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A rapid survey of myxomycetes associated with different substrates on Luzon Island, Philippines

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Abstract: Tropical habitats in the Philippines have many substrates (= microhabitats) that are suitable for myxomycetes. The present study looked at myxomycetes occurring in these substrates based on samples collected from selected sites on Luzon Island, Philippines. A total of 27 species belonging to 13 genera were identified from 142 positive moist chamber cultures which were prepared from ten substrate types. The highest number of species were recorded in grass litter with 14 species, followed by decayed woody vines or lianas (12), aerial leaf litter of *Musa paradisiaca* L. (8) and *Chromolaena odorata* (L.) King & Robinson (7), plant inflorescences (6), decayed fern fronds (5), ground leaf litter of *Swietenia macrophylla* King (4), decayed twigs (3), dung (3), pine-needle leaves (2), and broadleaf litter (2). *Arcyria cinerea*, *Diderma hemisphaericum* and *Stemonitis fusca* were the most abundant species of myxomycetes recorded.

Keywords: forest litter, microhabitats, moist chambers, slime molds, species list, substrate types

Introduction

Myxomycetes, also known as myxogastrids, are heterotrophic protist-like amoebozoans that are abundant in terrestrial habitats. Their occurrences and distribution are affected by abiotic and biotic factors such as temperature, pH, moisture, substrate type, and food availability, e.g., bacteria, yeasts, protists, and algae (Stephenson 1989; Everhart et al. 2008).

Substrate type is one of the important factors affecting the occurrences of myxomycetes in nature because dead and decomposing plants parts support moisture and microbial flora that provides suitable habitats for spore germination and food for the trophic stages of myxomycetes. Studies on preferences of myxomycetes on different substrates have been carried out in the tropical and temperate ecoregions and show varying distribution patterns. For instance, unique myxomycete assemblages have been observed on aerial structures of vascular plants (aerial leaf litter and other above-ground plant parts), inflorescences of neotropical herbs, ground leaf litter, dead barks of living trees, woody twigs, dung, and decaying wood (Schnittler 2001; Schnittler et al. 2002; Takahashi 2004; Novozhilov et al. 2007; Andrade-Bezerra et al.

2008; Everhart et al. 2008; Stephenson et al. 2008; Kilgore et al. 2009; Ko Ko et al. 2009; Macabago et al. 2010, 2012; Pecundo et al. 2021).

Other unique substrates for myxomycetes are submerged plant materials in water (Lindley et al. 2007) and on the skin of a living animal, *Corytophanes cristatus* Merrem (Townsend et al. 2005). In the Philippines, some of the interesting substrates so far studied for myxomycetes includes grass litter (Carascal et al. 2017), inflorescences (Pecundo et al. 2017), agricultural leaf litter (Alfaro et al. 2015; Redeña-Santos et al. 2017), and woody vines or lianas (Macabago et al. 2020; Pecundo et al. 2020). Ten different plant-based substrates including leaf samples of endemic plants were also surveyed for myxomycetes (dela Cruz et al. 2022). Synthetic materials such disposed old cloth, plastics, faux fur, and scouring pad also supported growth of myxomycetes (dela Cruz and Eloreta 2020; Garcia et al. 2022)

In addition, recent attempts have been made to survey ecosystems and other substrates commonly found in the country but often overlooked for myxomycetes including mangrove forests (Lim et al. 2021), beach forests (Cabutaje et al. 2021), and inland island ecosystems (Isagan et al. 2020). It is evident that the Philippines has abundant unique ecosystems supporting some of the global biodiversity hotspots with high plant endemism, yet it is interesting that some of the key microbial biodiversity components including myxomycetes associated with these locally available endemic plants are not well documented. Therefore, this study primarily aims to assess the occurrences of myxomycete species in different substrata collected within the main island of Luzon, Philippines.

Materials and methods

Study area

The main island of Luzon in the northern part of the Philippines is the largest inhabited and most populous island in the Philippine archipelago. It is bounded by the Philippine Sea in the east, the Sibuyan Sea in the south, the West Philippine Sea in the west, and the Luzon Strait in the north which separates the island from Taiwan. It has a land area of 104688 square km and a population of roughly 58 million as of May 2020. Vallejo (2014) noted Luzon Island as the oldest among the oceanic islands of the Philippine archipelago. Luzon Island, just like the rest of the country, is generally described as tropical and maritime with relatively high temperature, high humidity, and abundant rainfall. The specific study areas have Type I climate (Cavite, Ilocos Norte, Tarlac) with two pronounced seasons – dry from November to April and wet for the rest of the year, and Type II climate (Quezon) with no dry season but with a very pronounced maximum rain period from December to February (Philippine Atmospheric Geophysical and Astronomical Services Administration, PAGASA). The average temperature is between 25-30 ^{o}C while the average rainfall is between 78-142 mm.

Sampling localities and collected substrates

Eleven sites were visited in 2012 for the collection of 10 substrate types, also referred to as microhabitats, from different vegetation (Table 1). As used herein, the term microhabitat refers to some more limited portion of the total habitat (*sensu* Stephenson 1989). The sampled localities were found in the Luzon Island and were characterized by different land cover and use including secondary forest (a, c, d, e, g, h, i), agricultural plantation (b, f), lahar-affected area due to previous volcanic eruption (j), and sand dune (k). The specific microhabitats per sampling locality are listed in Table 1.

Moist chamber cultures and species identification

The moist chamber culture technique was used to grow myxomycetes. Preparation of the moist chambers follows the standard protocol described by Stephenson and Stempen (1994). The moist chamber cultures were kept at room temperature and regularly examined for a period of eight weeks, with an extension of another six weeks, to detect the presence of myxomycetes. Any visible fruiting bodies were removed from the moist chambers and placed in herbarium boxes. Species identification followed characterization of fruiting bodies and spore morphologies and comparison with published monographs (e.g., Stephenson and Stempen 1994; Keller and Braun 1999) and online identification guides (http://slimemold.uark.edu/). Taxonomic names were checked against the online nomenclatural database for the eumycetozoans (http://nomen.eumycetozoa.com; Lado 2005-2022).

Table 1. List of localities and substrates.

Localities and land use	Substrates (= microhabitats)
Cavite Province	
(a) Maragondon / secondary forest 14°13’16.14” N, 120°46’21.8” E	cow dung (CD)
(b) Indang / agricultural plantation 14°11’50.2” N, 120°51’58.8” E	aerial leaf litter of *Musa paradisiaca* var. *sapientum* (CBAL)
(c) Tagaytay / secondary forest 14°6’54.9” N, 120°54’35.6” E	ground leaf litter of *Swietenia macrophylla* (CMGL)
(d) Silang / open grassland 14°12’38.47” N, 121°1’37.9” E	grass litter of *Saccharum spontaneum* L. (CGR)
(e) Mt. Palay-Palay, Ternate / secondary forest 14°12’54” N, 120°38’46” E	woody vines or lianas (CL)
Quezon Province	
(f) Mulanay / agricultural plantation 13°32’0.8” N, 122°43’11.5” E	twigs of *S. macrophylla* King. (QMTW) aerial leaf litter of *M. paradisiaca* var. *sapientum* (QBAL) ground leaf litter of *S. macrophylla* (QMGL)
(g) Pitogo/ secondary forest 13°48’43.5” N, 122°6’25.3” E	twigs collected along marine habitat (QMTW)
(h) General Luna / secondary forest 13°39’31.7” N, 122°13’1.0” E	aerial leaf litter of *Chromolaena odorata* (QAL) inflorescences of *C. odorata* (QAI)
(i) Polilio Island / secondary forest 14°46’28.7” N, 121°5’17” E	woody vines or lianas (QPL)
Tarlac Province	
(j) Capas near Mt. Pinatubo area / grassland in lahar-affected area 15°21’2.76” N, 120°27’26.54” E	broadleaf litter (PTBL) fern fronds (PTFL) inflorescences (PTIN)
Ilocos Norte Province	
(k) Laoag / sand dune 18°11’45.65” N, 120°35’33.6” E	needle leaves of *Pinus insularis* (PN) grass litter of *Paspalum renggeri* Steud. (IGR)

Results

A total of 27 species of myxomycetes belonging to 13 genera were found during this study. These were derived from 142 moist chamber cultures that were all positive for myxomycetes. The highest number of species were recorded from Cavite Province (19 species) followed by Quezon with 16 species, Tarlac (10 species), and Ilocos Norte (7 species). The grass litter were the richest in species, followed by woody vines or lianas and aerial leaf litter (Figure 1). *Arcyria cinerea, Diderma hemisphaericum,* and *Stemonitis fusca* were the most abundant species.

Species list

The 27 myxomycetes taxa recorded in this rapid survey are listed below. In the list, the species are arranged in alphabetical order, accompanied with occurrence details of their localities and substrates. See Table 1 for substrate abbreviations.

Arcyria afroalpina Rammeloo [Cavite, Quezon; CBAL, QMTW, QAL]

A. cinerea (Bull.) Pers. [Cavite, Quezon, Tarlac, Ilocos Norte; CD, CBAL, CMGL, CGR, CL, QMTW, QBAL, QMGL, QAL, PTBL, PTFL, QPL, IPN, IGR]

A. pomiformis (Leers) Rostaf. [Cavite; CBAL, CGR]

Collaria arcyrionema (Rostaf.) Nann.-Bremek. ex Lado [Cavite, Quezon; CBAL, CGR, CL, QBAL, QAL, QPL]

Ceratiomyxa fruticulosa var. *fruticulosa* (O. F. Müll.) T. Macbr. [Quezon; QPL]

Comatricha tenerrima (M. A. Curtis) G. Lister [Cavite, Quezon, Ilocos Norte; CBAL, QBAL, IGR]

Clastoderma sp. [Cavite; CL]

Cribaria violacea Rex [Cavite, Quezon; CD, CL, QPL]

Diderma effusum (Schwein.) Morgan [Quezon, Tarlac; QAL, PTIN]

D. hemisphaericum (Bull.) Hornem [Cavite, Quezon; CMGL, CGR, QAL]

Didymium difforme (Pers.) Gray [Tarlac; PTFL]

D. nigripes (Link) Fr. [Cavite, Quezon; CGR, QAL]

D. squamulosum (Alb. & Schwein.) Fr. & Palmquist [Cavite, Quezon; CBAL, CGL, QAL]

Echinostelium minutum de Bary [Ilocos Norte; IGR]

Hemitrichia serpula (Scop.) Rostaf. ex Lister [Cavite, Tarlac; CL, PTFL]

Lamproderma scintillans (Berk. & Broome) Morgan [Cavite, Ilocos Norte; CMGL, CGR, IGR]

Perichaena chrysosperma (Curr.) Lister [Cavite, Quezon, Tarlac; CD, CL, PTIN, QPL]

P. depressa Lib. [Tarlac, Quezon; PTFL, PTIN, QPL]

P. pedata (Lister & G. Lister) G. Lister & E. Jahn [Cavite; CBAL, CL]

Physarum cinereum (Batsch) Pers. [Cavite, Tarlac; CGR, PTIN]

P. lakhanpalii Nann.-Bremek. & Y. Yamam. [Quezon; QPL]

P. leucophaeum Fr. & Palmquist [Tarlac; PTIN]

P. melleum (Berk. & Broome) Massee [Quezon, Ilocos; QBAL, IPN]

P. nutans Pers. [Cavite; CGR]

P. oblatum T. Macbr. [Cavite; CGR]

Physarum sp. [Quezon, Tarlac; QMTW, PTIN]

Stemonitis axifera (Bull.) T. Macbr. [Ilocos Norte; IGR]

S. fusca Roth [Cavite, Tarlac, Quezon, Ilocos Norte; CL, PTBL, PTFL, QPL, IGR]

S. nigrescens Rex [Cavite; CGR, CL]

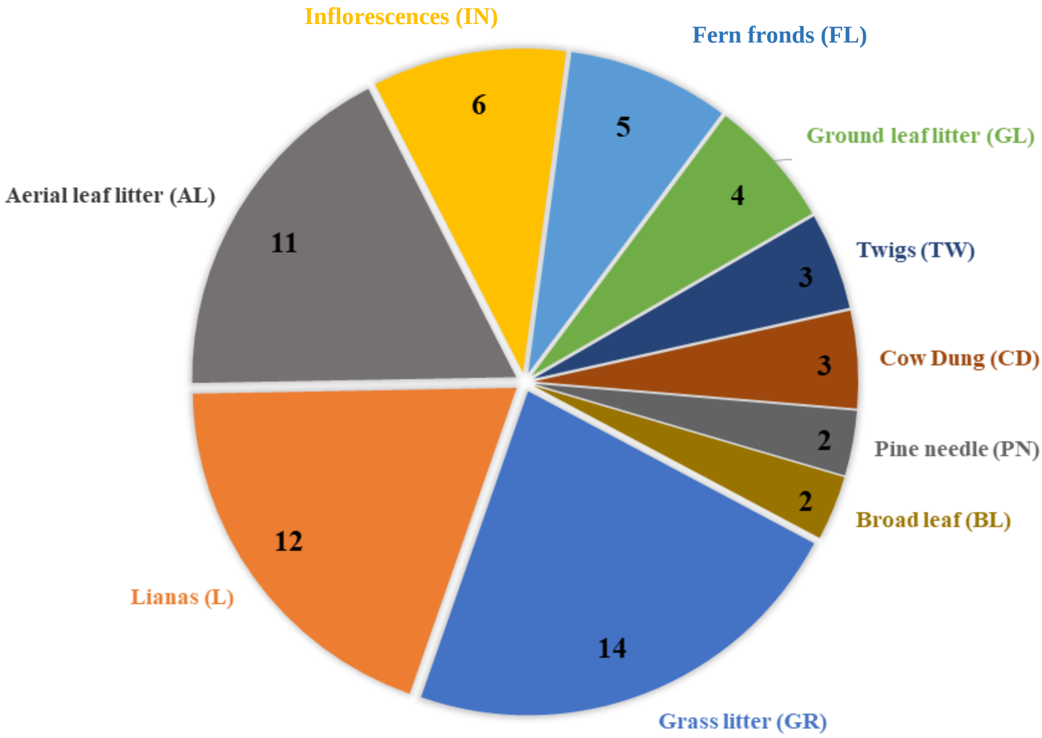

Figure 1. The number of species recorded per substrate. Substrate types: AL – aerial leaf litter of *Musa paradisiaca* and *Chromolaena odorata*, GL – ground leaf litter of *Swietenia macrophylla*, GR – grass litter of *Saccharum spontaneum* and *Paspalum renggeri*, TW – twigs, CD – cow dung, L – lianas or woody vines, IN - inflorescences, PN - needle leaves of *Pinus insularis*, BL – broadleaf trees, and FN - fern fronds.

Discussion

This rapid assessment survey recorded 27 myxomycetes species belonging to 13 genera from substrates collected within the main island of Luzon in Northern Philippines. The study relied exclusively on the moist chamber culture techniques, and hence, with a few localities studied herein, this could be attributed to the low number of species found. Thus, the number of myxomycete richness reported in the current study does not reflect the true species richness occurring in the main island of Luzon when compared with other studies elsewhere that reported a much higher number of species from a relatively fewer number of samples and substrates (Dagamac et al. 2012, 2014; Macabago et al. 2012; Cheng et al. 2013; dela Cruz et al. 2014; Eloreta et al. 2020).

For instance, Kuhn et al. (2013) in their study of three provinces in Luzon Island documented 25 species from samples of aerial and ground leaf litter, twigs, and barks. Corpuz et al. (2012) recorded 31 species from aerial and ground leaf litter and twigs from Mt. Palay-Palay in Cavite while woody vines or lianas collected from the same locality in this study harbored only nine species. Similarly, woody vines collected in Polilio Island herein had eight species of myxomycetes while Viray et al. (2014), with their study of ground leaf litter and twigs, recorded 34 species in the same island.

Meanwhile the study confirmed the diverse substrates for myxomycetes in the tropical regions. Some of the substrates that have received less attention for their myxomycete assemblages are aerial leaf litter of *Musa paradisiaca* (banana) and *Swietenia macrophylla* (mahogany), grass litter of *Saccharum* *spontaneum* and *Paspalum renggeri*, and decayed woody vines (also known as lianas), which had a high record of myxomycete species in comparison with other substrates (Fig. 1). These findings were consistent with other similar studies conducted in tropical forests (Wrigley de Basanta et al. 2008) including several forest sites of Southeast Asia in Thailand (Tran et al. 2008) and Vietnam (Tran et al. 2014; Nguyen et al. 2020).

The myxomycete assemblages were found to be partitioned between substrate types in relation to species composition, richness, and abundances. Aerial leaf litter are reported to be efficient in trapping spores due to their rough surface (Stephenson 1989). Ground-based organic matter harbors a high microbial load that phagotrophic stages of myxomycetes feed upon (Stephenson 2011). Woody substrates such as vines and twigs have high water holding capacity, creating a suitable moist environment in which slime molds develop (Snell and Keller 2003). Dead barks of living trees such as *S. macrophylla* and other dipterocarps support myxomycetes that are particularly adapted to grow on the bark surfaces (Policina and dela Cruz 2020a, 2020b). Grass litter provides ideal habitats for species that are easily dispersed by wind (Carascal et al. 2017; Pecundo et al. 2017).

Similar to other studies in the tropics, a low number of myxomycete assemblages were observed on fern fronds, herbivore dung, and needle leaves of *Pinus insularis*. Additionally, substantial number of myxomycetes were found in the aerial leaf litter of the herbaceous plant *Chromalaena odorata*. We also recorded eight species from aerial leaf litter of *Musa paradisiaca* while Buisan et al. (2019) reported only five species from dead ground leaves and decaying pseudostems of banana. Their other study also reported three cosmopolitan species of myxomycetes from aerial and ground leaf litter collected in rice fields (Buisan et al. 2020).

This finding suggests that dead plant parts of other tropical herbaceous plants should be further studied for the occurrence of myxomycetes. Overall, a detailed ecological study on the myxomycetes in tropical ecosystem is needed to increase our understanding of the relationships between the diverse microhabitats, environmental parameters and myxomycete assemblages.

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