp. L08-09, could increase the number of new records for the country. It is hoped that through this research interest in these organisms will be revived in the Philippines as many more sites await future exploration.

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