

Two new records of dictyostelids from five nature parks in Singapore

Keegan T.Y. Lee-Ng¹

¹ Department of Biological Sciences, Faculty of Science, National University of Singapore, 14, Science Drive 4, Singapore 117543, Singapore.

E-mail: e0878466@u.nus.edu

Received: 28 October 2024

Accepted for publication: 5 February 2025

Published: 10 February 2025

Editor: Thomas Edison dela Cruz

Abstract: Two new records of dictyostelid cellular slime molds are reported from 107 samples of ground soil collected between January and June 2024 from waste-woodland and native-dominated secondary tropical rainforests in Singapore. They are *Dictyostelium barbarae* and *Dictyostelium insulativitatis*. This adds to Cavender's (1976) survey of cellular slime molds in Southeast Asia, bringing the total records of dictyostelids in Singapore to seven described species.

Keywords: ground soil, cellular slime molds, Southeast Asia

This work is licensed under a Creative Commons Attribution 4.0 International License

Introduction

Singapore (1.3521°N, 103.8198°E) is an island with an aseasonal tropical climate characterized by copious rainfall, and high temperatures and humidity (Yee et al. 2019). As a small island, Singapore has an unusual number of nature parks and reserves. The predominant vegetation of these nature parks is waste-woodland and native-dominated secondary tropical rainforests, which features vascular plants from such genera as *Ficus*, *Macaranga*, and *Syzygium* (Neo et al. 2013; Wong and Ganesan 2019; Chan et al. 2023). In forest ecosystems, dictyostelids are primarily found in the top one cm of ground soil, immediately below a well-developed litter (Cavender 1973; Landolt and Stephenson, 1989). According to Cavender and Raper (1969), the assemblage of species associated with tropical forests is supposed to be highly diverse. As predators of soil bacteria, dictyostelids are involved in the removal of pathogenic bacteria from soils, and in so doing, modulate carbon and nitrogen dynamics (Stephenson and Feest 2012; Zahn et al. 2016).

The previous survey of dictyostelids from Singapore was carried out by Cavender (1976), who isolated five distinct species belonging to two genera from Bukit Timah soil (e.g., *Dictyostelium mucoroides*, *D. purpureum*, *D. rhizopodium*, *Polysphondylium pallidum*, and *P. violaceum*). The purpose of this study was to survey the dictyostelids of the soil/litter interface in Singapore and thus expand on the checklist of dictyostelids recorded in the country. Nomenclature used herein for dictyostelids follows Sheikh et al. (2018).

Materials and methods

The localities from which ground soil samples were obtained are listed below (Table 1). All samples were collected between January and June 2024.

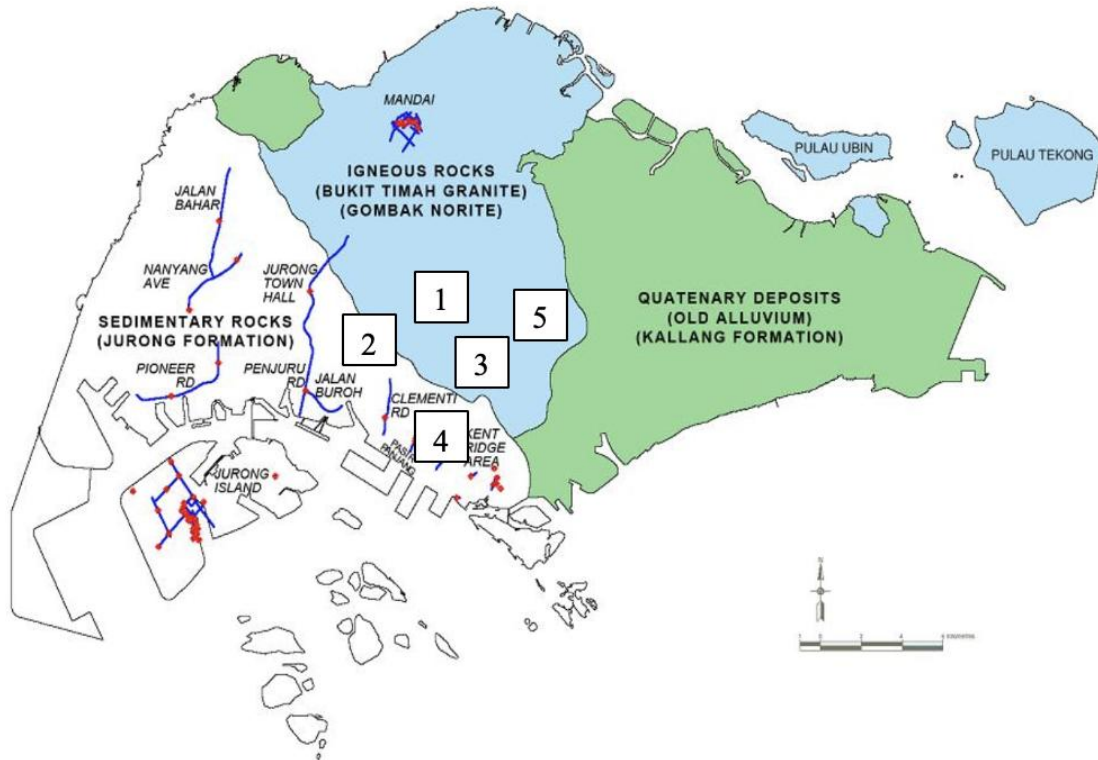


Figure 1. Geology of Singapore with study sites indicates. 1 = Dairy Farm Nature Park, 2 = Bukit Batok Hillside Park, 3 = Rifle Range Nature Park, 4 = National University of Singapore, 5 = Windsor Nature Park.

The collection methods were those described by Douglas et al. (2013). Using a drinking straw, approximately 1 cm of ground soil was excavated and transferred into microcentrifuge tubes. The straw was cut between collections, and the equipment (scissors and straw) was sterilized using 70% ethanol. Twenty-five of such samples were collected from Bukit Batok Nature Park, 16 samples from Dairy Farm Nature Park, 37 samples from National University of Singapore, Kent Ridge Campus, 20 samples from Rifle Range Nature Park, and nine samples from Windsor Nature Park, for a total of 107 samples. The samples were brought back to the laboratory at National University of Singapore and processed immediately or stored at 4°C for no longer than four days.

The isolation methods were modified from those of Cavender and Raper (1965). Each sample was weighed and enough sterile reverse osmosis water, adjusted to pH 7 with hydrochloric acid (HCl), was added to form a 1:100 dilution. The tubes were vortexed on the lowest setting for 1 hour to disperse the soil material and suspend the dictyostelids. Aliquots (each 100 µL) of soil suspension were added to each of three 9 mm x 9 mm Petri dishes pre-filled with hay (sundried and leached *Phleum*) infusion agar. A streak of *Escherichia coli* strain DH10B, maintained on Luria agar and suspended in 1x PBS solution, was

applied to the surface of the agar. The plates were incubated in a shaded area at 22-25°C for 14 days. Each inoculated plate was examined once on days 1, 3, 7, 10, and 14.

Table 1. Details of Study Sites.*

Locality	GPS	Secondary Forest Type	Geology	Major Wood Types	Vascular Plant Diversity
Dairy Farm Nature Park	1°21'48"N 103°46'25"E	Waste-woodland	Igneous	<i>Artocarpus integer</i> , <i>Cinnamomum iners</i> , <i>Durio zibethinus</i> , <i>Ficus</i> spp., <i>Hevea brasiliensis</i> , <i>Macaranga bancana</i> , <i>Spathogea campunulata</i> ,	No Data
Bukit Batok Hillside Park	1°21'31"N 103°44'45"E	Waste-woodland	Sedimentary and Igneous	<i>Ficus</i> spp., <i>Hevea brasiliensis</i> , <i>Macaranga</i> spp., <i>Syzygium</i> spp.	88 species from 54 families
Rifle Range Nature Park	1°20'36"N 103°46'46"E	Waste-woodland	Igneous	<i>Cinnamomum iners</i> , <i>Durio zibethinus</i> , <i>Macaranga heynei</i> , <i>Syzygium</i> spp., <i>Trema</i> spp.	401 species from 106 families
National University of Singapore, Kent Ridge Campus	1°17'47"N 103°46'35"E	Waste-woodland, and native-dominated	Sedimentary	<i>Adenanthera pavonina</i> , <i>Adrinandra dumosa</i> , <i>Albizia saman</i> , <i>Alstonia angustifolia</i> , <i>Cyrtophyllum fragrans</i> , <i>Peltophorum pterocarpum</i> , <i>Pterocarpus indicus</i> , <i>Rhodamnia cinerea</i>	420 species from 116 families
Windsor Nature Park	1°21'23"N 103°49'17"E	Waste-woodland, and native-dominated	Igneous	<i>Durio zibethinus</i> , <i>Hevea brasiliensis</i> , <i>Nephelium lappaceum</i> , <i>Rhodamnia cinerea</i> , <i>Syzygium filiforme</i>	311 species from 97 families

*The sites surveyed were secondary, anthropogenic, non- or limitedly managed young forests with comparatively level tree crowns (Chan and Davison 2019; Yee et al. 2011, 2019). Due to historical land-use where forests were cleared for cash crops, the soils have become poorly aerated, higher in temperature, highly degraded (e.g., low in calcium, nitrogen, and potassium), disturbed, and acidic (Yee et al. 2019). The information and data presented is non-exhaustive. It has been collated from personal observations and the listed studies: Chan et al. (2023), Neo et al. (2013, 2014), Tan et al. (2019), Wong and Ganesan (2019) and Yee et al. (2011, 2016, 2019).

Sorocarps were collected immediately for morphological and molecular work. DNA identification was performed using the Extract-N-Amp™ Tissue PCR Kit. GoTaq® Green Master Mix (Promega M7123) was used in place of Extract-N-Amp™ PCR Reaction Mix. Upon extraction, the genomic DNA solution was immediately subjected to small subunit (SSU) PCR amplification using the primer pairs 18SF-A (AACCTGGTTGATCCTGCCAG) and 18SR-B (TGATCCTTCTGCAGGTTTAC) (Medlin et al. 1988) and D542F(ACAATTGGAGGGCAAGTCTG) and D1340R (TCGAGGTCTCGTCCGTTATC) (Cavender et al., 2022). The PCR condition was: initial denaturation (95°C, 5 mins), 35 cycles (95°C, 30 s; 55°C, 20 s; 72°C, 45 s), final elongation (72°C, 10 mins) (Cavender et al. 2022). DNA products were verified via gel electrophoresis on a 3% DNA agarose gel stained with Gel Red® Nucleic Acid Gel Stain at 80V for 30 minutes. Viable PCR products were sent to Bio-Basics Asia for Sanger Sequencing. Identification was confirmed through NCBI Nucleotide BLAST.

Results

A total of 321 dictyostelid germination cultures were established from 107 collection of soil, but only seven yielded dictyostelids. In each case, only one of the triplicates was successful, and in all cases, both species were present together. Ultimately, two distinct species representing new records of dictyostelids in Singapore from the genus *Dictyostelium* were recovered.

Dictyostelium barbarae Stephenson, Liu, Li & Zou, 2019 (Fig. 2).

New Records. BBS18_24-6-24 [GPS: 1°21'35.0"N 103°44'38.9"E; Elevation: 68 m]; NUSS2_6-3-24 [GPS: 1°17'41.8"N 103°46'17.4"E; Elevation: 37 m]; NUSS3_6-3-24 [GPS: 1°17'41.4"N 103°46'17.6"E; Elevation: 49 m]; RRS11_17-6-24 [GPS: 1°20'47.9"N 103°47'05.0"E; Elevation: 49 m]; RRS6_17-6-24 [GPS: 1°20'56.6"N 103°47'03.1"E; Elevation: 65 m]; WS24_17-5-24 [GPS: 1°21'32.2"N 103°49'26.7"E; Elevation: 49 m]; WS32_17-5-24 [GPS: 1°21'27.5"N 103°49'23.3"E; Elevation: 32 m].

Identification. Sorocarps: white, clustered, unbranched, varied in height but commonly between 0.2 and 2 mm. Sorophores: white with several tiers of cells. Sori: white, globose. Spores: 5-8 x 3-5 µm, hyaline to oblong. Sanger Sequencing for both pairs of primers (18SF-A/18SR-B & D542F/D1340R) returned *Dictyostelium barbarae* with Query Cover = 100%, 98%; E-value = 0, 0; Per. Ident. = 100%, 99.35%, GenBank Accession No. MK322959.1, respectively (Liu et al. 2019). It is distinguished from *D. insulativitatis* by the multiple layers in the sorophore, and from *D. macrocephalum* by the base of the sorophore, which is clavate as opposed to round (Liu et al. 2019).

Dictyostelium insulativitatis Stephenson, Liu, Li & Zou, 2019 (Fig. 3).

New Record. BBS18_24-6-24 [GPS: 1°21'35.0"N 103°44'38.9"E; Elevation: 68 m]; NUSS2_6-3-24 [GPS: 1°17'41.8"N 103°46'17.4"E; Elevation: 37 m]; NUSS3_6-3-24 [GPS: 1°17'41.4"N 103°46'17.6"E; Elevation: 49 m]; RRS11_17-6-24 [GPS: 1°20'47.9"N 103°47'05.0"E; Elevation: 49 m]; RRS6_17-6-24 [GPS: 1°20'56.6"N 103°47'03.1"E; Elevation: 65 m]; WS24_17-5-24 [GPS: 1°21'32.2"N 103°49'26.7"E; Elevation: 49 m]; WS32_17-5-24 [GPS: 1°21'27.5"N 103°49'23.3"E; Elevation: 32 m].

Identification. Sorocarps: white, gregarious, unbranched, and, on average, smaller than *D. barbarae*, commonly 0.4 to 1.2 mm tall. Sorophore: white, with one or two tiers of cells only, the base is likewise composed of one tier. Sori white, globose. Spores: 6-10 x 4-7 μm , hyaline or oblong, but are, on average, larger than *D. barbarae*. Sanger Sequencing for both pairs of primers (18SF–A/18SR–B & D542F/D1340R) returned *Dictyostelium insulativitatis* with Query Cover = 100%, 99%; E-value = 0, 0; Per. Ident. = 100%, 99.09%, GenBank Accession No. MK322958.1 (Liu et al. 2019).

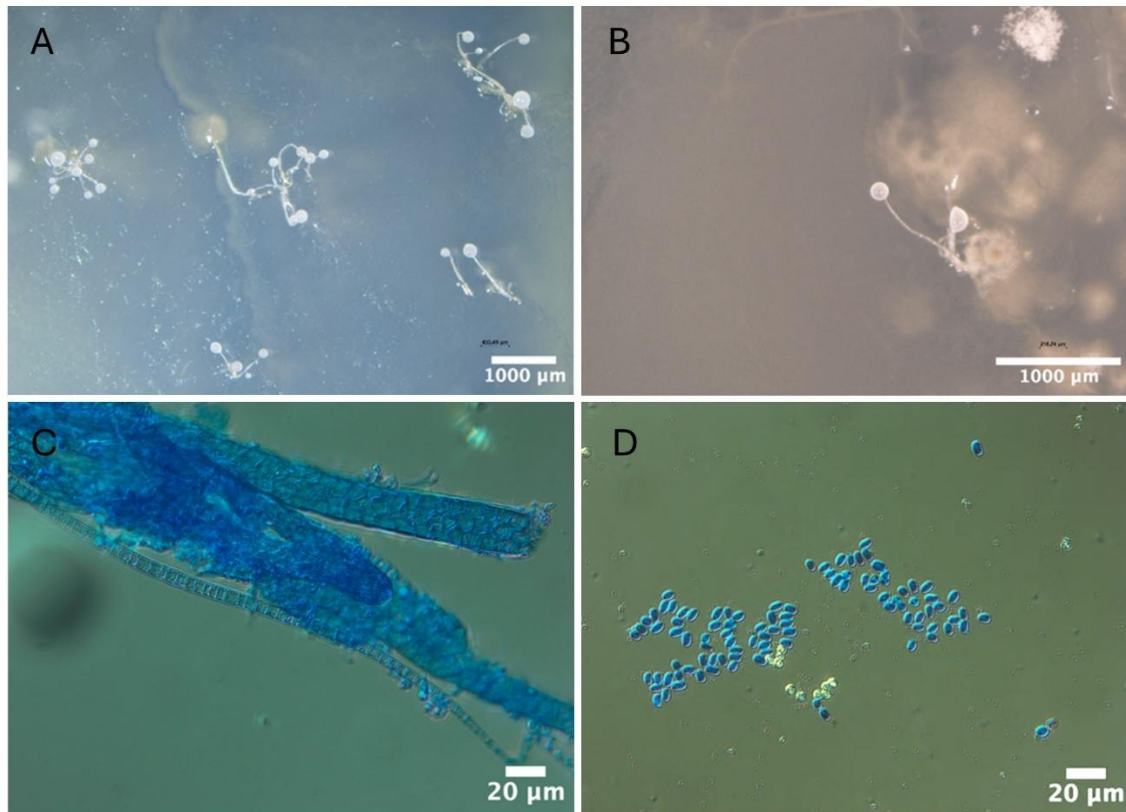


Figure 2. Morphological features of *Dictyostelium barbarae* Stephenson, Liu, Li & Zou, 2019. (A-B) Stereozoom images of sorocarps growing on hay-infusion agar; (C). Details of sorophore, stained with Lactophenol Cotton Blue and visualized with DIC, x400; (D) Spores stained with Lactophenol Cotton Blue and visualized with DIC, x400.

Discussion

The biodiversity of dictyostelid is supposed to be highest in forest soils occurring at tropical latitudes (Swanson et al. 1999), so the recovery of only two species in the present study is markedly low. Interestingly, neither *Dictyostelium mucoroides* nor *Heterostelium pallidum* (previously known as *Polysphondylium pallidum*), the two most abundant species worldwide, was recovered in this survey. In fact, none of the species previously recorded in Cavender (1976) was recovered. This may be because Cavender (1976) examined soils from “Bukit Timah”, a region where primary forests (or older, less disturbed secondary forests) still existed at the time of the study. In contrast, the present study only

examined soils from well-beaten nature trails in degraded secondary forests. Although no study has chronologically assessed the changes to the population dynamics of microbes in Singapore's forests' soils, it is likely that anthropogenic activities in the last 40 years have affected the top-soil microbiome.

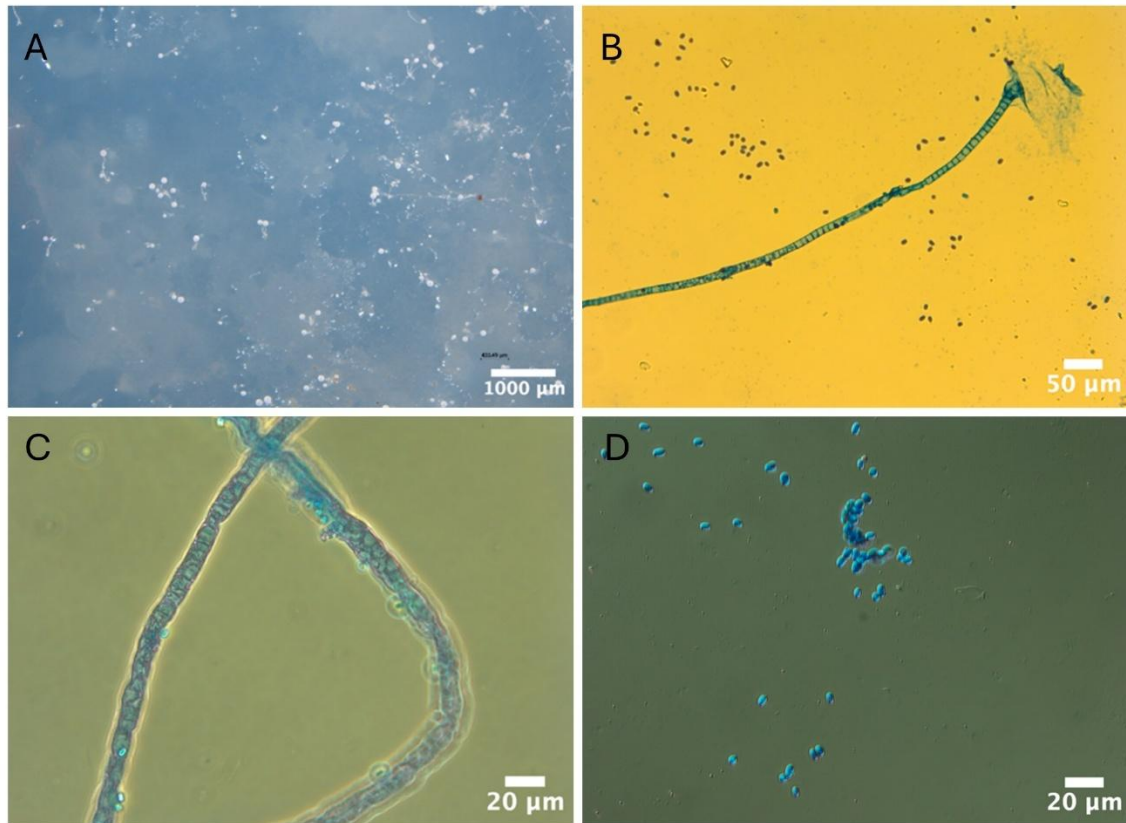


Figure 3. Morphological features of *Dictyostelium insulinativitatis* Stephenson, Liu, Li & Zou, 2019. (A). Stereozoom image of sorocarps emerging from the surface of HA-culture plates; (B). Details of sorophore, stained with Lactophenol Cotton Blue, x100; (C). Details of sorophore, Phase Contrast x400; (D). Spores, DIC x400.

Since Singapore does not possess significant difference in longitude, latitude, elevation/altitude, temperature, rainfall, soil pH or composition, comparing parameters classically demonstrated to affect the diversity and community composition of soil protists is unlikely to reveal productive results (Sun et al. 2024). Indeed, it is difficult to explain the scarcity and unpredictability of the dictyostelid's occurrence.

Nevertheless, several hypotheses come to light. First, while the dictyostelid prefer slightly acidic conditions (pH 6.5) (Yulo and dela Cruz 2012), Singapore's soil pH ranges between 3 and 5. Second, the top-layer is usually characterized by near-complete decomposition due to chemical and physical weathering (Rohardjo et al. 2004). Therefore, competition between members of the decomposer community is extremely tight. Met with unfavorable living conditions and high competition from prolifically reproducing microbes and diverse fungi, the dictyostelids, which do not form extensive

nutrient gathering networks, may be a disadvantaged minority in the soil microbiome. Finally, the fact that only Group 4 dictyostelids—incapable of microencystment—have been found in Singapore, and form the bulk of species isolated from wet-tropical Southeast Asian areas (Yulo and dela Cruz 2011, 2012), appears to bolster the hypothesis that unfavorably high-osmolarity selects against groups of dictyostelids that encyst into a dormant stage incapable of outlasting extended durations of wetness. Furthermore, this indicates that Group 4 dictyostelids may possess alternative mechanisms that allow them to survive in these harsh conditions. Of course, a caveat to this hypothesis is that *Polyspondylium pallidum*, reported in Cavender (1976) has been moved to the genus *Heterostelium* (Group 2); the members of this group, should be able to encyst since it is a common trait in the Amoebozoa (Kin and Schaap 2021).

In summary, the present study supplements the data on the dictyostelids for the island of Singapore, but these data are quite limited. Additional sampling and further investigations into the soil microbiome's complex dynamics and the mechanisms behind adaptations that the dictyostelids possess are warranted to develop a better understanding of the biodiversity and ecology of these organisms in Singapore, and in Southeast Asia.

Acknowledgements

The project was made possible because of the funding and research facilities provided by the Department of Biological Sciences (National University of Singapore) and the research permit NP/RP24-002(a) granted by the National Parks Board. I am grateful for the guidance and feedback provided by Amy Choong Mei Fun, Ying Chang, Geoffrey Zahn, and Steven L. Stephenson. Finally, I thank Neville Ho Zi Hergn and Spencer Heng Ek Khun for helping in sample collection.

References

- Cavender JC. 1976. Cellular slime molds of Southeast Asia. II. Occurrence and distribution. *Am J Bot.* 63(1): 71.
- Cavender JC, Raper KB. 1965. The Acrasieae in nature. Isolation. *Am J Bot.* 52: 294-296.
- Cavender JC, Raper KB. 1969. The occurrence and distribution of Acrasieae in forests of subtropical and tropical America. *Am J Bot.* 55: 504-513.
- Cavender JC, Vadell EM, Perrigo AL, Landolt JC, Stephenson SL, Liu P. 2022. Four new species of dictyostelids from soil systems in Northern Thailand. *J Fungi.* 8(6): 593.
- Chan L, Davison GWH. 2019. Introduction to the comprehensive biodiversity survey of Bukit Timah Nature Reserve, Singapore, 2014–2018. *Gard Bull Singapore.* 71(suppl.1): 3-17.
- Chan L, Ng D, Lim LK. 2023. Handbook on habitat restoration: general principles and case studies in Singapore. National Parks Board, Singapore. 340 p.
- Douglas TE, Brock DA, Adu-Oppong B, Queller DC, Strassmann JE. 2013. Collection and cultivation of dictyostelids from the wild. In: Eichinger L, Rivero F, editors. *Dictyostelium discoideum* Protocols. New Jersey: Humana Press. p. 113-124

- Kin K, Schaap P. 2021. Evolution of multicellular complexity in the dictyostelid social amoebas. *Genes* 12(4): 487.
- Landolt JC, Stephenson SL. 1989. Cellular slime molds in forest soils of West Virginia. *Mycologia* 82: 114-119.
- Liu P, Zou Y, Li W, Li Y, Li X, Che S, Stephenson, SL. 2019. Dictyostelid cellular slime molds from Christmas Island, Indian Ocean. *mSphere* 4:10.1128/msphere.00133-19.
- Neo L, Yee ATK, Chong KY, Kee CY, Lim RCJ, Ng WQ, Ng XY, Tan HTW. 2013. The vascular plant flora of Bukit Batok, Singapore. *Nature in Singapore* 6: 265-287.
- Neo L, Yee ATK, Chong KY, Yeoh YS, Tan HTW. 2014. The vascular plant flora of abandoned plantations in Singapore IV: Windsor Forest. *NiS*. 7: 93-109.
- Rohardjo H, Aung KK, Leong EC, Rezaur RB. 2004. Characteristics of residual soils in Singapore as formed by weathering. *Eng Geol.* 73(1-2): 157-169.
- Sheikh S, Thulin M, Cavender JC, Hernández E, Kawakami S-I, Lado C, Landolt JC, Nanjundiah V, Queller D, Shaap P, Strassmann J, Spiegel F, Stephenson SL, Vadell EM, Baldauf SL. 2018. A new classification of the dictyostelids. *Protist* 169: 1-28.
- Stephenson SL, Feest A. 2012. Ecology of soil eumycetozoans. *Acta Protozool.* 51(3): 201–208.
- Swanson AR, Vadell EM, Cavender JC. 1999. Global distribution of forest soil dictyostelids. *J Biogeogr.* 26: 133-148.
- Wong KM, Ganesan SK. 2019. The plant diversity of Singapore. In Middleton DJ, editor. *Flora of Singapore*, Vol. 1. Singapore: National Parks Board. p. 37-46.
- Yee ATK, Chong KY, Neo L, Tan HTW. 2016. Updating the classification system for the secondary forests of Singapore. *RBZ.* 32: 11-21.
- Yee ATK, Chong KY, Seah WW, Lua HK, Yang S. 2019. Vegetation of Singapore. In Middleton DJ, editor. *Flora of Singapore*, Vol. 1. Singapore: National Parks Board. p. 47-70.
- Yee ATK, Corlett RT, Liew SC, Tan HTW. 2011. The vegetation of Singapore—an updated map. *Gard Bull Singapore* 63(1&2): 205-212.
- Yulo PRJ, dela Cruz TEE. 2011. Cellular slime molds isolated from Lubang Island, Occidental Mindoro, Philippines. *Mycosphere* 2(5): 575-582.
- Yulo PRJ, dela Cruz TEE. 2012. Bacterial and yeast food preferences of cellular slime molds (Dictyostelids) isolated from Lubang Island, Occidental Mindoro, Philippines. *Philipp J Syst Biol.* 6: 46-53.
- Zahn G, Wagai R, Yonemura, S. 2016. The effects of amoebal bacterivory on carbon and nitrogen dynamics depend on temperature and soil structure interactions. *Soil Biol Biochem.* 94: 133-137.