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First records of protosteloid amoebae isolated from leaf litter in Singapore

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Abstract: Nine new records of protosteloid amoebae are reported from 88 samples of leaf litter collected between January and June 2024 from waste-woodland and native-dominated secondary tropical rainforests in Singapore. They are *Acanthamoeba pyriformis*, *Cavostelium apophysatum*, *Ceratiomyxella tahitiensis/Nematostelium gracile*, *Protostelium mycophaga*, *Schizoplasmodiopsis amoeboidea*, *S. pseudoendospora*, *S. vulgaris*, *Tychosporium acutostipes*, and *Vannella fimicola*. This is the first survey conducted on the occurrence of protosteloid amoebae in Singapore and the second in Southeast Asia.

Keywords: leaf litter, protosteloid amoebae, Southeast Asia

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 as they represent secondary tropical rainforests with 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, protosteloid amoebae are found terrestrially on dead plant matter, and occasionally, in streams and water bodies, but are only known to fruit at the substrate/air interface (Lindley et al. 2007; Spiegel et al. 2017). According to Aguilar et al. (2011, 2014), Zahn et al. (2014) and Zahn (2015), while elevation, precipitation, and latitude are often inversely proportional to species richness and abundance, microhabitat variability is the most important determining factor at the species level.

There appears to be no previous records of protosteloid amoebae from Singapore. Only two studies have investigated the distribution and assemblages of these amoebae in the Paleotropics (e.g., Shadwick and Stephenson 2004, in northern India and Ocenar-Bautista et al. 2024, in the Philippines). The latter study represented the first survey conducted in Southeast Asia; they recovered 12 species belonging to nine genera from coastal litter. The purpose of the present investigation was to survey the protosteloid amoebae from leaf litter in Singapore and contribute to the checklist as a consequence.

Materials and methods

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

Table 1. Details of Study Sites.*****

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

*The sites surveyed were secondary, anthropogenic, non- or limited 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).

The collection methods were those described by Spiegel et al. (2007). Three or four leaves of a similar decay stage were collected in plastic bags. Eighteen of such samples were collected from Bukit Batok Nature Park, nine from Dairy Farm Nature Park, 29 from National University of Singapore Kent Ridge Campus, 18 from Rifle Range Nature Park, and 14 from Windsor Nature Park, for a total of 88

samples. Samples were brought back to the laboratory at National University of Singapore and processed immediately or stored at 4ºC for no longer than one week.

The isolation methods were modified from those of Spiegel et al. (2007). Ground litter was cut into approximately 2 cm squares; they were spread out in a pentagonal or octagonal shape into each of two 9 mm x 9 mm Petri dishes prepared with 1.5% water agar. The plates were incubated in a shaded area at 22-25ºC for 14 days. Each plate was examined once on days 7 and 14 using the stereomicroscope (Nikon, SMZ1270). Characterization and species identification were performed through morphological comparison using the "A Beginner's Guide to Identifying the Protostelids" (Spiegel et al. 2007) and other publications, since protosteloid amoebae—at least the 37 described species—are still identified via morphospecies concept (Spiegel et al. 2017).

Results

A total of 176 protosteloid amoebae germination cultures were established from 88 collections of ground litter. Nine species of protosteloid amoebae from eight genera were recovered. One undescribed species was included as observational data since no identification was confidently ascribed to it.

Species list

Acanthamoeba pyriformis (Olive & Stoian.) Spiegel & Shadwick 2016 (Fig. 1).

New Records. BBG6_24-6-24 [GPS: 1°21'39.2"N 103°44'42.5"E; Elevation: 18 m]; BBG22_24-6-24 [GPS: 1°21'33.3"N 103°44'39.3"E; Elevation: 71 m]; NUSG1_15-2-24 [GPS: 1°17'42.9"N 103°46'15.9"E; Elevation: 38 m]; NUSG7_11-6-24 [GPS: 1°17'55.3"N 103°46'18.2"E; Elevation: 71 m]; RRG2_17-6-24 [GPS: 1°20'40.6"N 103°46'52.5"E; Elevation: 46 m].

Identification. Sporocarp: variable in size, resembling *Protostelium mycophaga*. Stalk: narrow, erect to gently curved and slightly tapered, with a knob-like apophysis, stiffer than *P. mycophaga*. Spore: obpyriform, with socket in narrow basal part of the spore (arrowed in Figure A, and, especially prominent in B).

Comments. *Acanthamoeba pyriformis* (= *Protostelium pyriformis*) is commonly found in the tropics (Spiegel et al. 2017).

Additional References. Ocenar-Bautista et al. (2024), Spiegel et al. (2017), and Tice et al. (2016).

Cavostelium apophysatum L.S. Olive 1965 (Fig. 2).

New Records. BBG2_7-5-24 [GPS: 1°21'36.6"N 103°44'46.4"E; Elevation: 18 m]; BBG3_24-6-24 [GPS: 1°21'37.7"N 103°44'44.3"E; Elevation: 33 m]; BBG13_24-6-24 [GPS: 1°21'32.7"N 103°44'43.0"E; Elevation: 62 m]; NUSG1_15-2-24 [GPS: 1°17'42.9"N 103°46'15.9"E; Elevation: 38 m].

Identification. Sporocarp: sizes may vary considerably from isolate to isolate, but the proportion of spore-to-stalk is consistent, often fruits in dense patches (Figure B). Stalk: reduced and wide, with a

cup-like apophysis (arrowed in Figure A). Spore: roughened in appearance, appearing less refractile than expected (arrowed in Figure C).

Additional References. Ocenar-Bautista et al. (2024) and Tice et al. (2016).

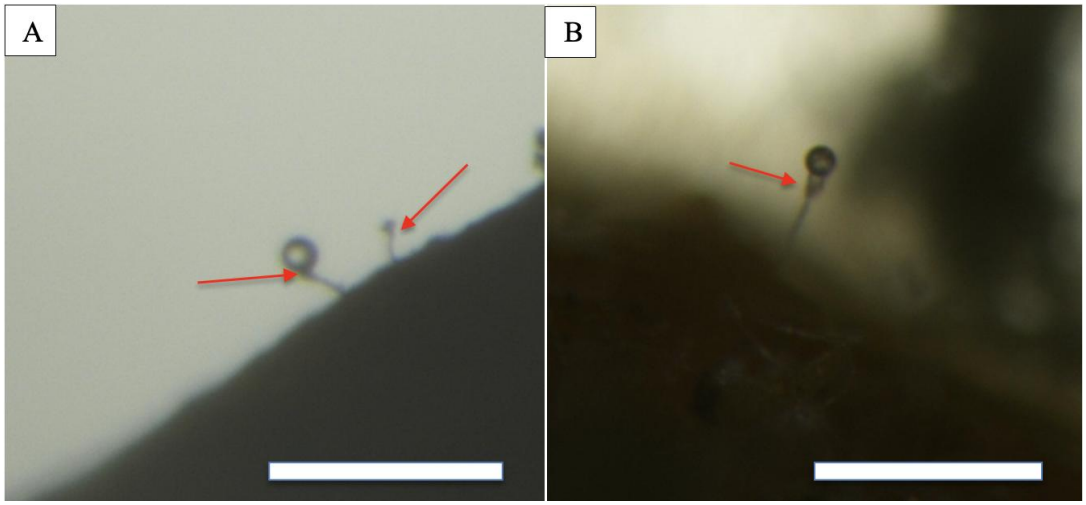

Figure 1. *Acanthamoeba pyriformis* (Olive & Stoian.) Spiegel & Shadwick 2016. Stereozoom Images, x80. Bar = 100 µm. *Note: the scale bars are estimates.

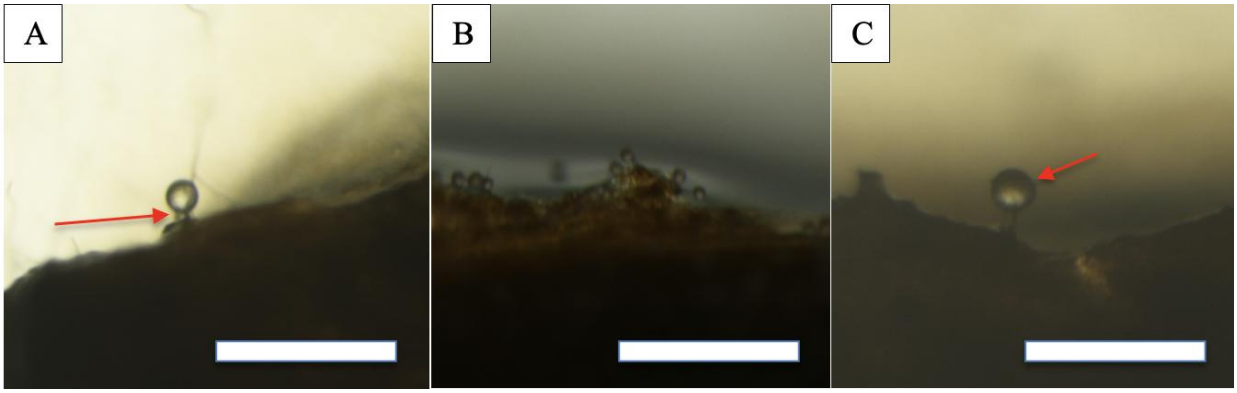

Figure 2. *Cavostelium apophysatum* L.S. Olive 1965. Stereozoom Images, x80. Bar = 100 µm. *Note: the scale bars are estimates.

Ceratiomyxella tahitiensis Olive & Stoianovitch/*Nematostelium gracile* (Olive & Stoian.) Olive & Stoianovitch Complex (Fig. 3).

New Records. BBG9_7-5-24 [GPS: 1°21'32.5"N 103°44'44.0"E; Elevation: 70 m]; BBG13_24-6-24 [GPS: 1°21'32.7"N 103°44'43.0"E; Elevation: 62 m]; BBG29_7-5-24 [GPS: 1°21'35.3"N 103°44'40.4"E; Elevation: 69 m]; DFG11_17-6-24 [GPS: 1°21'47.2"N 103°46'37.9"E; Elevation: 56 m]; NUSG6_22-4-24 [GPS: 1°17'51.9"N 103°46'14.6"E; Elevation: 49 m]; NUSG7_11-6-24 [GPS:

1°17'55.3"N 103°46'18.2"E; Elevation: 71 m]; RRG7_17-6-24 [GPS: 1°20'45.3"N 103°46'57.3"E; Elevation: 50 m].

Identification. Sporocarps: often gregarious. Stalk: extremely long, not refractile along its entire length. Spore: spherical to slightly turbinate.

Comments. *Ceratiomyxella tahitiensis* and *Nematostelium gracile* share identical morphotypes; they can only be distinguished by spore-to-spore culture or by molecular methods. *Nematostelium gracile* produces only plasmodia; it will not germinate amoeboflagellates. The species complex has a worldwide distribution and is commonly found in lowland tropics (Spiegel et al. 2017).

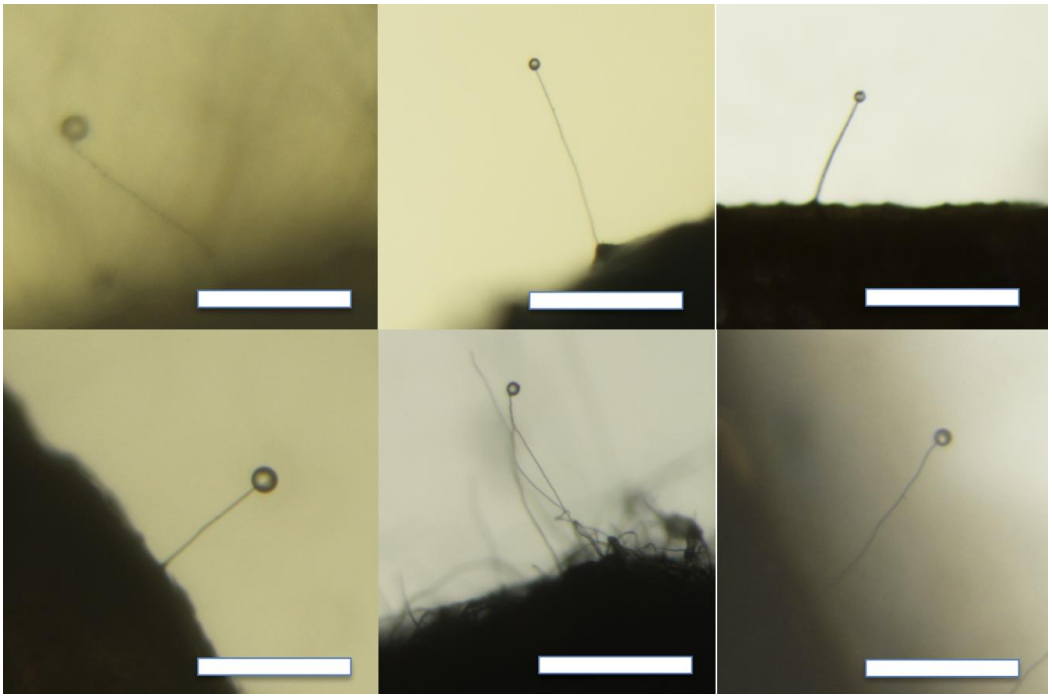

Figure 3. *Ceratiomyxella tahitiensis* Olive & Stoianovitch/*Nematostelium gracile* (Olive & Stoian.) Olive & Stoianovitch Complex. Stereozoom Images, x80. Bar = 100 µm. *Note: the scale bars are estimates.

Protostelium mycophaga Olive & Stoianovitch Species Complex (Fig. 4).

New Records. BBG2_7-5-24 [GPS: 1°21'36.6"N 103°44'46.4"E; Elevation: 18 m]; BBG3_7-5-24 [GPS: 1°21'35.4"N 103°44'43.2"E; Elevation:55 m]; BBG6_24-6-24 [GPS: 1°21'39.2"N 103°44'42.5"E; Elevation: 18 m]; BBG7_7-5-24 [GPS: 1°21'36.1"N 103°44'46.9"E; Elevation: 17 m]; BBG9_7-5-24 [GPS: 1°21'32.5"N 103°44'44.0"E; Elevation: 70 m]; BBG22_24-6-24 [GPS: 1°21'33.3"N 103°44'39.3"E; Elevation: 71 m]; DFG10_17-6-24 [GPS: 1°21'52.0"N 103°46'31.0"E; Elevation: 37 m]; DFG11_17-6-24 [GPS: 1°21'47.2"N 103°46'37.9"E; Elevation: 56 m]; NUSG1_15-2-24 [GPS: 1°17'42.9"N 103°46'15.9"E; Elevation: 38 m]; NUSG1_18-3-24 [GPS: 1°17'38.4"N 103°46'16.0"E; Elevation: 29 m]; NUSG1_22-4-24 [GPS: 1°17'50.1"N 103°46'43.5"E; Elevation: 21 m]; NUSG6_22-4-24 [GPS: 1°17'51.9"N 103°46'14.6"E; Elevation: 49 m]; NUSG7_11-6-24 [GPS: 1°17'55.3"N 103°46'18.2"E;

Elevation: 71 m]; NUSG9_18-3-24 [GPS: 1°18'02.1"N 103°46'20.9"E; Elevation: 16 m]; RRG2_17-6-24 [GPS: 1°20'40.6"N 103°46'52.5"E; Elevation: 46 m]; RRG2_25-1-24 [GPS: 1°20'37.2"N 103°46'57.9"E; Elevation: 58 m]; RRG7_17-5-24 [GPS: 1°20'44.7"N 103°46'52.2"E; Elevation: 41 m]; RRG8_17-6-24 [GPS: 1°20'44.5"N 103°46'58.7"E; Elevation: 51 m]; WG2_17-5-24 [GPS: 1°21'34.6"N 103°49'36.2"E; Elevation: 15 m]; WG12_13-2-24 [GPS: 1°21'34.5"N 103°49'29.5"E; Elevation: 46 m].

Identification. Sporocarps: considerable variation in size but constant proportions with degreed spore deciduousness. Stalk: tapered at maturity (arrowed in Figures A and C), often with a kink (arrowed in Figure C) that separates the upper, shrivelled portion from the lower straight-stalked region. Spore: spherical smooth (Figure E).

Comments. By far the most abundant and common species in the world (Spiegel et al. 2017) and also in Singapore (as noted herein). The morphological variability of *Protostelium mycophaga/mycophagum* suggests that the species is a complex including the 'rare' *Protostelium aurantium* and others (e.g., *Protostelium nocturnum*) (Shadwick et al. 2018; Spiegel et al. 2017).

Additional References. Ocenar-Bautista et al. (2024).

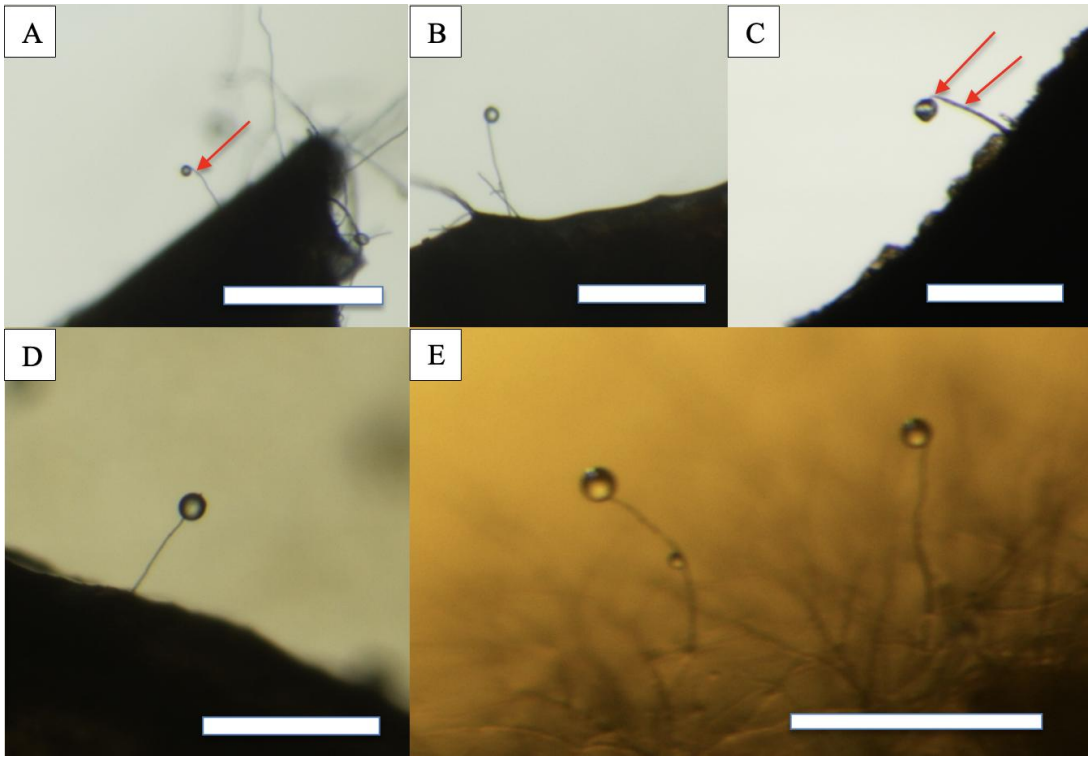

Figure 4. *Protostelium mycophaga* Olive & Stoianovitch Species Complex. Stereozoom Images, x80. Bar = 100 µm. *Note: the scale bars are estimates.

Schizoplasmodiopsis amoeboidea (Olive & Stoian.) Spiegel 1994 (Fig. 5).

New Records. BBG13_24-6-24 [GPS: 1°21'32.7"N 103°44'43.0"E; Elevation: 62 m]; NUSG1_18-3-24 [GPS: 1°17'38.4"N 103°46'16.0"E; Elevation: 29 m]; NUSG1_22-4-24 [GPS: 1°17'50.1"N 103°46'43.5"E; Elevation: 21 m]; NUSG2_15-4-24 [GPS: 1°17'42.4"N 103°46'17.1"E; Elevation: 47 m]; NUSG6_22-4-24 [GPS: 1°17'51.9"N 103°46'14.6"E; Elevation: 49 m]; NUSG9_11-6-24 [GPS: 1°17'49.9"N 103°46'26.5"E; Elevation: 58 m]; NUSG9_18-3-24 [GPS: 1°18'02.1"N 103°46'20.9"E; Elevation: 16 m]; RRG8_17-6-24 [GPS: 1°20'44.5"N 103°46'58.7"E; Elevation: 51 m]; RRG10_25-1-24 [GPS: 1°20'45.3"N 103°47'03.1"E; Elevation: 40 m]; WG2_17-5-24 [GPS: 1°21'34.6"N 103°49'36.2"E; Elevation: 15 m]; WG25_17-5-24 [GPS: 1°21'30.7"N 103°49'25.7"E; Elevation: 39 m].

Identification. Sporocarps: in groups or singly. Stalks: short, with a taper at the apex. Spore: spherical, smooth, and disproportionately large.

Additional Reference. Ocenar-Bautista et al. (2024).

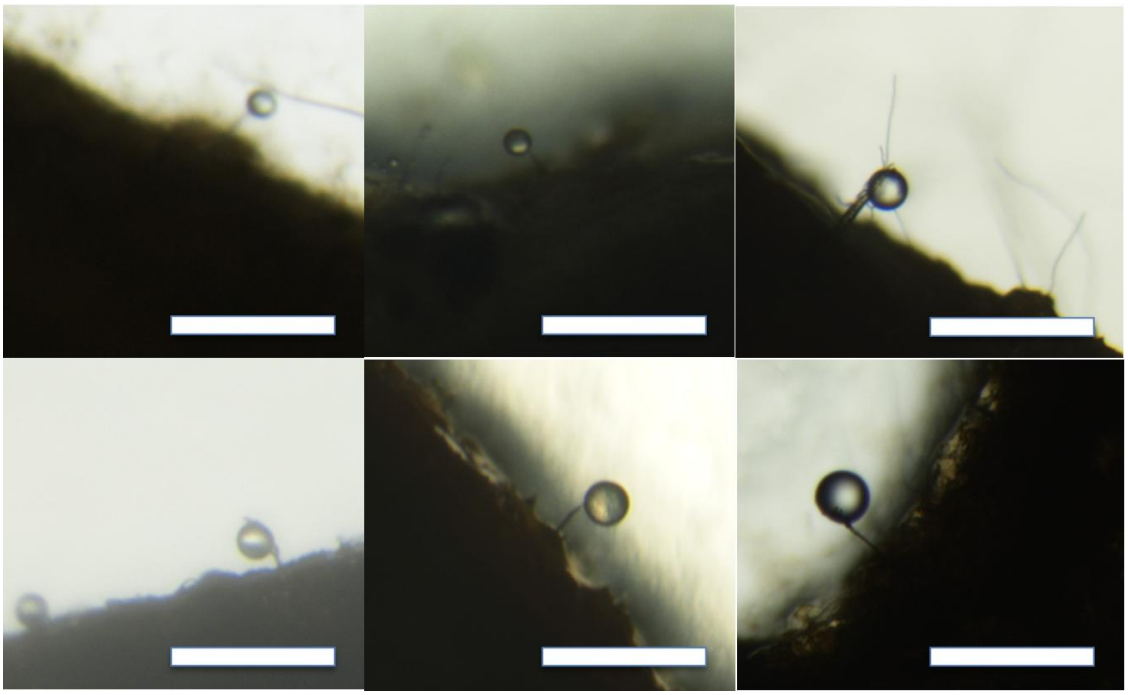

Figure 5. *Schizoplasmodiopsis amoeboidea* (Olive & Stoian.) Spiegel 1994. Stereozoom Images, x80. Bar = 100 µm. *Note: the scale bars are estimates.

Schizoplasmodiopsis pseudoendospora Olive, Martin & Stoian. 1967 (Fig. 6).

New Records. BBG3_7-5-24 [GPS: 1°21'35.4"N 103°44'43.2"E; Elevation:55 m]; BBG7_7-5-24 [GPS: 1°21'36.1"N 103°44'46.9"E; Elevation: 17 m]; BBG11_7-5-24 [GPS: 1°21'32.7"N 103°44'43.0"E; Elevation: 67 m]; BBG13_24-6-24 [GPS: 1°21'32.7"N 103°44'43.0"E; Elevation: 62 m]; BBG29_7-5-24 [GPS: 1°21'35.3"N 103°44'40.4"E; Elevation: 69 m]; BBG34_7-5-24 [GPS: 1°21'30.6"N 103°44'45.5"E;

Elevation: 78 m]; DFG10_17-6-24 [GPS: 1°21'52.0"N 103°46'31.0"E; Elevation: 37 m]; NUSG1_18-3-24 [GPS: 1°17'38.4"N 103°46'16.0"E; Elevation: 29 m]; RRG2_25-1-24 [GPS: 1°20'37.2"N 103°46'57.9"E; Elevation: 58 m]; RRG4_17-6-24 [GPS: 1°20'41.8"N 103°46'51.3"E; Elevation: 40 m]; RRG6_17-6-24 [GPS: 1°20'44.7"N 103°46'52.2"E; Elevation: 41 m]; RRG7_17-5-24 [GPS: 1°20'44.7"N 103°46'52.2"E; Elevation: 41 m]; WG4_13-2-24 [GPS: 1°21'34.1"N 103°49'33.1"E; Elevation: 28 m]; WG18_17-5-24 [GPS: 1°21'34.0"N 103°49'31.7"E; Elevation: 50 m]; WG29_17-5-24 [GPS: 1°21'24.7"N 103°49'22.5"E; Elevation: 40 m]; WG33_17-5-24 [GPS: 1°21'35.9"N 103°49'25.3"E; Elevation: 16 m].

Identification. Stalk: short, consistent width throughout. Spore: spherical, small, highly refractile.

Comments. *S. pseudoendospora* is one of the smallest yet most commonly reported species of protosteloid amoebae; their distribution is worldwide with little to no difference in abundance between the tropics and temperate regions; they are most abundant in the leaf litter layer (Spiegel et al. 2017).

Additional Reference. Ocenar-Bautista et al. (2024).

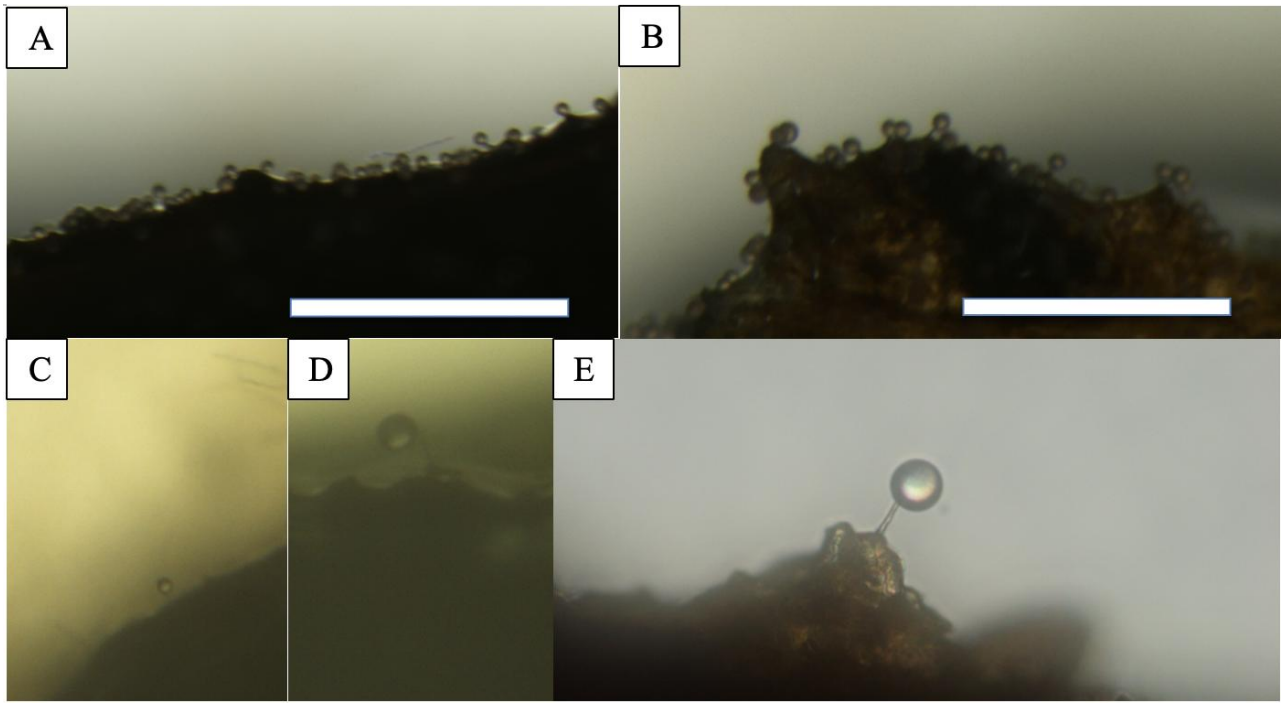

Figure 6. *Schizoplasmodiopsis pseudoendospora* Olive, Martin & Stoian. 1967. (A-D). Stereozoom Images, x80; (E). Compound Microscope, x400. Bar = 100 µm. *Note: the scale bars are estimates.

Schizoplasmodiopsis vulgaris Olive & Stoian. 1976 (Fig. 7).

New Records. BBG3_7-5-24 [GPS: 1°21'35.4"N 103°44'43.2"E; Elevation: 55 m]; BBG22_24-6-24 [GPS: 1°21'33.3"N 103°44'39.3"E; Elevation: 71 m]; NUSG1_18-3-24 [GPS: 1°17'38.4"N 103°46'16.0"E; Elevation: 29 m]; NUSG6_22-4-24 [GPS: 1°17'51.9"N 103°46'14.6"E; Elevation: 49 m]; NUSG9_18-3-24 [GPS: 1°18'02.1"N 103°46'20.9"E; Elevation: 16 m]; RRG6_17-6-24 [GPS:

1°20'44.7"N 103°46'52.2"E; Elevation: 41 m]; RRG10_25-1-24 [GPS: 1°20'45.3"N 103°47'03.1"E; Elevation: 40 m]; WG2_17-5-24 [GPS: 1°21'34.6"N 103°49'36.2"E; Elevation: 15 m]; WG5_17-5-24 [GPS: 1°21'32.9"N 103°49'34.5"E; Elevation: 36 m].

Identification. Sporocarps: usually gregarious; though the size may vary, the stalk, spore, and taper are always proportionate. Stalk: long, thick along its entire length, may be undulate, often has ridges (arrowed in Figure C), tapering slightly (Figure C). Spore: spherical, but coarse-looking.

Comments. This species has a worldwide distribution, most commonly found on decaying primary tissue (Spiegel et al. 2017), and appears to be associated with higher moisture levels. It resembles *Tychosporium acutostipes* (e.g., Figure A), but can be distinguished by the thinness of the stalk proportional to the taper.

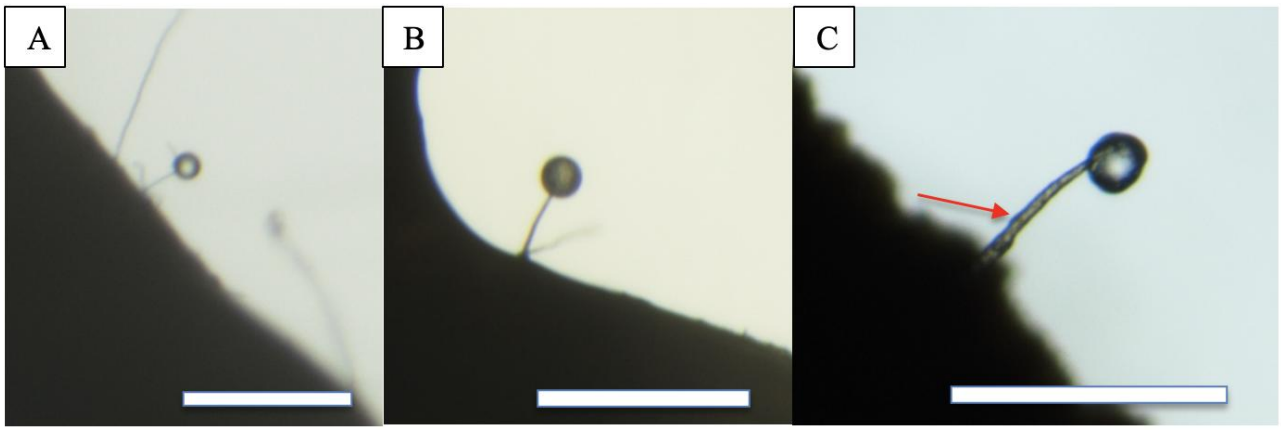

Figure 7. *Schizoplasmodiopsis vulgaris* Olive & Stoian. 1976. Stereozoom Images, x80. Bar = 100 µm. *Note: the scale bars are estimates.

Tychosporium acutostipes Spiegel, Moore & Feldman 1995 (Fig. 8)

New Records. BBG29_7-5-24 [GPS: 1°21'35.3"N 103°44'40.4"E; Elevation: 69 m]; BBG34_7-5-24 [GPS: 1°21'30.6"N 103°44'45.5"E; Elevation: 78 m]; NUSG10_11-6-24 [GPS: 1°17'48.1"N 103°46'31.2"E; Elevation: 74 m].

Identification. Stalks: usually appearing stiff, tapering sharply at 90% of its entire length. Spore: spherical.

Comments. This species is relatively uncommon and tends to fruit in dense strands on agar, though it can fruit singly (Figure B) or in small groups (Figure A) (Spiegel et al. 2017). It is distinguished from *P. mycophaga* because its spores are non-deciduous.

Additional References. Ocenar-Bautista et al. (2024).

Vannella fimicola (Olive & Stoianovitch) Kang et al. 2017 (Fig. 9).

New Records. BBG2_7-5-24 [GPS: 1°21'36.6"N 103°44'46.4"E; Elevation: 18 m]; BBG13_24-6-24 [GPS: 1°21'32.7"N 103°44'43.0"E; Elevation: 62 m]; BBG22_24-6-24 [GPS: 1°21'33.3"N 103°44'39.3"E; Elevation: 71 m]; BBG34_7-5-24 [GPS: 1°21'30.6"N 103°44'45.5"E; Elevation: 78 m]; RRG4_17-6-24 [GPS: 1°20'41.8"N 103°46'51.3"E; Elevation: 40 m]; RRG7_17-6-24 [GPS: 1°20'45.3"N 103°46'57.3"E; Elevation: 50 m]; WG12_13-2-24 [GPS: 1°21'34.5"N 103°49'29.5"E; Elevation: 46 m]; WG18_17-5-24 [GPS: 1°21'34.0"N 103°49'31.7"E; Elevation: 50 m]; WG21_17-5-24 [GPS: 1°21'33.3"N 103°49'27.3"E; Elevation: 44 m]; WG29_17-5-24 [GPS: 1°21'24.7"N 103°49'22.5"E; Elevation: 40 m].

Identification. Stalks: long, usually refractile along its entire length with a taper that does not come to a point (arrowed in Figure A), with noticeably broadened/swollen base (arrowed in Figures B and C). Spore: distinctly spherical (all Figures).

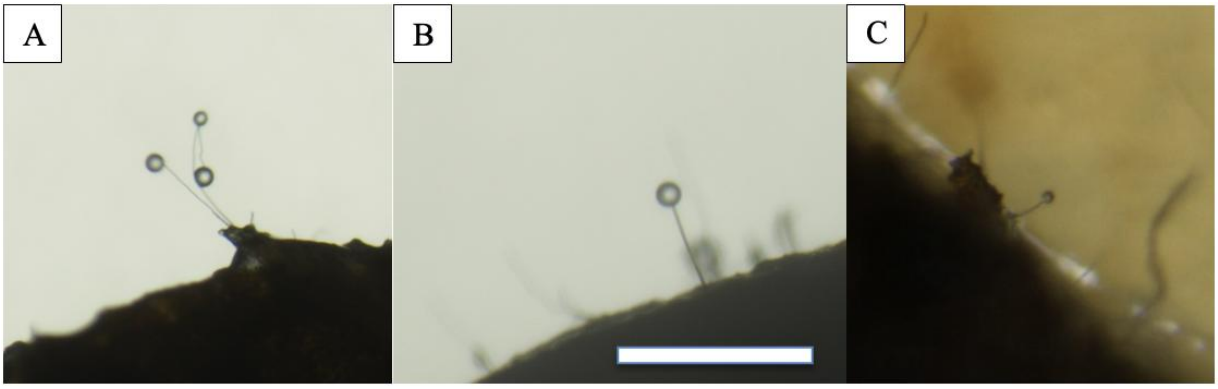

Figure 8. *Tychosporium acutostipes* Spiegel, Moore & Feldman 1995. Stereozoom Images, x80. Bar = 100 µm. *Note: the scale bars are estimates.

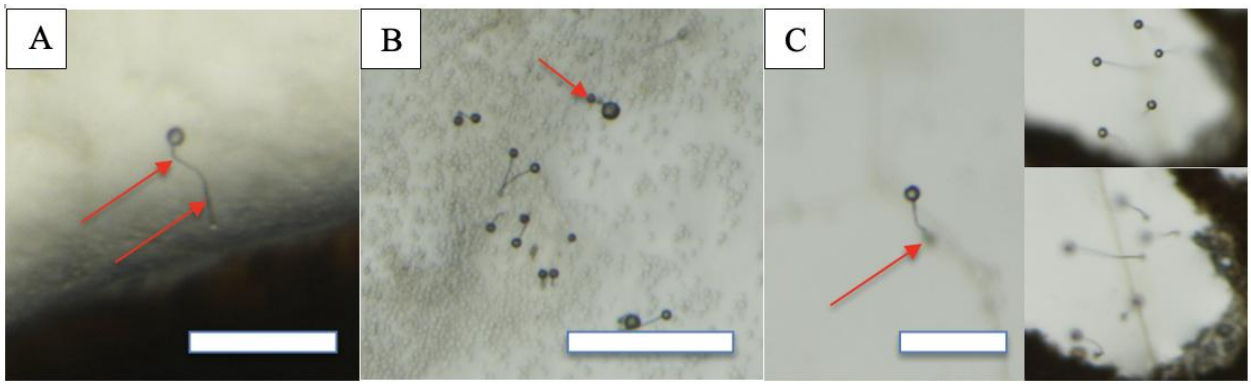

Figure 9. *Vannella fimicola* (Olive & Stoianovitch) Kang et al. 2017. Stereozoom Images, x80. Bar = 100 µm. *Note: the scale bars are estimates.

Comments. *Vannella fimicola* was formerly circumscribed to genus *Protostelium*, then moved to genus *Protosteliopsis* on the basis of sporocarpic morphology and non-deciduousness of spores (Olive & Stoianovitch 1966; Ozment 2019); however, molecular evidence in Shadwick et al. (2009) illustrated that the amoebal morphology is archetypical of vannellids. The species was moved into genus *Vannella* in Kang et al. (2017). Further confirmation of this species will require germination from spores and photographic evidence of trophic cells.

Undescribed species

New Records. RRG8_17-6-24 [GPS: 1°20'44.5"N 103°46'58.7"E; Elevation: 51 m]; WG29_17-5-24 [GPS: 1°21'24.7"N 103°49'22.5"E; Elevation: 40 m].

Identification. Stalk: bipartite with a thick basal portion (arrowed in Figure C). Spore: spherical, proportionally large.

Comments. The specimens identified were frequently found with a smaller-than-expected spore; Figure C is reminiscent of *Ceratiomyxa hemispherica*, but the stalks are too thick.

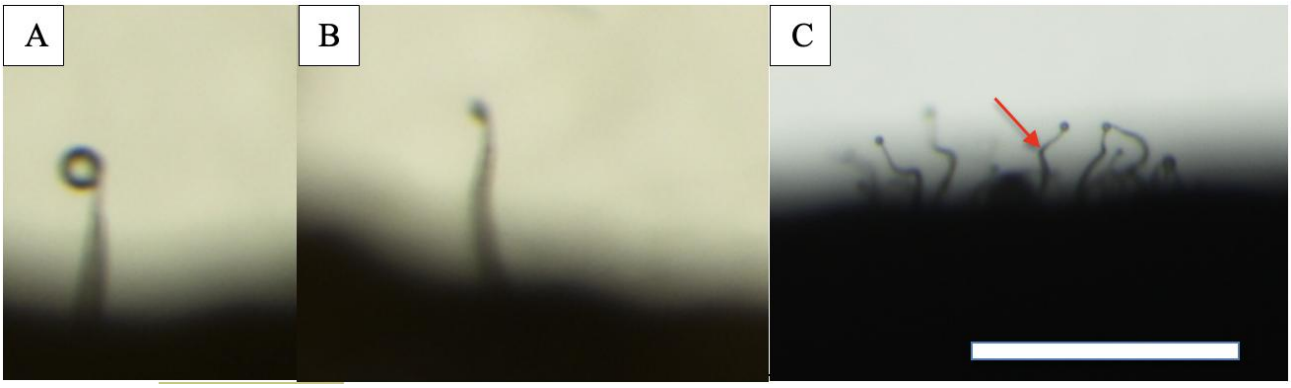

Figure 10. Undescribed species. Stereozoom Images, x80. Bar = 100 µm. *Note: the scale bars are estimates.

Discussion

Isolation cultures recovered nine species from eight genera of protosteloid amoebae, all of which are new records of protosteloid amoebae in Singapore. Moreover, Figure 10 provides observational evidence for a possibly new-to-science protosteloid amoeabae; it was initially identified as *Soliformovum expulsum* based on the assertion that no other species possesses a “reflex, bipartite stalk” according to Spiegel et al. (2017). However, the presence of an empty stalk rules *S. expulsum* out as the species is ballistosporous and the stalks disappear upon spore discharge.

According to Ocenar-Bautista et al. (2024), Stephenson et al. (1999), and Stephenson and Moore (1998), the diversity of vascular plant speecies may be correlated with species richness as they provide more microhabitats that are suitable for protosteloid amoebae. Although Singapore’s rainforests are highly

diverse—it was found that 2.4 acres of land in Bukit Timah Nature Reserve contains more tree species than the whole of North America (Lee and Lim 2015)—the surveyed regions in this study (i.e., along trails of Nature Parks) had considerably lower variety in plant species.

Nevertheless, the recovery of several species is in line with established trends: 1) *A. pyriformis* has a worldwide distribution though its climatic preference remains unelucidated; 2) *Cavostelium apophysatum* may, indeed, prefer tropical conditions (Stephenson and Moore 1998; Moore and Stephenson 1998; Stephenson et al. 1999; Moore and Spiegel 2000; Powers and Stephenson 2006); 3) the *Protostelium mycophaga* species complex is cosmopolitan, with abundant occurrence and widespread distribution; 4) *Schizoplasmodiopsis pseudoendospora* occurs frequently and dominantly in the leaf-litter layer (Powers and Stephenson 2006; Ndiritu et al. 2009; Aguilar et al. 2011; Spiegel et al. 2017).

This study is not without limitations. First, at 80x magnification, it is likely that many more species were present, but unidentifiable, especially when portions of the sporocarp where in different planes. Second, the infrequent examination of the germination plates may have led to instances were sporocarps were obfuscated by fungi. Third, some ballistosporous species like *Protostelium nocturnum*, reported in the Philippines by Ocenar-Bautista et al. (2024), were presumably present; however, since the entire stalk disappears after spore discharge (Spiegel et al. 2017), it is likely that their presence went unnoticed. Finally, species like *Nematostelium ovatum*, *Protosporangium bisporum*, and *Schizplasmodium cavostelioides* were suspected, but could not be confidently identified based on morphological observations made with the employed imaging techniques.

Ultimately, to unravel the latent species diversity and their biogeographical patterns, microhabitat preferences, and ecological roles, further work is required to form pure isolation cultures so that the spore-to-spore life cycles of these protists can be formally documented. Since over 100 species of protosteloid amoebae are estimated to have been observed, but not officially described, it is likely that many of these identifications will require revision.

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