Occurrence and distribution of myxomycetes (plasmodial slime molds) in three provinces of Luzon Island, Philippines

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Myxomycetes (slime molds) are ideal model organisms for population dynamics studies in terrestrial forest habitats. This research assessed the occurrence and distribution of myxomycetes in selected highland areas of three provinces in Luzon Island, Philippines. Myxomycetes present in the moist chambers of aerial and ground leaf litter, twigs, and dead barks of living trees were recorded and used to assess their abundance and diversity for each of the study sites. The obtained results showed that 51% of prepared moist chambers yielded myxomycetes. Identification of the collected myxomycetes resulted in 25 species belonging to 7 genera including *Arcyria, Comatricha, Diderma, Didymium, Perichaena, Physarum,* and *Stemonitis*. *Physarum decipiens* is reported as a new record for the Philippines. Four species including *Arcyria cinerea*, *Comatricha nigra, Physarum album*, and *Stemonitis fusca* were the most abundant. Many species were reported as rare. Our research showed similarities in the distribution of myxomycete assemblages in the study areas with closer proximities.

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INTRODUCTION

One of the less explored groups of organisms in the Philippines are the myxomycetes or plasmodial slime molds. This small, relatively homogenous but morphologically diverse group of eukaryotic, phagotrophic, fungus-like organisms occurs as common inhabitants of forest ecosystems where they feed on bacteria, protozoa, and yeast cells (Stephenson and Stempen 1994). For most of their life cycle, they exist as free-living plasmodium that typically thrives in cool and shady moist places. One or more minute-sized fruiting bodies then develop from this plasmodium in a drier and more exposed location after a period of feeding and growth. These fruiting bodies exhibit elaborate and intricate structures, each containing numerous spores which can be dispersed by wind and will eventually germinate and develop into a plasmodium under suitable conditions (Stephenson and Stempen 1994). This remarkable transformation from an animal-like to a fungus-like form makes myxomycetes ideal model systems for cellular development in lower life forms.

Studies on myxomycete assemblages have been done in tropical and temperate regions. Areas in the tropics studied for their myxomycetes included the Maquipucuna Cloud Forest Reserve in Ecuador (Schnittler et al. 2002), the Cocos Island in Costa Rica (Rojas and Stephenson 2008), and in Chile (Lado et al. 2007). Myxomycetes were also studied in temperate zones, e.g. in Southwestern Virginia (Stephenson 1989) and in Everglades National Park, Florida (Keller 1973) both in the United States, and in the Lower Volga River Basin in Russia (Novozhilov et al. 2006). However, many areas remain understudied or unexplored, particularly in Asia. In Southeast Asia, myxomycetes were recorded from Thailand and Burma or Myanmar (Reynolds and Alexopoulos 1971; Tran et al. 2006, 2008), and in Singapore (Rosing 2009). In the Philippines, myxomycetes were first reported from collections in Mindanao (Davao, Cotabato and Zamboanga) by E. B. Copeland, and in Luzon by A. D. E. Elmer (Benguet) and E.D. Merrill (Bataan, Manila, Cavite and Laguna) (Reynolds 1981). Their collections could only be found in the British Museum in London. Uyenco (1973) also reported 18 species belonging to 10 genera while Dogma (1975) listed 46 species of myxomycetes from 20 genera. To date, the most extensive species list of Philippine myxomycetes was that of Reynolds (1981). The annotated list of 107 species was based on published and unpublished records and included species of *Arcyria* (8), *Badhamia* (1), *Ceratiomyxa* (1), *Clastoderma* (1), *Comatricha* (5), *Craterium* (3), *Cribaria* (5), *Diachea* (4), *Dictydium* (1), *Diderma* (4), *Didymium* (10), *Echinostelium* (1), *Fuligo* (1), *Hemitrichia* (4), *Lamproderma* (3), *Licea* (1), *Lycogala* (2), *Metatrichia* (1), *Perichaena* (5), *Physarella* (1), *Physarum* (26), *Stemonitis* (6), *Trichia* (5), and *Tubifera* (3). Recently, Dagamac et al. (2011) and Macabago et al. (2012) reported eleven new records for the Philippines from Mt. Arayat National Park, Pampanga and Lubang Island, Occidental Mindoro, respectively. Dagamac et al. (2010) also included five new records of corticolous myxomycetes associated with *Samanea saman* (Jacq.) Merr. in the Philippines. Moreno et al. (2009) reported one new species from a specimen collected in Anda Island, Pangasinan. These publications bring the total number of myxomycetes in the Philippines to 124. Still, the recorded number is considered small, indicating that many more species of myxomycetes remain undiscovered in the Philippines and a vast tract of the country's tropical habitats remained un- or

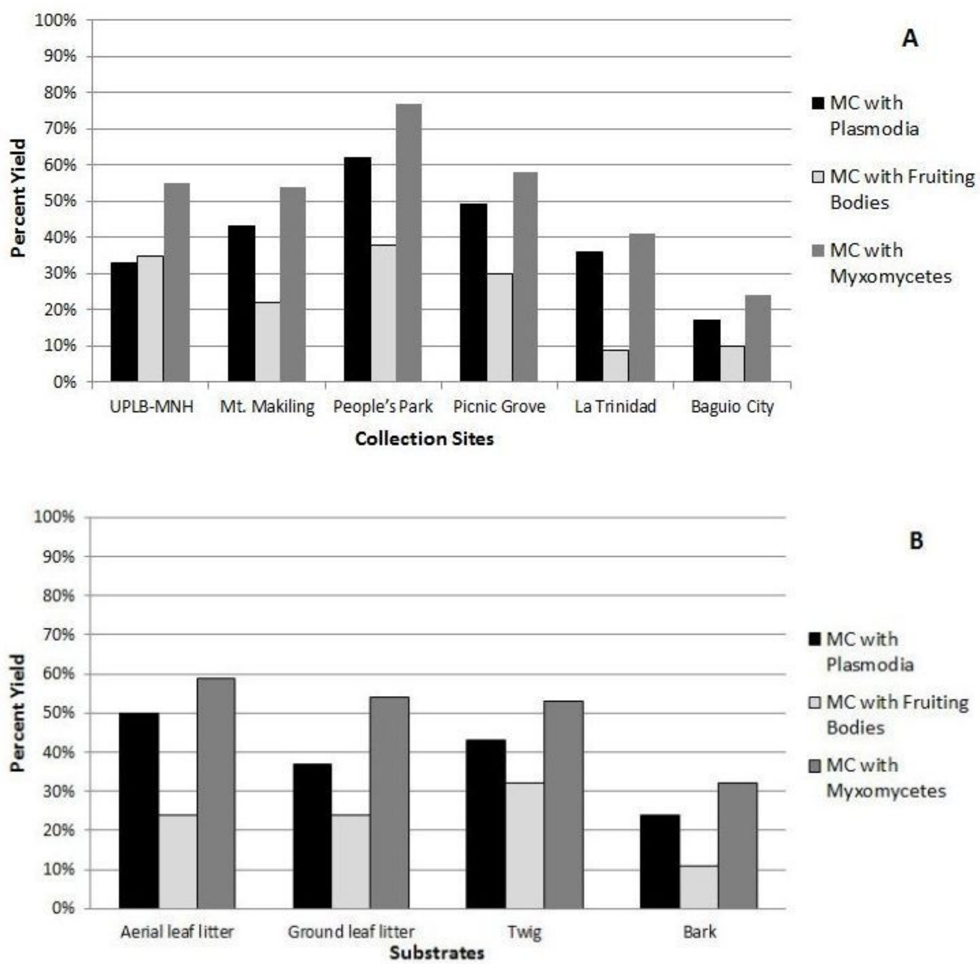

Figure 1. Percent yield of myxomycetes from different study sites (A) and different substrates (A) in three provinces in Luzon Island, Philippines.

under-explored. In this study, six study sites from 3 provinces (Cavite, Laguna, and Benguet) in Luzon Island were assessed for the occurrence and distribution of slime molds. These highland areas often have cool and moist conditions ideal for growth of myxomycetes. This research constitutes part of the more comprehensive study aimed in documenting the myxomycete assemblages in the Philippines.

MATERIALS AND METHODS

Sample Collection and Moist Chamber Preparation.

Aerial and ground leaf litter, dead twigs, and dead barks from living trees were randomly collected within six collection sites from three provinces with different elevations ranging from 600 – 1,500 meters above sea level (masl) during the months of April to May (Table 1) and air-dried prior to the preparation of moist chambers (MC). At least 5 samples for each substrate per site were collected. Moist chambers were set up following the protocol of Stephenson and Stempen (1994). The moist chambers were then placed inside wooden cabinets, not directly exposed to sunlight, incubated at room temperature for up to 8 weeks, and observed at least twice a week for the presence of plasmodia and/or fruiting bodies. The number of moist chambers with plasmodia and/or fruiting bodies was noted and recorded. Fruiting bodies with their respective substrate were then glued on herbarium boxes. All vouchers of collected specimens were deposited at the Pure and Applied Microbiology Laboratory, Research Center for the Natural and Applied Sciences, University of Santo Tomas.

Characterization and Identification of Plasmodial Myxomycetes.

Species identification of the collected myxomycetes was based on the morphologies of fruiting bodies, i.e. type, size, shape, appearance, and color, and spores, e.g. spore size, shape, texture, and color, presence and appearance of internal structures such as capillitium, and the presence or absence of lime ($CaCO_3$). The fruiting body and spore morphology descriptions were then compared with published literatures (Stephenson and Stempen 1994; Keller and Braun 1999), web-based identification keys (http://slimemold.uark.edu/), and an electronic, computer-based identification key, *SynKey* (Mitchell 2008). Current and valid names follow the on-line nomenclatural information system: (http://nomen.eumycetozoa.com/). Representative specimens were sent to Prof. Dr. Steven L. Stephenson, University of Arkansas, USA for the confirmation of their identities.

Ecological Analysis.

Productivity of the moist chambers per site and substrate was initially obtained by dividing the number of MC with plasmodia/fruiting bodies by the total number of MC prepared multiplied by 100. Abundance Indices (AI) were also assigned to all of the species represented among the collections from each of the given sites. A moist chamber with a myxomycete species is considered here as one collection. To determine the abundance of each species, the percentage of a particular species among the total number of collections was initially determined per study site. Then, a "breaking point" was assigned based on these percentage values as described in Dagamac et al. (2012). Abundance for each species per site was then noted or categorized as follows: abundant (A) if their relative abundance is ≥10% of the total collections; common (C) if RA is ≥5% but <10%; occasionally occurring (O) if RA is ≥3 but <5; and rare (R) if RA is <3%.

The myxomycete communities associated with the different collection sites were also compared using the coefficient of community (CC) and the percentage similarity (PS) indices as

Table 1. Description of the study sites.

Province	Collection Sites	Habitat Description	Elevation (in masl)	Monthly Rainfall (in mm)
Cavite	Picnic Grove, Tagaytay City	eco-park dominated by dipterocarp trees	640 masl	6.7 mm (February) to 481.9 mm (August)
	People's Park, Tagaytay City	eco-park with small trees and grasslands		
Laguna	UPLB Museum of Natural History, Los Baños	mahogany tree-dominated park area	1,090 masl	26.3 mm (February) to 321.4 mm (October)
	Mt. Makiling Botanic Garden, Los Baños	along walk trail to Raptor's Area dominated by dipterocarp trees		
Benguet	Teacher's Camp, Baguio City	pine forests inside camp	1,500 masl	11.7 mm (February) to 911.8 mm (August)
	Benguet State University Campus, La Trinidad	inside the campus		

previously described by Stephenson (1989). For the CC index, the equation was based solely on the presence or absence of species and is as follows:

$$CC = \frac{2c}{a + b} \qquad (1)$$

where a = total number of species in the first community being considered, b = total number of species in the second community, and c = number of species common to both communities. To interpret results, CC values close to 1.0 indicate both communities have the same species of myxomycetes. For the PS index, in which relative abundance of species and not only their presence is considered, the following equation was used:

$$PS = \Sigma \min (a, b, \dots x) \qquad (2)$$

where min = the lesser of the two percentage compositions of species a, b, …x in the two communities. The values for PS range from 0 for communities without common species to 1.0 for communities with similar species present both in composition and in quantitative values.

RESULTS AND DISCUSSION

Productivity of the Moist Chambers

Generally, little is known for myxomycete assemblages associated with tropical forests in the Southeast Asian region. A few published studies reported 124 species for the Philippines (Reynolds 1981; Moreno et al. 2009; Dagamac et al. 2010, 2011; Macabago et al. 2012). In the present study, assemblages of myxomycetes were assessed in six highland areas in Luzon Island using the moist chamber culture technique. Moist chambers were effective in reflecting myxomycete assemblage occurring in a region and were considered as an essential technique in surveying whole myxomycete diversity in tropical forests (Schnittler et al. 2002). The artificially maintained humidity in moist chambers and the moisture content of the substrates contribute to the formation of myxomycetes (Ing 1994; Wrigley-de Basanta et al. 2008). Of the 420 moist chambers prepared in the study, 51% yielded myxomycetes either as plasmodium and/or fruiting bodies (data not shown). Plasmodia (40%) were observed more than the fruiting bodies (23%). Many of these plasmodia did not eventually develop into fruiting bodies, and hence the lower number of moist chambers with fruiting bodies. However, their presence still indicates the existence of myxomycetes. A similar productivity was also observed from moist chambers of substrates collected in La Mesa Ecopark in the Philippines (Macabago et al. 2010) and in agricultural and forest sites in Thailand (Tran et al. 2008).

Among the different collection sites, People's Park in Tagaytay City (Cavite) yielded the most number of myxomycetes (77%) and also had the highest number of MC with plasmodia (62%) and fruiting bodies (38%) (Fig. 1A). Substrates collected from Baguio City (Benguet) gave the least number of myxomycetes (24%). The number of MC with plasmodia was also lowest in Baguio City (17%), while fruiting bodies were least counted from samples collected in La Trinidad (9%). In our study, higher percent yield was generally observed at 650 masl elevation (Cavite) than at 1,500 masl elevation (Benguet). These results was supported by previous studies of Rojas and Stephenson (2008) and Stephenson et al. (2004) which reported decreasing myxomycete diversity with increasing elevations.

Among the four substrates collected, aerial leaf litter gave the highest myxomycete yield (59%) followed by ground leaf litter (54%), twigs (53%) and barks (32%) (Fig. 1B). Plasmodia were recorded highest on aerial leaf litter while fruiting bodies were found more on twigs. Macabago et al. (2010) also recorded higher percentage yield in aerial leaf litter compared to ground leaf litter. These results were similar with the studies of Novozhilov et al. (2000) where high productivity (35%) were recorded from leaf litter collected from different forest types in Puerto Rico. On the other hand, our results with the bark samples contradicted that of Stephenson (1989) where the percentage yield ranged from 70 to 90%, which is higher than other reports. The rough, thick, and furrowed barks may presumably be better spore traps for myxomycetes, and thus, yield better results (Stephenson 1989). Our collected bark samples may have trapped only few spores of myxomycetes, and thus, resulted in lower percentage yield.

Species Abundance and Distribution of Myxomycetes in Three Provinces of Luzon Island

Twenty five species of myxomycetes were identified in this study as belonging to the genera *Arcyria* (4 species), *Didymium* (6 species), *Perichaena* (2 species), *Physarum* (11 species), and one species each for *Comatricha*, *Diderma*, and *Stemonitis* (Table 2). In the Philippines, a total of 43 *Physarum* species were so far recorded (Reynolds 1981; Macabago et al. 2010; Dagamac et al. 2011). All but one, *i.e. Physarum decipiens* reported in this study is known to the Philippines. The identified species of *Comatricha, Diderma, Didymium, Perichaena,* and *Stemonitis* in this study were also reported in other sites from the Philippines such as Mt. Arayat (Dagamac et al. 2011), Lubang Island (Macabago et al. 2012), and La Mesa Ecopark in Quezon City (Macabago et al. 2010). Most of the myxomycetes recorded here were also previously reported in the country (Reynolds 1981). However, this is the first report that documented the presence of *P. decipiens*, bringing the total number of myxomycetes in the Philippines to 125.

With regards to the species abundance, *Arcyria cinerea, Comatricha nigra, Physarum album,* and *Stemonitis fusca* were

Table 2. Occurrence of myxomycetes in the different highland areas of Luzon Island, Philippines.

Selected Luzon Highlands	Cavite		Laguna		Benguet	
Taxa	People's Park	Picnic Grove	UPLB-MNH	Mt. Makiling Botanic Garden	La Trinidad	Baguio City
Arcyria cinerea (Bull.) Pers.	A	A	A	A	A	A
Arcyria denudata (L.) Wettst.	O					
Arcyria pomiformis (Leers) Rost., Mon.		R	R			
Arcyria sp. 1		O	O	R		
Comatricha nigra (Pers.) J. Schröt.	R	C			C	A
Diderma effusum (Schwein) Morgan				R		
Didymium iridis (Ditmar) Fr.		R		C		C
Didymium minus (Lister) Morgan			R			
Didymium nigripes (Link) Fr.	O	O				
Didymium squamulosum (Alb. & Schwein) Rabenh.		R				
Didymium sp. 1	O		R			
Perichaena chrysosperma (Curr.) Lister			C	R	C	
Perichaena depressa Lib.			R			
Physarum album (Bull.) Chevall.				R	A	
Physarum bivalve Pers.	R			R		
Physarum cinereum (Batsch) Pers.			R			
Physarum compressum Alb. & Schwein	O	O				
Physarum decipiens Curtis		R				
Physarum flavicomum (Berk.)				R		
Physarum magnum		R				
Physarum sp. 1	R	R		R		
Physarum sp. 2		R				
Physarum sp. 6				R		
Physarum sp. 7			R			
Stemonitis fusca Roth.	O	R	C	R		A
Unidentified Myxo 1					C	
Unidentified Myxo 2					C	
Unidentified Myxo 3	R					
Unidentified Myxo 10		R				
No. of Species	**9**	**13**	**10**	**11**	**4**	**4**
No. of Genera	**5**	**5**	**5**	**6**	**4**	**4**

[a] Abundance Indices (% occurrence in all collected specimens per site):

A = abundant (>10) C = common (5 – 10) O = occasional (3- 5) R = rare (<3)

found to be abundant among the six sites in this study (Table 2), with *A. cinerea* as the most abundant. Similar results were also obtained in other studies. Ndiritu et al. (2009) noted the abundance of *A. cinerea* together with other myxomycete species in Big Bend National Park, USA. In a study conducted by Tran et al. (2006) in tropical forests of northern Thailand, *A. cinerea* was also noted in all the study plots. Abundance of *A. cinerea* together with other species of myxomycetes belonging to the order Physarales was also reported in the Maquipucuna Cloud Forest Reserve in Ecuador (Stephenson et al. 2004).

Among the species reported in this study, six species, *i.e. Physarum compressum, Arcyria denudata, C. nigra, Didymium iridis, Didymium nigripes,* and *Perichaena chrysosperma,* were common or occasionally occurring (Table 2). The remaining identified species were considered rare. Some of the rare species collected from the sampling sites were reported as abundant or common in temperate regions of the world. *Perichaena depressa*, *Physarum cinereum,* and *Diderma effusum* were known to occur abundantly in five study sites in Northern Virginia (Ndiritu et al. 2009; Stephenson 1989). These results show the differences in species composition and abundance between the two distinct eco-regions: the temperate and the tropical regions. Stephenson et al. (2008) noted that there are many limiting factors that may affect myxomycete diversity. These included temperature and moisture (Alexopolous 1963) and pH (Keller et al. 2008). Schnittler and Stephenson et al. (2000) reported that litter and bark substrates with higher pH tended to be positively correlated with higher species diversity of myxomycetes. Thus, these factors might also contribute to the rarity of some myxomycete species collected in the different sites in Luzon Island, Philippines. Also, in constantly moist, higher elevation forests, myxomycetes may not complete their life cycle, as sporulation is only induced when the substrates dry out (Schnittler and Stephenson, 2000). This may explain the lower yield recorded for Benguet province. At higher elevation, Benguet received higher amount of rainfall (Table 1).

Comparison of Myxomycete Assemblages in the Study Sites

Community analysis was also conducted to assess the similarities or differences in myxomycete assemblages in relation to the sampling sites. The myxomycete communities in each collection site were then compared using the Sorensen's Coefficient of Community (CC) and the Percentage Similarity (PS) values (Table 3). These indices are commonly used in studying myxomycete diversity. The CC takes only into account the presence or absence of a myxomycete species in the communities being compared while PS considers both the presence and relative abundance. In this study, Picnic Grove and People's Park, both in Tagaytay City, Cavite province, have more species in common as shown by their higher CC value (0.54). Higher CC value (0.50) was also observed between Baguio City and La Trinidad in the Benguet Province (Table 3). It was not surprising to observe high similarities among the myxomycetes present between these sites as these sites are within closer proximity. The lowest CC value (0.24) was reported between La Trinidad in Benguet and Picnic Grove in Cavite.

When the relative abundance is considered in addition to the species composition, lower PS values were obtained between Picnic Grove and People's Park, and between Baguio City and La Trinidad (Table 3). This indicates that though species composition may be similar in the communities being compared, the relative abundance of these similar species in each of the community varies. Myxomycete distribution ranges can also be large and its occurrence and dispersal potential can be influenced by factors related to microclimate and vegetation (Stephenson et al. 2000). Some myxomycete species also demonstrate distribution patterns that are related to micro-environmental parameters such as moisture and pH (Novozhilov et al. 2005). This explains further the differences in species abundance noted in the present study. Further studies are still needed to assess the distribution patterns of myxomycetes, especially at high elevation forests in the tropics. These studies should also relate the assemblages of myxomycetes and their abundance with the different microhabitats in each of the forest types.

CONCLUSIONS

A total of 25 species of myxomycetes were identified from three provinces in Luzon Island, Philippines from this study. Species identified belonged to the genera *Arcyria, Comatricha, Diderma, Didymium, Perichaena, Physarum*, and *Stemonitis.* Aerial leaf litter remained a very good substrate for myxomycetes as shown by the highest percent yield in this study. Many species of myxomycetes reported here were already known in the country, but some rare species were previously reported as abundant in temperate forests. Community analysis showed a high value between the two sites from Benguet Province and between the two sites in Cavite. This further indicated that areas of closer proximity have high similarities in species composition and abundance. This research study reports for the first time *Physarum decipiens* in the Philippines, bringing the total number of myxomycetes in the country to125.

CONFLICTS OF INTEREST

None

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Dr. Thomas Edison dela Cruz was involved in the planning

Table 3. Coefficient of community (upper right) and percentage similarity (lower left) values for myxomycetes from the highland areas in Luzon Island, Philippines.

	Picnic Grove	People's Park	UPLB-MNH	Mt. Makiling Botanic Garden	La Trinidad	Baguio City
Picnic Grove	X	0.54	0.35	0.42	0.24	0.47
People's Park	0.30	X	0.32	0.30	0.31	0.46
UPLB-MNH	0.26	0.24	X	0.38	0.29	0.29
Mt. Makiling Botanic Garden	0.23	0.24	0.28	X	0.40	0.40
La Trinidad	0.15	0.20	0.20	0.19	X	0.50
Baguio City	0.20	0.27	0.19	0.21	0.20	X

of the research study, collection of the substrates, identification of the collected specimens, and writing the manuscript. The experiments were performed by the remaining authors as part of their undergraduate thesis. All authors read and approved the final manuscript.

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