

ORIGINAL ARTICLE

# Dictyostelids isolated from the soils of San Fernando City, La Union, Philippines

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## Summary

To document the first dictyostelid report in San Fernando City, La Union, Philippines, soils from upland and coastal areas were randomly collected and processed in the laboratory using Hay Infusion Agar. After several weeks of incubation, the spore touch technique was employed to isolate the six determinable morphospecies of dictyostelids from 38% of positive plates: *Heterostelium pallidum*, *Dictyostelium discoideum*, *Dictyostelium purpureum*, *Dictyostelium rosarium*, *Dictyostelium* sp.1 (the most abundant), and *Raperostelium minutum*. This is the first attempt to isolate dictyostelids using the coastal soils in the tropical part of the world.

**Key words:** cellular slime molds, eumycetozoans, pseudoplasmodium, social amoebae, Paleotropics

## Introduction

Dictyostelids are a diverse group of single-celled eukaryotic organisms ubiquitously found in most soils. Like other eumycetozoan counterparts that have phagotrophic nutrition, which allows them to engulf microorganisms, these micro-predators are vital in maintaining ecological balance in soil microhabitats. These social amoebae have a unique

asexual life cycle consisting of two phases. The vegetative feeding phase is represented by a starvation period when these organisms feed on bacteria. The following aggregation phase is represented by a chemotactic response when they form large groups that later differentiate into multicellular slugs called pseudoplasmodium. As social amoebae, they have also been utilized as experimental models for cancer studies (Mathavarajah et al., 2021), extracellular

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vesicle messengers (Tatischeff, 2019), phototaxis (Claudio-Paragas et al., 2023), and spatial gradient sensing (Shams et al., 2020). As to their global distribution, according to Liu et al. (2020), the subtropical zone is the area where the cellular slime molds are observed to thrive better compared to temperate, alpine, and tropical zones.

Historically, the seminal work on dictyostelids by Dogma and Blancaver (1965) primarily identified *Dictyostelium discoideum*, *D. mucoroides*, *Polysphondylium pallidum* (currently known as *Heterostelium pallidum* (Olive) S. Baldauf, S. Sheikh and Thulin), and *P. violaceum* as the first dictyostelid isolates for the Philippines. This was followed by the report by Dogma (1975) on the effective isolation of the species *D. rosarium*. Cavender (1976) then reported five additional species, namely, *Acytostelium subglobosum*, *D. lacteum* var. *papilloideum* (currently known as *Tieghemostelium lacteum* (Tiegh.) S. Baldauf, S. Sheikh and Thulin), *D. mucoroides* var. *stoloniferum* (*D. mucoroides* Bref.), *D. polycephalum* (*Coremiostelium polycephalum* (Raper) S. Baldauf, S. Sheik, Thulin and Spiegel), *D. purpureum*, and one unidentified species of *Dictyostelium* sp. Since then and until the study by Yulo and dela Cruz (2011), research on dictyostelids had been stagnant for more than three decades. In the latter paper, two out of seven species, *D. aureostipes* (*Cavenderia aureostipes* (Cavender) S. Baldauf, S. Sheikh and Thulin) and *D. laterosorum* (*Polysphondylium laterosorum* (Cavender) S. Baldauf, S. Sheikh and Thulin), were reported for the first time in the country. Currently, the total number of dictyostelids identified in the Philippines is 12 (Dagamac and dela Cruz, 2019).

In comparison with the myxomycetes that have been more or less well studied in the Philippines (see Alfaro et al., 2014; Dagamac and dela Cruz, 2015; Pecundo et al., 2017), studies on dictyostelids remain relatively scarce, with breakthroughs and research only being initiated over a decade ago (Dagamac and dela Cruz, 2019). With the increasing discovery of new dictyostelid species over the years, it can be inferred that many different habitats and ecosystems in the country may still harbor such peculiar groups of protists. This assumption is supported by a previous study by Balaoro-Banzuela et al. (2023) on the occurrence of other slime molds, specifically myxomycetes, in the reef-to-ridge terrain of San Fernando City, La Union. Hence, the current research project evolved from this previous report by isolating and determining dictyostelids from soil samples collected in upland and coastal areas of the same region.

## Material and methods

### STUDY SITE AND SOIL COLLECTION

Samples were collected in June 2022 at random sites in upland and coastal terrains in San Fernando City, La Union, Philippines (16.6159° N, 120.3210° E). The city is located in about 270 km north of Manila (Fig. 1). Annual temperature ranges from 24 °C to 32 °C. The site belongs to Cluster 3 of the Philippine redefined climate zones, which identifies June to December as wet months and January to May as dry months (Corporal-Lodangco and Leslie, 2017)

Due to having distinct coastal and upland sites that support many species of microfauna and microflora, this city is an ideal location for isolating slime molds (Balaoro-Banzuela et al., 2023). Based on the accessibility of sampling sites, five random collecting points were selected for the upland, and the other five random collecting points along the coastal areas were selected for soil sampling. For each, a 5m×5m plot was randomly selected for collecting 3 soil replicate samples. This makes 15 soil samples for each of the terrains and a total of 30 soil samples for the whole study. The samples were initially air dried and kept inside a sterile Ziploc® bags placed in room temperature until further laboratory procedure was conducted.

### LABORATORY PROCEDURE

Isolation of dictyostelids followed the protocol established by Cavender and Raper (1965a, 1965b), in which 10 g of each soil sample was initially diluted in 90 mL of distilled water to yield a 1:10 dilution. Following this, 5 ml of the soil suspension was further diluted in 7.5 mL of distilled water to yield a 1:25 dilution. Lastly, 5 mL from this suspension was transferred to Hay Infusion Agar (HIA, boiled 10 g of hay in 1 L of distilled water for 20 min) to reach a final dilution of 1:50. Subsequently, 0.4 mL of the 24-h old suspension of *E. coli* was added to the suspension as a food source, which rendered the culture two-membered (Yulo and dela Cruz, 2012; Guyer et al., 2017). From this, each soil sample was plated in duplicates amounting to 60 plates, wherein the presence and growth of dictyostelids were regularly observed while under incubation in diffuse light and room temperature for a maximum of six weeks. For further isolation, dictyostelids were transferred to freshly prepared HIA plates by using the spore touch technique established by Cavender and Raper (1965a). This was done by gently dabbing



**Fig. 1.** Map of the Philippines, showing the exact location of the sampling site in San Fernando City, La Union, with inset photos of the actual soil collection at random sites in the city.

on the spores using a modified glass Pasteur pipette, which was then tapped on top of the new agar plate for dispersal.

#### DETERMINATION OF DICTYOSTELIDS

The presence of dictyostelids was recorded regularly throughout the 4 weeks' incubation period. The dictyostelids were then identified by characterizing their morphological traits such as spore characteristics, aggregation patterns, shape and sequence of their sorophore base, stalk, plasmodium and sorocarp in their various life cycle stages based on the data from different publications (Vadell et al., 2018; Perrigo et al., 2020; Cavender et al., 2022) and a slime mold database (<http://slimemold.uark.edu/index.htm>). Photography for documentation was done using a smartphone taking a picture directly from the microscope ocular lens. Currently accepted names were checked against the online nomenclatural database available for eumycetozoans (<http://nomen.eumycetozoa.com>).

#### Results

There were 23 out of 60 (38%) plates positive for dictyostelids. From those positive plates, six morphologically identifiable species from three different genera of dictyostelids were found to be present in the sample replicates from both upland and coastal ecosystems. *Dictyostelium* sp.1 had the broadest distribution among the species present in the records. The species *D. purpureum*, on the other hand, was only found in the upland soil samples.

An explicate list of 6 taxa of cellular slime molds are described below, with details of the records such as the species name, total number of records seen inside the brackets and their different morphological characteristics such as spores, spore shape, branching pattern, stalk length and width. In addition, two new dictyostelids have also been found, *Raperostelium minutum* and one unidentified *Dictyostelium* species adding to the overall species count to the current 12 previously discovered species.

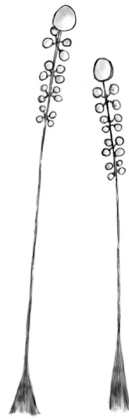


Fig. 2. Fruiting bodies of *Heterostelium pallidum*.

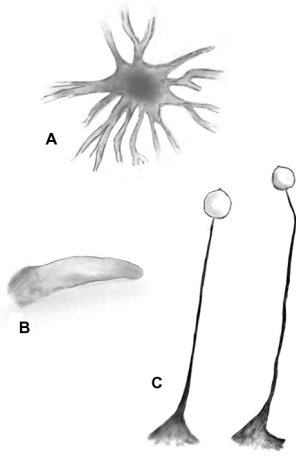


Fig. 3. A – Aggregating amoebae, B – slug, and C – fruiting bodies of *Dictyostelium discoideum*.

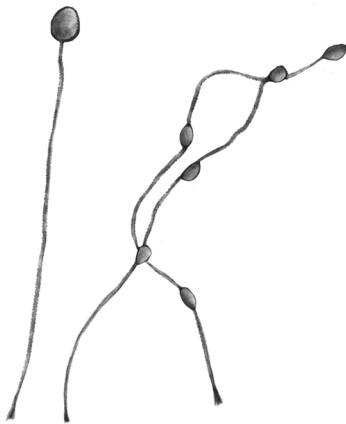


Fig. 4. Fruiting bodies of *Dictyostelium purpureum*.

*Heterostelium pallidum* (Olive) S. Baldauf, S. Sheikh and Thulin. Upland: 23 / Coastal: 4. Spores multiple; spore shape globose; branching pattern abstriction with regular whorls; stalk long and wide; spore size = 5 to 7  $\mu\text{m}$ ; sorocarp height = 3 to 8 mm (Yulo and dela Cruz, 2011).

*H. pallidum* (Fig. 2) Has spores that are multiple, unconsolidated, and oblong in shape. *H. pallidum* represents one of the twelve genera under the new classification of monophyletic entities based on both molecular phylogeny (18S rRNA sequencing) and morphological recognition (Sheikh et al., 2018).

*Dictyostelium discoideum* Raper. Upland: 29/Coastal: 7. Spores single; spore shape oblong; branching pattern unbranched; stalk long and wide; spore size = 8  $\times$  3  $\mu\text{m}$ ; mature sorocarp height = 1.8 to 3.8 mm; basal disc = 10–20  $\mu\text{m}$  diameter (Cotter and Raper, 1966).

As seen in Fig. 3, *D. discoideum* have vertical sorocarps, unbranched, with long and wide stalk. Spores were observed to be singular and oblong in shape. *D. discoideum* is one of the most commonly studied species of dictyostelid slime molds in terms of its morphology and life cycle; as such, it serves an important model organism in a variety of ecological studies (Sunderland, 2009).

*Dictyostelium purpureum* Olive. Upland: 1/ Coastal: 0. Spores single; spore shape capsule; branching pattern unbranched; stalk long and wide; spore size = 5 to 8  $\mu\text{m}$ ; sorocarp height = 4 to 5 cm (Yulo and dela Cruz, 2011).

Fig. 4 shows *D. purpureum* having long and wide unbranched stalked sorocarps. Spores are singular, oblong in shape, and have visible purple pigmentation. *D. purpureum* is similar to *D. discoideum* in terms of initial life cycle, differing only in the final stages of slug to fruiting body formation, when *D. purpureum* sterile stalks form alongside migration to its final location (Jack et al., 2008).

*Dictyostelium rosarium* Raper and Cavender, J. Elisha Mitchell. Upland: 7/Coastal: 1. Spores multiple; spore shape spherical; branching pattern sessile; stalk long and wide; spore size = 0.1 to 0.4 mm; sorocarp height = 3 to 6 mm; basal disc = 0.5 mm in diameter (Cavender et al., 2002).

As illustrated in Fig. 5, *D. rosarium* are vertically upright, colorless, have long and wide branching stalks, with a number of sessile lateral sori. Spores are multiple, smooth, and spherical in shape. Its fruiting body base is clavate or acuminate. *D. rosarium*, though widely distributed, were found to have a restricted distribution in nature, increasing in rarity outside of the southern hemisphere (Romeralo et al., 2010). Its occurrence in the Philippines could suggest implications for new information on life cycle, sporulation mechanism, as well as adaptability.

***Dictyostelium* sp.1.** Upland: 15/Coastal: 5. Spores multiple; spore shape spherical; branching pattern abstriction with regular whorls; stalk long.

*Dictyostelium* sp.1 as observed in Fig. 6, are seen to have a single spore on top and multiple spores around the stalk just below the singular one. Other than those parts, there are distinguishable similarities to *D. discoideum* for the rest of its features. Minimal information is available for this species, and even its specific epithet has not been identified yet.

***Raperostelium minutum*** (Raper) S. Baldauf, S. Sheikh and Thulin. Upland: 2/Coastal: 1. Spores single; spore shape elliptical; branching pattern one branched and unbranched; spore size =  $5$  to  $7\ \mu\text{m} \times 3$  to  $3.5\ \mu\text{m}$ ; sorocarp height =  $550$  to  $850\ \mu\text{m}$ ; basal disc =  $12$  to  $20\ \mu\text{m}$  in diameter (Raper, 2018).

*Raperostelium minutum* have short and narrow stalks, as well as present branching. Its morphological features are evident in Fig. 7. Spores are singular and oblong in shape. They are distinguished from other members of the group by having notably diminutive branched sorocarps that are more common within this species than in any other *Dictyostelium* group. They also have conspicuously smaller radiate pseudoplasmodiums as well as differentiating patterns following complete fructification (Raper, 2018).

## Discussion

This taxonomic report on six dictyostelid species is the first documentation for this relatively unexplored province in the Philippines. So far, among the eumycetozoans, only myxomycetes (Balaoro-Banzuela et al., 2023) and protosteloid amoebae (Zahn et al., 2014) have been reported on the litter substrates collected haphazardly in the reef-to-

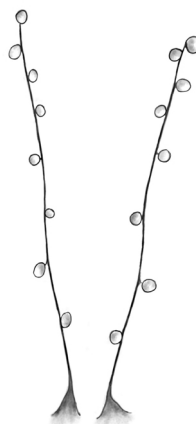


Fig. 5. Fruiting bodies of *Dictyostelium rosarium*.



Fig. 6. Fruiting bodies of *Dictyostelium* sp.1.

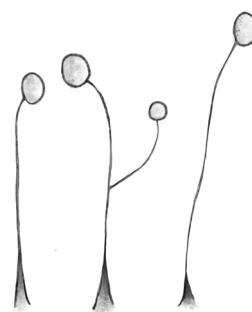


Fig. 7. Fruiting bodies of *Raperostelium minutum*.

ridge terrains. Dictyostelids and myxomycetes are the two groups of slime molds that are commonly present and frequently abundant in terrestrial ecosystems because of their similarity in the usage of

resources (Clark et al., 2002). Compared to the study by Balaoro-Banzuela et al. (2023), carried out at a similar study site in La Union, myxomycetes were found to have more species present than the dictyostelids identified in our study. This may happen due to factors such as their difference in nutrient utilization techniques, microhabitat preferences, and life cycle adaptations (Landolt and Stephenson, 1986; Schnittler and Stephenson 2000; Schnittler and Stephenson, 2002). One of the most abundant species (registered in 27 out of 60 plates) was *H. pallidum* – a finding consistent with a previous study conducted in Puerto Rico, a tropical country (Stephenson et al., 1999). In contrast, *R. minutum* was noted as one of the less prevalent species, registered only in three plates out of the whole set. This is unsurprising given its primary distribution in temperate regions, making it rare in tropical or subtropical countries (Cavender et al., 2010). Another less prominent species is *D. purpureum*, which was only found once from an upland soil sample. Their occurrence was also registered in temperate regions in the US and China (Landolt et al., 2009; Singh, 1947). Additionally, *D. discoideum* is known to be a cosmopolitan species (Langousis, 2008), and in our study, it was present in 36 out of the 60 plates. They are known to have a disjunct distribution pattern, which indicates the possibility that the species might constitute a part of the few remaining populations of a once widely distributed species (Watson et al., 1995). *D. rosarium* was first discovered in the Philippines in the province of Laguna based on the report of Dogma (1975). There were 8 plates positive for *D. rosarium* whereas this is the first time the species have been found in the coastal area of a tropical country. An unknown species of *Dictyostelium* was also present in both upland and coastal soils. No published records for its morphology has been found so far; thus, there is a possibility that it is a species new to science.

Nonetheless, efforts to uncover biogeographic distribution of cellular slime molds, especially in the ASEAN countries, have been slowly progressing. Besides these studies in the Philippines, other ASEAN countries such as Thailand, for example, have already reported 22 species from studies carried out in Chiang Mai, Nakhon Si Thammarat, and Songkhla provinces (Cavender, 1976; Seephueak and Petcharat, 2014; Vadell et al., 2018). With the current number of 12 dictyostelids in the Philippi-

nes, this is still relatively low. However, this fact calls for more taxonomic training and field surveys that should be conducted in major biodiversity hotspots in the Philippines so that the patchy occurrence records of dictyostelids in the country could match up with the relatively good species account of myxomycetes in the Philippines (Dagamac et al., 2012; Bernardo et al., 2018; Isagan et al., 2020).

Moreover, the anecdotal reports of dictyostelids in the Philippines were usually isolated from upland soils. This is due to the fact that the nature of the soil of most tropical mountainous ecosystems already points out the diversity of dictyostelids that can thrive (see Yulo and dela Cruz, 2012). However, to the best of our literature search, this is the first attempt to isolate dictyostelids using the coastal soils that are of marine origin and are seen along the coastal plains and basin land as a narrow strip formed from fluvial sediments of lacustrine or riverine sediments. Interestingly, five out of 6 species reported herein were found on coastal alluvial-forming soils, which now allows us to think of the high potential of such protists to tolerate marine environments. Interestingly, it would also be a good prospect to check for dictyostelids in other soils known to have similar profiles, such as those in the estuarine environments like the mangrove ecosystems.

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