A comparative diversity study of myxomycetes in the lowland forests of Mt. Malasimbo and Mt. Siburan, Mindoro Island, Philippines

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Abstract

Million years ago, the island of Mindoro separated from mainland Asia. Its geologic origin led to many species distinct from Asia and the other islands of the Philippines. In this study, two lowland mountain forests – Mt. Malasimbo (MM) in Puerto Galera, Oriental Mindoro, and Mt. Siburan (MS) in Sablayan, Occidental Mindoro – were surveyed for myxomycetes. The combined opportunistic sampling in the field and the setting up of 1,260 moist chamber cultures retrieved a total of 1,007 fruiting body collections representing 50 species from 17 genera. A relatively higher number of taxa (49 species) was recorded in Mt. Siburan than in Mt. Malasimbo (36). Seventeen species were classified as rare with only four taxa that were widely distributed in both study sites, namely, *Arcyria cinerea*, *Perichaena pedata*, *Diderma hemisphaericum*, and *Lamproderma scintillans*. Higher species diversity and richness were noted for Mt. Siburan than Mt. Malasimbo, but a clear similarity in species composition (CC = 0.80)

and abundance (PS = 0.72) can be observed between forest sites. This suggest that lowland natural forest habitats of Mt. Malasimbo and Mt. Siburan are hotspots of myxomycete diversity. This research represents the most comprehensive survey of myxomycetes in Mindoro Island.

Introduction

Mindoro Island in Southwest of Luzon was rifted from the Asian mainland millions of years ago. Together with Palawan and Busuanga, it was believed that Mindoro was once part of the North Palawan Block, a micro-continent that was separated when the South China Sea marginal basin opened during the mid-Oligocene (Sarewitz & Karig 1986). In addition, the variations in geographical attributes of Mindoro's interior have suggested that the south-western portion of the island has a separate origin (Hamilton, 1989). Owing to its geologic origin, the island is considered as a distinct biogeographical region in the Philippine archipelago which strongly influenced the evolution of present biological diversity. In fact, Mindoro ranks as the seventh largest island and one of the 117 key conservation sites in the Philippines (De Alban et al. 2004). Based on the previous report, a total of 941 plant species under 179 families occur on Mindoro, with many notable plant species that are unique or endemic to the island including *Pinus merkusii* Jungh & de Vriese, *Calamus mindorensis* Becc., *Centrolepis philippinensis* Merr., *Elatostema halconense* C.B. Rob., *Mezoneuron mindorensis* Merr., *Leucosyke mindorensis* C.B. Rob., and *Strobilanthes halconensis* Merr (Villanueva & Buot 2015). This makes the geographically isolated island an ideal site to study other organisms such as the eukaryotic plasmodial slime molds or myxomycetes.

The interior structure of the island is basically rugged with vegetation types ranging from dipterocarp (at 500 meters above sea level, masl) to mossy forests (at 1,000 masl) to subalpine forests (2,470–2,587 masl) (Merill 1907, Mandia 2001). Here, we chose two lowland mountain forests from the eastern and western sides of the island. The first lowland mountain forest is Mt. Malasimbo which towers the Puerto Galera shore, a profound coastal scenery in Mindoro that is threatened due to unregulated tourism activities and commercial logging. Four decades ago, these activities led to the peninsula being declared as a biosphere reserve under the United Nations Educational, Scientific, and Cultural Organization (UNESCO) - Man and Biosphere Programme to protect Puerto Galera's marine and terrestrial biodiversity. Another lowland forest is the Mt. Siburan in Sablayan Watershed Forest Reserve in the southwestern part of Mindoro where the forest has been selected as an important biodiversity conservation area by Haribon Foundation and BirdLife International. Mt. Malasimbo and Mt. Siburan were just among the identified sites as highly critical under conservation priorities. In terms of vegetation, Mt. Siburan apparently displayed richer and more healthy vegetation than Mt. Malasimbo. The former is the largest and the only one left with intact lowland forest in Mindoro while the latter has a logged-over secondary growth forest with remnants of the primary lowland dipterocarp forest. Myxomycetes have also been previously reported in Mindoro Island in coastal and community forests of Puerto Galera with 42 species belonging to 16 genera (Dagamac et al. 2015) and in the island of Lubang with 44 species and 13 genera (Macabago et al. 2016). Lubang is a distinct island near the main island of Mindoro, exactly 136 km away, albeit politically listed under the province of Occidental Mindoro.

Myxomycetes are eukaryotic protists characterized by uninucleated amoeba, multinucleated plasmodium, and fungal-like fruiting bodies which produces spore mass (Clark & Haskins 2016). The number of studies about the distribution of myxomycetes has been increasing during the last years in the tropical Philippines as surveys of enormous habitat types [grasslands (Carascal et al. 2017), agricultural lands (Alfaro et al. 2015, Redeña-Santos et al. 2017)] and intensive regional surveys [Neotropics vs Paleotropics (Dagamac et al. 2017a)] were conducted. The country has also shown a great increase in the number of newly recorded taxa as indicated in the study of Mt. Arayat in Pampanga (5 records new to the country, Dagamac et al. 2011), Lubang Island (7 records, Macabago et al. 2012), Hundred

Islands in Pangasinan (3 records, Kuhn et al. 2013), Bataan, Cavite and Zambales (19 records, dela Cruz et al. 2014), the Bicol peninsula (8 records, Dagamac et al. 2017b), Bohol Islands (8 records, Macabago et al. 2017), and Laguna province (1 record, Bernardo et al. 2018). One species new to science, *Craterium retisporum* G. Moreno, D.W. Mitch. & S.L. Stephenson was described from specimens collected in Anda Island, Pangasinan (Moreno et al. 2009). Furthermore, the first local habitat suitability modeling using the tropical cosmopolitan species *Diderma hemisphaericum* (Bull) Hornem has demonstrated range expansion of the species on various climate change scenarios (Almadrones-Reyes & Dagamac 2018). Recently, the Paleotropical Asia-Pacific zone had also gained its momentum of more exhaustive myxomycete surveys wherein Southeast Asian countries like Vietnam (174 species, Nguyen et al. 2019, Novozhilov et al. 2020) and Thailand (145 species, Dagamac et al. 2017a) had shown an increased number of recorded species and identification of new species [*Comatricha spinispora* Novozh. et D.W. Mitch. (Novozhilov & Mitchell 2014), *Diderma cattiense* Novozh. & D.W. Mitch. and *D. pseudotestace-*

um Novozh. & D.W. Mitch. (Novozhilov et al. 2014), and *Perichaena echinolophospora* Novozh. & S.L. Stephenson (Novozhilov & Stephenson 2015)]. To contribute to this growing trend in myxomycete distribution studies, the primary objective of the present study was to investigate the assemblages of myxomycetes in two lowland forests of Mindoro Island. In addition, the possible influence of rainfall events during the field sampling, vegetation composition, and/or forest disturbance in the study area on the assemblages of myxomycetes was evaluated.

Materials and Methods

Sampling expeditions and study sites

Two sampling expeditions with rainfall variations were conducted in the study areas, one in October 2014 (end of rainy season) and the other in June 2015 (onset of rainy season). Mindoro Island, where
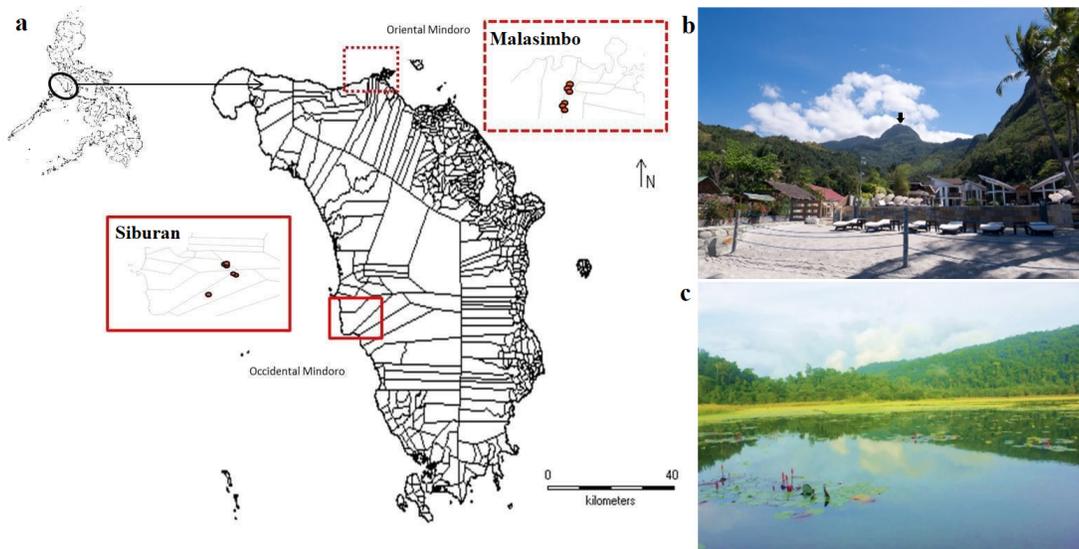

Figure 1. Map of the study sites. **A:** Map of the entire Mindoro Island, displaying the plotted collecting points (generated using the Quantum GIS, v1.8) and view **B:** of Mt. Malasimbo (black arrow: peak) in Puerto Galera, Oriental Mindoro and **C:** Mt. Siburan, Sablayan, Occidental Mindoro

Table 1. Description and geographic coordinates of the two lowland forests in Mindoro Island.

Forest site	Lat.	Long.	Elev. (masl)	Dominant plant spp.	Climate type	Average rainfall (Oct 2014)	Jun (2015)
Mt. Malasimbo (MM)	N13°28'30"	E120°54'59"	702	*Dipterocarpus spp, Ficus, Cleidon, Aglaia*	Type III	300 mm	268 mm
Mt. Siburan (MS)	N12°49'40.7"	E120°54'57.1"	333	*Dipterocarpus, Ficus, Clerodendrum, Ixora, Lunasia*	Type I	201 mm	131 mm

the collecting localities were identified, is bounded on the north by the Verde Island passage and is about 123 km south of Manila. Two forest sites: Mt Malasimbo in Puerto Galera, Oriental Mindoro, and Mt. Siburan in Sablayan, Occidental Mindoro, were chosen in this study (**Figure 1**). Generally, both areas were characterized as lowland mountain forests dominated by dipterocarp trees, e.g. *Shorea* spp., *Dipterocarpus gracilis* Blume, *D. grandiflorus* Blanco, and *D. validus* Blume. Other plant species observed in the areas were listed in **Table 1**. Within these study sites, six collecting plots, ca. 25 m² each, were randomly selected. The two coasts of the island experience different weather types due to the high mountain range in the central island (Collins et al. 1991). Mt. Malasimbo has a Type III climate type with no distinct dry or wet season, i.e., maximum rain period is not pronounced with a short dry season lasting from one to three months (December to February or from March to May) (PAGASA). The highest rainfall was observed for the months of October to December in 2014 (300–386 mm), during the first sampling expedition, with the annual mean relative humidity of about 84%. A slightly lower average rainfall of 201 mm was observed during the second expedition in June 2015. We also observed some anthropogenic disturbance in this area as evident in the presence of a golf course, zipline, and commercial establishments near the forest edge. The remaining forests here are somewhat threatened by agricultural expansion and extensive development of coastal areas for tourism.

On the other hand, Mt. Siburan is located near the Sablayan Prison and Penal Farm and is part of the Sablayan Watershed Forest Reserve. The lowland forest of Mt. Siburan comprises almost half of the total forest cover of Mindoro. Some of the key plant species in this forest include *Shorea contorta* Vidal (White Lauan) and *Calamus* spp. (Rattan) (De Alban et al. 2004). It has a Type I climate with two pronounced seasons, dry from November to April and wet throughout the rest of the year. The monthly rainfall was noted to be 268 mm (October 2014) and 131 mm (June 2015) during sampling expeditions with the annual mean relative humidity of 84%. Mt. Siburan is recognized as the largest tract of lowland forest in Mindoro. The forest trees were up to 25 m height or more, almost uniform in girth, and formed a three-layered canopy (De Alban et al. 2004). Lianas and other climbing plants were also observed in this study area. Adjacent to the northwestern edge of Mt. Siburan is a freshwater Lake Libuao. The presence of the penal colony near the area confers protection thereby restricting access to the forest. Hence, the not-so-frequent visits by the prisoners of the penal colony to the forest is the only human interference noted in the area.

Collection of field specimens and substrates

Field collection was made at the different sampling plots throughout the general study area. We spent 1-2 hours surveying each study plot for myxomycete

fruiting bodies and collection of substrates. Samples were collected by harvesting fruiting bodies of myxomycetes directly observed during the two sampling expeditions. All collected specimens of myxomycetes were processed for permanent storage following the methods of Stephenson (1989). Note that the term "collection or record" here refers to one or more fruiting bodies that originated from a single plasmodium (Stephenson 1989). In addition to the field collections, aerial leaf litter (AL), woody vines (VN), ground leaf litter (GL), and twigs (TW) were also collected for each sampling plot in Mt. Malasimbo and Mt. Siburan. According to the collecting protocol of Stephenson & Stempen (1994), AL and VN were described as dead/dried but still attached plant parts that had not been in contact with the ground while TW and GL were plant parts that had already been dropped on the forest floor. AL also included those leaves that have been detached but remained suspended above the ground by any manner like being trapped by twigs and/or vines. Note that due to their abundant occurrences, woody vines were collected only in Mt. Siburan. Ten samples of each substrate were randomly collected at each of the sampling plots, giving a total of 180 samples for Mt. Malasimbo and 240 for Mt. Siburan. Each sample was then placed in a medium-sized (12 cm x 20 cm) clean paper bag labeled with the study site, type of substrate, and date of collection. The samples were then transported to the laboratory and air-dried for seven to ten days prior to the preparation of moist chambers (MC).

Preparation of moist chambers

Myxomycete cultivation using a moist chamber technique was reported to be an excellent technique to assess the diversity of myxomycetes in a habitat type or study site (Dagamac et al. 2012, 2014). In our study, each bag with a plant sample was used to prepare three moist chamber cultures. For the aerial (AL) and ground (GL) leaf litter, the substrates were cut into postage-stamp-sized pieces and 8–10 pieces of the cut leaves were placed in disposable Petri dishes (90 mm) lined with tissue paper. Similarly, twigs (TW) or woody vines (VN) were cut into 4–7 cm length pieces and 5–8 pieces of the cut material were placed in a Petri dish in the

same manner (Stephenson & Stempen 1994). Substrates in each moist chamber were soaked with distilled water overnight and pH measurements were carried out on the substrates using an electrode pH meter (Sartorius PB-11). Subsequently, the excess water was then poured off from the samples and the moist chambers were incubated at room temperature (23–26ºC) under indirect ambient light (Macabago et al. 2010). All moist chambers were checked for the presence of fruiting bodies and/or plasmodia at least twice a week under an Olympus stereomicroscope (model:SZ2-ILST) for a duration of 8 - 12 weeks. The moisture of each moist chamber was maintained by adding a small amount of water occasionally during the observation period (Stephenson & Stempen 1994). Moist chamber cultures were checked and regarded as a positive collection when fruiting bodies, plasmodia, and/or sclerotia of a given species developed; otherwise, it was considered as negative. Sporocarps of the same species that were observed more than once in the same culture plate were considered as one single collection.

Preparation of voucher specimens and identification of myxomycetes

Fruiting bodies of myxomycetes attached to a portion of the substrate were initially dried and harvested from the moist chamber cultures and carefully glued to paper boxes (48 x 33 x 12 mm) (Keller & Braun 1999). Voucher specimens of representative collections were prepared and assigned with the UST Myxomycete Collection (USTMC) accession numbers and deposited at the Mycology Laboratory, Research Center for the Natural and Applied Sciences, University of Santo Tomas in Manila, Philippines. Fruiting bodies of each recovered myxomycete species were studied using the traditional morphology-based techniques wherein the details of the morphological characteristics of fruiting bodies were observed using a stereomicroscope. Moreover, spores of each species were examined with a compound microscope (Olympus CX3112C04). The identification of the myxomycete collections was based on published literature (Mitchell 1980) and identification keys (SYNKey, Mitchell 2008). Lastly, online web-based electronic databases, e.g. the Eumycetozoan Project (slimemold.uark.edu) and

nomen.eumycetozoa.com (Lado 2005–2020), were used to check the validity of the nomenclature of myxomycetes species.

Data evaluation

To assess the completeness of the sampling effort, a series of species accumulation curves was plotted using the downloadable program EstimateS (Version 9.0, Colwell 2013, with 100 randomizations). In accordance with the study of Dagamac et al. (2015) and Alfaro et al. (2015), the Chao 2 estimator was used to calculate the percentage of completeness for the study. Moreover, the productivity of the moist chambers (MC) for each of the substrate types and study sites was also computed by dividing the total number of positive collections to the total number of MC prepared multiplied by 100. The Abundance Index (AI) of each myxomycete species was also calculated. This was done by dividing the total number of collections per species to the total number of collections in a specific study site. Following the computation, each species was ranked based on ACOR criteria given by Stephenson et al. (1993): abundant (A) [RA value is > 3%], common (C) [RA value is > 1.5–3%], occasional (O) [RA value is > 0.5–1.5%], and rare (R) [RA value is < 0.5%].

In addition, the diversity analysis was conducted by calculating species diversity values for the study sites as described in Stephenson (1989) and Dagamac et al. (2012). Five diversity indices were used in this study. They include the Shannon-Weiner (HS) Index, which takes into account the heterogeneity (both abundance and evenness) of the species present, the Gleason Index (HG), which measures species diversity in relation to species richness (variety of different species in a biota), and the Pielou's species evenness index (E), which quantifies how numerically equal are the communities in a given sampling area. In addition, the more intuitive indices like Fisher's Alpha Index (FAI) and Simpson's diversity index (SID) were calculated using the software SPADE. All diversity values resulting from the computation hereafter were statistically compared using a modified t-test (Magurran 2004). Aside from determining the alpha diversity, similarities of the communities of myxomycetes (beta-diversity) in the study areas and substrate types were also analyzed.

In this aspect, Sorensen's coefficient of community (CC) and the percentage similarity (PS) indices were calculated as described by Stephenson (1989): CC= 2c / (a + b) where a corresponds to the total number of species in the first community being considered, b is the total number of species in the second community, and c is the number of species common to both communities. The values of this index range from 0 to 1. A CC value close to one (1.0) indicates that both communities have a high degree of similarity in myxomycete composition while a low CC value (close to zero) is an indicator that a few or no species are shared by the communities being evaluated. Finally, the PS index was computed based not only on the presence of species but including also their relative abundance: PS = ∑ min $(a, b, … x),$ where min is the lesser of the two percentage compositions of species a, b, …x in the two communities.

Results

Myxomycete records in the two sampling sites

A total of 1,007 determinable fruiting bodies of myxomycetes were recorded in this study using the combined field collections (FC) and moist chamber (MC) techniques. Of these, 108 collections were represented by field collections while 899 collections were retrieved from the 71% positive moist chamber cultures (611 as fruiting body records) as opposed to 290 plasmodial records (**Figure 2**). The dataset of our field-collected myxomycetes showed that between the two forest sites, more records of myxomycetes were noted in Mt. Siburan (69 collections with 23 species) than in Mt. Malasimbo (39 collections with 18 species). Combining FC and MC collections, of the 36 species recorded in Mt. Malasimbo, 18 species were observed only in the moist chamber cultures whereas nine were recorded only from the field. Nine species were noted using both collecting techniques. Interestingly, a higher total number of species (49) was noted in Mt. Siburan. Of these, 26 species were recovered from MC only, six from FC only, and 17 taxa appeared in both FC and MC. The
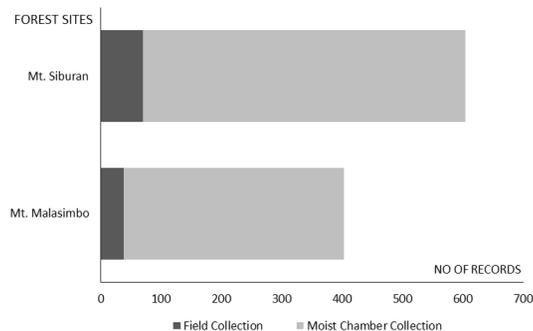
Figure 2. Bar graph showing the number of records from the two forest sites obtained using field collections and the moist chamber technique.

myxomycetes that were recorded from field collections but not in moist chamber cultures included *Arcyria incarnata* (Pers. ex J.F. Gmel.) Pers.*, Craterium leucocephalum* (Pers. ex J.F. Gmel.) Ditmar*, Cribraria cancellata* (Batsch) Nann.-Bremek.*, Didymium iridis* (Ditmar) Fr., and *Physarum viride* (Bull.) Pers.

Species composition and extensiveness of the survey

This study reports a total of 50 species of myxomycetes representing 17 genera: *Physarum* (15 species), *Arcyria* (5), *Stemonitis* (4), *Comatricha* (3), *Cribraria* (3), *Didymium* (3), *Hemitrichia* (3), *Perichaena* (3), *Diachea* (2), *Diderma* (2), *Ceratiomyxa* (1), *Clastoderma* (1), *Craterium* (1), *Echinostelium* (1), *Lamproderma* (1), *Lycogala* (1), and *Metatrichia* (1). Fruiting bodies of two taxa designated here as *Stemonitis* sp. and *Comatricha* sp. formed immature fruiting bodies or were completely damaged by molds, which resulted in difficulty assigning them to any described species. From these records, a total of 171 representative specimens were preserved, stored, and assigned with the accession numbers USTMC 2456 to USTMC 2626.

The abundance index (AI) ranking of each myxomycete species per study site is provided in **Table 2.** The species accumulation curve showed that

Mt. Siburan was more exhaustively sampled than Mt. Malasimbo, though from the plateau seen in both graphs the study seems to have recovered most myxomycete species present in the collection area using field survey and moist chamber preparations (Figure 3). In fact, the calculated survey completeness of 85.14% for Mt. Malasimbo and 94.92% for Mt. Siburan and the values of the computed Chao 2 mean of 41.11 for Mt. Malasimbo and 50.57 for Mt Siburan showed that the survey was almost complete when compared to the actual morphospecies of myxomycetes identified in the respective areas (36 for Mt. Malasimbo, 49 for Mt. Siburan). Therefore, the differences in the number of samples collected, i.e., 180 for Mt. Malasimbo vs 240 for Mt. Siburan, did not evidently affect the study as the species accumulation curve for both study sites showed almost complete recovery of species present and that the number of samples collected was already sufficient.

Species diversity and community assemblages

In this study, datasets from the field collections and moist chamber cultures were combined and used to assess myxomycete diversity. Results showed that Mt. Siburan had higher species diversity (HS = 1.33, FAI = 12.60), richness (HG = 7.50), and evenness (E = 0.35, D = 0.10) as opposed to Mt. Malasimbo (HS = 1.13, FAI = 9.20, HG = 5.83, E = 0.36, D = 0.15) (**Table 3**). The differences between diversity values were proved statistically significant using the modified t-test of Magurran (2004) at 95% level of confidence. Comparing now the community assemblages of myxomycetes in the two study sites, a relatively high CC value (0.80) and PS value (0.72) were recorded indicating similarities in their species composition and abundance. Venn diagram (**Figure 4**) showed a high percentage of shared species (70% of the identified taxa), though one species, *Hemitrichia pardina* (Minakata) Ing, was recorded only in Mt. Malasimbo while 14 species were reported only in Mt. Siburan.

Discussion

The Philippine archipelago has a surprisingly rich

Table 2. The number of records from field survey (FC) and moist chambers (MC), the Abundance index (AI) of myxomycetes collected from the two forest sites, and the number of records on different substrates: aerial leaf litter (AL), ground leaf litter (GL), twigs (TW), and vines (VN).

Taxon	Mt. Malasimbo				Mt. Siburan				Substrates			
	FC	MC	Total	AI	FC	MC	Total	AI	AL	GL	TW	VN
Arcyria cinerea	4	137	141	A	8	156	164	A	100	42	89	62
Arcyria denudata	4	1	5	O	11	9	20	A	-	-	4	6
Arcyria incarnata	1	-	1	R	2	-	2	R	-	-	-	-
Arcyria pomiformis	-	-	-	-	-	1	1	R	-	-	-	1
Arcyria sp.	-	-	-	-	-	1	1	R	-	-	-	1
Ceratiomyxa fruticulosa	6	2	8	C	7	6	13	C	-	-	6	2
Clastoderma debaryanum	-	1	1	R	1	7	8	O	1	1	1	5
Comatricha pulchella	-	3	3	O	-	7	7	O	5	2	2	1
Comatricha tenerrima	-	12	12	C	-	11	11	C	2	-	13	8
Comatricha sp.	-	-	-	-	-	1	1	R	-	1	-	-
Craterium leucocephalum	3	-	3	O	2	-	2	R	-	-	-	-
Cribraria cancellata	-	-	-	-	2	-	2	R	-	-	-	-
Cribraria microcarpa	3	-	3	O	4	28	32	A	-	-	13	15
Cribraria violacea	-	6	6	O	-	14	14	C	2	-	9	9
Diachea bulbillosa	-	5	5	O	1	-	1	R	3	2	-	-
Diachea leucopodia	2	10	12	C	-	4	4	O	4	10	-	-
Diderma effusum	-	15	15	A	-	11	11	C	8	16	2	-
Diderma hemisphaericum	-	42	42	A	-	49	49	A	57	32	1	1
Didymium iridis	2	-	2	R	1	-	-	R	-	-	-	-
Didymium nigripes	1	-	1	R	1	3	4	O	2	-	-	1
Didymium squamulosum	1	10	11	C	3	10	13	C	12	7	-	1
Echinostelium minutum	-	-	-	-	-	5	5	O	-	-	1	4
Hemitrichia calyculata	2	-	2	R	5	4	9	O	1	-	2	1
Hemitrichia pardina	-	2	2	R	-	-	-	-	1	1	-	-
Hemitrichia serpula	2	2	4	O	3	11	14	C	1	-	7	5
Lamproderma scintillans	1	19	20	A	-	27	27	A	17	6	16	7
Lycogala exiguum	-	-	-	-	-	2	2	R	-	-	-	2

Taxon	Mt. Malasimbo				Mt. Siburan				Substrates			
	FC	MC	Total	AI	FC	MC	Total	AI	AL	GL	TW	VN
Metatrichia vesparia	-	-	-	-	-	2	2	R	-	-	2	-
Perichaena chrysosperma	-	12	12	C	1	18	19	A	7	1	18	4
Perichaena depressa	-	3	3	O	-	8	8	O	1	-	8	2
Perichaena pedata	-	14	14	A	-	39	39	A	28	6	14	5
Physarum album	2	2	4	O	2	4	6	O	2	2	1	1
Physarum cinereum	-	2	2	R	-	3	3	R	-	2	1	2
Physarum compressum	-	-	-	-	-	3	3	R	-	-	2	1
Physarum crateriforme	-	-	-	-	-	6	6	O	3	-	-	3
Physarum decipiens	-	11	11	C	-	9	9	O	1	-	13	6
Physarum echinosporum	-	1	1	R	-	9	9	O	-	7	3	-
Physarum javanicum	-	-	-	-	-	1	1	R	-	-	1	-
Physarum leucophaeum	-	-	-	-	-	2	2	R	-	1	1	-
Physarum melleum	-	35	35	A	2	15	17	C	22	8	13	7
Physarum oblatum	-	1	1	R	-	3	3	R	2	-	1	1
Physarum roseum	-	-	-	-	-	2	2	R	-	-	-	2
Physarum stellatum	2	3	5	O	1	9	10	C	6	1	3	2
Physarum superbum	-	-	-	-	1	1	2	R	-	-	1	-
Physarum tenerum	1	-	1	R	7	1	8	O	-	-	-	1
Physarum viride	1	-	1	R	2	-	2	R	-	-	-	-
Stemonitis axifera	-	-	-	-	1	2	3	R	-	-	1	1
Stemonitis fusca	-	4	4	O	1	14	15	C	-	1	8	9
Stemonitis splendens	1	-	1	R	-	9	9	O	-	-	1	8
Stemonitis sp	-	10	10	C	-	7	7	O	-	-	12	5
Total No of records	404				603				288	149	270	192
No of Morphospecies	36				49				24	20	33	35
No of Genera	14				17				11	11	13	14
S/G ratio	2.57				2.88				2.1	1.8	2.5	2.5

Table 3. Different species diversity indices and the values of coefficient of community (CC) and percentage similarity (PS) between the two lowland forests, Mt. Malasimbo (MM) and Mt. Siburan (MS).

Forest site	Morphospecies	Shannon Diversity Index (H')	Gleason Species Richness (S)	Pielou's Evenness (E)	Fisher's Alpha Diversity Index (FID)	Simpson's Index (D)	CC	PS
MM	36	1.13	5.83	0.36	9.20	0.15	0.80	0.72
MS	49	1.33	7.50	0.35	12.60	0.10		

myxobiota as the number of records remarkably increased from 107 to 159 just in the span of the last decade (Dagamac & dela Cruz 2015, 2019, Macabago et al. 2020). In Mindoro, the first survey of myxomycetes was carried out in Lubang Island, Occidental Mindoro (Macabago et al. 2012). From that study, a total of 45 species were reported, with six taxa new to the Philippines, namely, *Arcyria globosa* Schwein., *Collaria rubens* (Lister) Nann.-Bremek., *Comatricha robusta* (T.N Lakh et K.G. Mukerji) Nann.-Bremek. et Y. Yamam., *Craterium atrolucens* Flatau, *Lamproderma cacographicum* Bozonnet, Mar Mey et Poulain, and *Perichaena microspora* Penz et Lister. However, it should be noted that Lubang Island is exactly 136 km away from the main island of Mindoro but falls within the political jurisdiction of the province of Occidental Mindoro. This study was followed by the report of Dagamac et al. (2015) with 42 species identified from Puerto Galera. The number of species recorded from these previous studies in Mindoro along with the present report of 50 species from 17 genera is comparable to the number of species reported for Palawan, another remote island with geographical proximity to Mindoro. The number of taxa from Palawan has been recently updated to 56 species with the addition of *Badhamia macrocarpa* (Ces) Rostaf as new record for the country (Macabago et al. 2020). Interestingly, these records are

significantly higher than the number reported from other forest sites, e.g. Mt. Kanlaon in Negros Island (28 species, Alfaro et al. 2015), Mt. Arayat in Pampanga (30 species, Dagamac et al. 2014), and Mt. Makulot in Batangas (21 species, Cheng et al. 2013, 35 species, Isagan et al. 2020) and in island ecosystems in the country, e.g. Puerto Princesa in Palawan (33 species, Pecundo et al. 2017) and Anda Island in Pangasinan (24 species, Kuhn et al. 2013). In fact, the number of myxomycete species presented herein is one of the highest for the Philippines, following Dagamac et al. 2017b (with 57 species from the Bicol Peninsula) and Macabago et al. 2017 (with 54 species from Bohol Island). Our study reported 49 species and 17 genera in Mt. Siburan and 36 species and 14 genera in Mt. Malasimbo. Despite these records, a large part of Mindoro Island can still be explored for myxomycetes.

Interestingly, a total of 108 field-collected fruiting bodies was obtained in this study. Among the two collection periods, the June collection was more productive than the October collection for both study sites. Looking now at the data of the mean monthly rainfall from each study site, a higher amount of rainfall was recorded during the sampling expedition of October 2014 (300 mm for Mt. Malasimbo, 268 mm for Mt. Siburan) than in June 2015 (201 mm for Mt. Malasimbo, 131 mm for
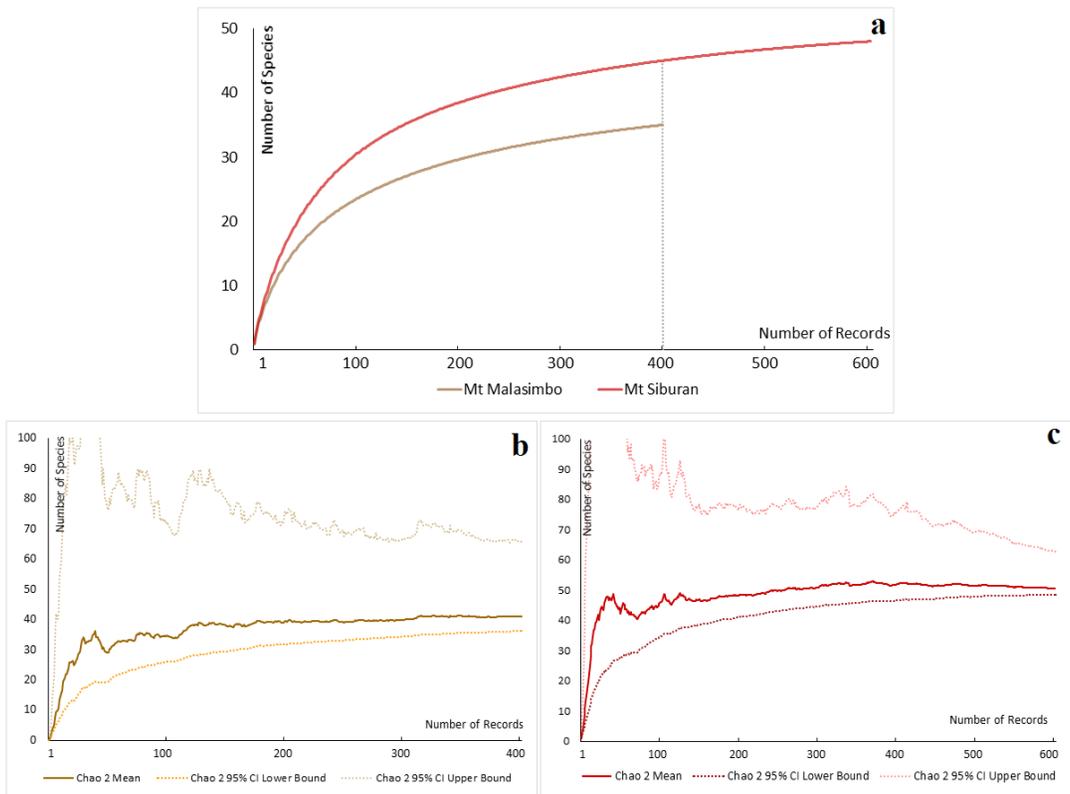
Figure 3. A: Rarefaction curve showing that Mt. Siburan (MS) is more species-rich than Mt. Malasimbo (MM).
B–C: Species accumulation curve generated with the number of records obtained from the two study sites:
B: Mt. Malasimbo – Chao 2 mean: 41.11±6.06; % covered: 85.14 **C:** Mt. Siburan – Chao 2 mean: 50.57 ± 2.81; % covered: 94.92

Mt. Siburan). Rainfall is considered as one of the main factors in the development of myxomycetes in the field since slime molds require moisture for their amoebal and plasmodial stages (Schnittler 2001). However, frequent, high rainfall consequently makes the substrate excessively wet which may prolong the plasmodial stage, therefore hindering or delaying sporocarp formation (Ogata et al. 1996, Keller & Everhart 2010). In addition, excessive rainfall can also destroy and wash away rapidly the delicate fruiting bodies resulting in their disappearance on the substrate in the field. Heavy rains were reported to clear almost all myxomycete plasmodia and spores from the substrate's surface (Stephenson 2003). The above scenarios could probably explain why there was scarcity of fruiting bodies in the

field during the first sampling expedition. A similar case was observed in one year-period investigation of myxomycete occurrence in Thailand, a country with climatic conditions almost similar to the Philippines, and revealed that the month of June when the rain is on and off gave the highest number of field collections and species of myxomycetes, i.e. 45 species (Tran et al. 2006). The number of field specimens collected in this study, i.e., 108 records, outnumbered the other field-collected myxomycetes reported in previous surveys conducted in the country, i.e. Bicol Peninsula with only 33 records (Dagamac et al. 2017b) and Mt. Makulot in Batangas with 68 records (Cheng et al. 2013). However, the records from field survey alone reported a lower number of species, i.e. 23 taxa. It is therefore important to aug-
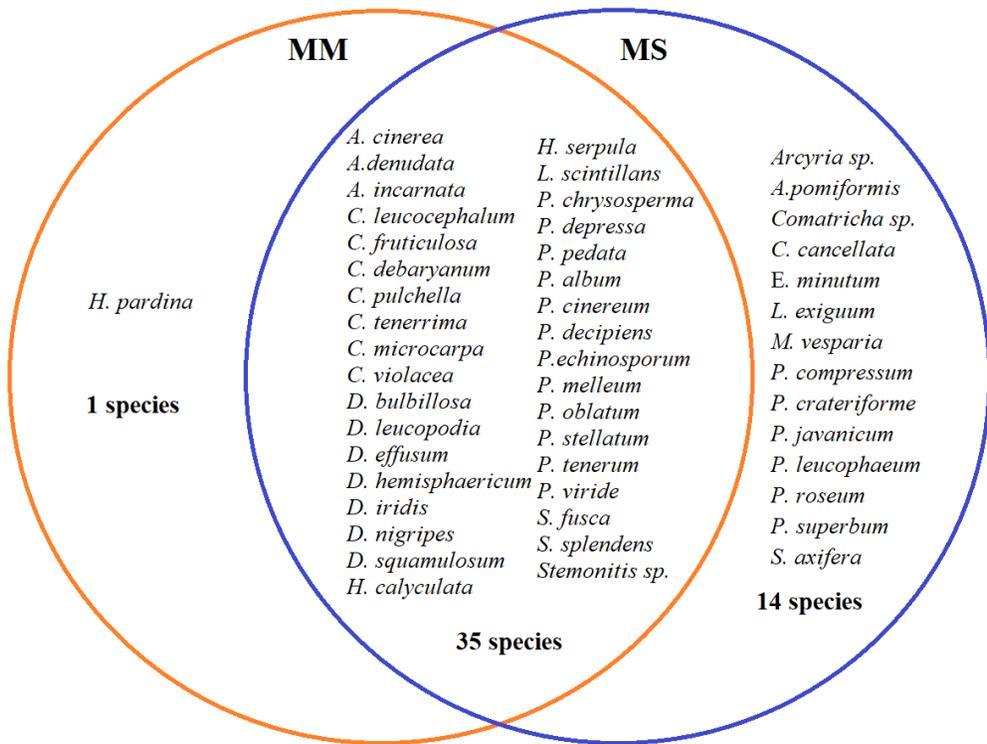
Figure 4. Venn diagram showing the assemblages of myxomycetes between Mt. Malasimbo (MM) and Mt Siburan (MS) with 35 common species.

ment field collections with additional records derived from moist chambers. In fact, field collections and moist chamber cultures are complementary approaches for the study of myxomycetes, thereby providing useful information in the ecological analysis (Dagamac et al. 2012, Novozhilov et al. 2017). As shown in this study, the number of species was augmented (adding 18 species for Mt. Malasimbo and 26 species for Mt. Siburan) when the MC technique was employed together with field collections.

Myxomycete occurrences are known to be affected by the condition of their substrates. As such, each microhabitat is known to support a different assemblage of myxomycete species (Stephenson 2003). Generally, the myxomycetes recovered in this study fruit preferentially on woody substrates, in congruence with the study of Rea-Maminta et

al. (2015) and Pecundo et al. (2017) where twigs had the highest number of records. The most abundant, common and occasional species that were encountered in TW and VN samples include *Arcyria denudata* (L.) Wettst., *Clastoderma debaryanum* A. Blytt, *Cribraria cancellata*, *C. microcarpa* (Schrad.) Pers., *C. violacea* Rex, *Physarum compressum* Alb. & Schwein., *Stemonitis axifera* (Bull.) T. Macbr., *S. splendens* Rostaf., and *S. fusca* Roth. Most of these are members of Cribrariales, and Stemonitidales, which are known as common inhabitants of woody substrates (Everheart et al. 2008). Three myxomycete species, *Arcyria cinera* (Bull.) Pers., *Lamproderma scintillans* (Berk. & Broome) Morgan, and *Perichaena pedata* (Lister & G. Lister) G. Lister ex E. Jahn, did not show any substrate preference as they were noted across all substrates in this study. The cos-

mopolitan *A. cinerea* has been reported abundant in almost all substrates surveyed for myxomycetes in the temperate and tropical regions (Stephenson et al. 2004, Rea-Maminta et al. 2015) including the present study. On the other hand, among the common species recorded for leaf litter were *Diachea bulbillosa* (Berk. & Broome) Lister, *D. leucopodia* (Bull.) Rostaf., *Diderma effusum* (Schwein.) Morgan, *D. hemisphaericum, Didymium squamulosum* (Alb. and Schwein.) Fr., *Perichaena chrysosperma* (Curr.) Lister, and *Physarum melleum* (Berk. & Broome) Massee. Some of these were also common species appearing in moist chambers with leaf litter from other tropical lowland forest sites (Nguyen et al. 2019) and agricultural sites (Tran et al. 2008, Redeña-Santos et al. 2017). Of interest*, Hemitrichia pardina* was regarded as rare species and only noted in the leaf litter. A relatively low number of records for this species was also noted in other lowland forests in the country but merely occurring in woody substrates such as twigs (dela Cruz et al. 2014, Pecundo et al. 2017), although one record was recovered in GL collected from Palawan island (Pecundo et al. 2017). Moreover, some species such as *Metatrichia vesparia* (Batsch) Nann.-Bremek. ex G.W. Martin & Alexop., *Physarum javanicum* Racub, and *Physarum superbum* Hagelst. were recorded in twigs only. *P. superbum* has been reported as occurring in lianas in Australia and Peru (Wrigley de Basanta et al. 2008) but this has been recorded in AL in the Philippines (dela Cruz et al. 2014) and was collected also from *Ficus* fruit during our field expedition. *Arcyria pomiformis* (Leers) Rostaf.*, Echinostelium minutum* de Bary, *Lycogala exiguum* Morgan, and *Physarum roseum* Berk. & Broome were recorded from VN but not from other microhabitats. *E. minutum* is known as a corticolous myxomycete which has been the most abundant species in bark and grapevine as reported by Everhart & Keller (2008). In addition, the structure of the woody vines offers promising microhabitat for myxomycetes as it rapidly absorbs water and prolongs its moisture over longer periods of time. However, the myxomycetes associated with this substrate are often less explored in a tropical country like the Philippines, and therefore, merit further study.

Of all species collected in the study, a relatively high number of collections was recorded for *Arcyria cinerea* with 305 collections (30%). *A. cinerea* had been reported as a cosmopolitan species and known to inhabit almost all kinds of plant materials or substrates. This was followed by *Didymium hemisphaericum* (91 collections, 9%), *Perichaena pedata* (53 collections, 5%), *Physarum melleum* (52 collections, 5%), and *Lamproderma scintillans* (47 collections, 4%). "Singleton" species represented by a single collection included *Arcyria* sp., *Arcyria pomiformis*, and *Comatricha sp.* from moist chambers. Of note, one collection of *Stemonitis axifera* was observed to inhabit living grass during field survey. *Arycria pomiformis* was also recorded as rare in Puerto Galera (Dagamac et al. 2015) whereas *Stemonitis axifera* was abundant in different substrates collected from Lubang Island (Macabago et al. 2012).

Species diversity analysis considers factors such as species richness, abundance, and evenness in a community being compared (Stephenson et al. 1989). In this study, Mt. Siburan evidently showed a higher species diversity as opposed to Mt. Malasimbo (**Table 3**). Even by merely considering the number of species recovered from a moist chamber, still, a higher number of species was recorded in Mt. Siburan (43) than in Mt. Malasimbo (27). However, the evenness of species in the two study sites was not statistically different (p-value $\geq$ 0.05), indicating that the two mountain sites have similar species evenness. Interestingly, comparing the myxomycete assemblages, a relatively high CC value (0.80) was recorded between the two study sites indicating a high similarity in their species composition. This is obviously observed in the number of shared species between the two study sites, i.e. 35 species of the 50 recorded species (**Figure 4**). Interestingly, Mt. Siburan harbored more "unique" species than Mt. Malasimbo, i.e., 14 species, of which 4 species were singleton (unique species means that the species was recorded only in one site). Furthermore, when the abundance values of each species were included in the community analysis, a higher PS value of 0.72 was recorded.

Despite the difference in the sample sizes, the values of the survey completeness clearly showed that most of the myxomycete species were already recovered in the two study sites. The differences in species diversity between the two forest sites could therefore be attributed to varied factors. The lower

species diversity recorded in Mt. Malasimbo may be attributed to the failure of many plasmodia in moist chambers to develop into identifiable fruiting bodies, decreasing the number of taxa and records reported in that area. However, the persistence of plasmodia in MC is not even surprising since myxomycetes may not always undergo fructification even under natural conditions (Stephenson et al. 1989). The vegetation between the two study sites also showed some degree of differences which could lead to differences in recorded taxa. Although Mt. Malasimbo has a larger forest cover (71% of its estimated land area) than Mt. Siburan (57% of its land area) (Gatumbato, 2009), among the two sites, Mt. Siburan displays a more healthy and intact lowland forest vegetation which probably supports a significantly higher number of plant species. In contrast, evident disturbance, e.g. presence of recreational areas near the forest edge, may somewhat negatively impact its vegetation composition, and thereby its myxomycete communities. Such observation has been already demonstrated by Macabago et al. (2017) where forest structure, specifically the number of trees and biomass of vascular plants, had a direct correlation to the diversity of myxomycetes. Interestingly, forest disturbance brought by human movement had been speculated to affect myxomycetes in the study of protected and unprotected plantation forests in Vietnam (Redeña-Santos et al. 2018) and in the forest edges of Mt. Isarog (Eloreta et al. 2020). Their findings reported that taxonomic and species diversity is higher in unprotected habitats than in protected area where moderate forest disturbance brought by anthropogenic activities could add habitat heterogeneity resulting in a more species-rich and diverse myxomycete community. This has been further an observed trend in the Philippine setting when fragmented forest with moderate level of anthropogenic activities showed higher taxonomic and species diversity than from forest patches with high exposure to human induced activities (Bernardo et al. 2018). The results of the present study may follow and support the outcomes of these previous reports, however, we believe that any conclusions about the direct impact of anthropogenic disturbance on the assemblages of myxomycetes require further verification across a range of habitats.

Acknowledgements

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