



First report of myxomycetes in the karst forest of Minalungao National Park, Nueva Ecija, Philippines with updates on the limestone-inhabiting myxomycetes of the Philippines

Pecundo MH^{1*}, Espiritu LM¹, Carillaga RLG¹, Samaniego AFE¹, Cabutaje EM³ and dela Cruz TEE^{2,3}

¹Department of Biological Sciences, Institute of Arts and Sciences, Far Eastern University, Nicanor Reyes Sr. Street, Sampaloc 1015 Manila, Philippines

²Department of Biological Sciences, College of Science, University of Santo Tomas, España Blvd. 1008 Manila, Philippines

³Fungal Biodiversity, Ecogenomics, and Systematics (FBeS) Group, Research Center for the Natural and Applied Sciences, University of Santo Tomas, España Blvd. 1008 Manila, Philippines

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Abstract

The karst forest landscape is a unique terrestrial ecosystem where myxomycetes have not been extensively studied. Herewith, we report the first formal listing and ecological assessment of myxomycetes on the limestone forest in Minalungao National Park, Nueva Ecija, Central Luzon, Philippines. The integrated field and moist chamber techniques gave a total of 318 identifiable fruiting bodies classified into 28 species and 14 genera, nine of which are recorded as rare, and five as abundant. Moist chambers with above-ground plant materials, i.e., aerial leaf litter and woody vines (78%) were more productive than the forest floor litter – twigs (72%) and ground leaf litter (63%). The species accumulation curve determined that 80% of the species were recovered from the area. We also determined the general taxonomic diversity (TDI = 2.07) and species diversity (HG = 4.86, HS = 0.91, FAI = 7.76, E = 0.37) of myxomycetes associated with the limestone forest habitat. However, among substrates, woody vines and twigs were the most “favored” substrates with the highest taxonomic and species diversity. Furthermore, Bray Curtis (BC) community analysis of myxomycete assemblages showed high similarities in species composition between similar types of microhabitats, i.e., between aerial and ground leaf litter and between woody vines and twigs. Our study provided baseline information on the composition of limestone-inhabiting myxomycetes and gives updates on the status of myxomycetes in limestone forests in the Philippines.

Keywords – Central Luzon – limestone forest – litter myxomycetes – microhabitats – plasmodial slime molds

Introduction

One distinctive type of ecosystem in a tropical region that stretches over a hill area is a forest underlain by limestone. It has an extraordinary landform sculpted by karstification where a natural

excavation of carbonate material from the surface of the earth occurred followed by leeching and dissolution in water (Veress 2020). This makes the condition on the above-ground rich in minerals like calcium and magnesium (Voroney 2019). Karst landscape has also been commonly characterized by unique features of sinkholes, caves, fissures, and aquifers (Tolentino et al. 2020). Interestingly, the distinct substrate condition in the karst areas along with its unique microclimatic conditions provides a perfect niche allowing the existence of a rich biological constituent and can lead to a center of species endemism. Through the years, studies revealed that the limestone forests offered unique habitats for plants and animals with the possibility of new and/or endemic species. In Asia, studies showed that limestone forests harbor a low number of species but a relatively unique community of pteridophytes (Phouthavong et al. 2019). Limestone karst vegetations in Laos and Malaysia were also reported to hold high diversity of orchids and mosses, respectively (Kumar et al. 2016, Norhazrina et al. 2019). Floristic investigations in the Philippines presented several new and limestone-restricted species in the peninsula of Bohol, Cebu, and Palawan (Barcelona 2006, Pelser et al. 2016). For instance, a new species of *Amorphophallus* was recovered from the limestone hill in El Nido, Palawan (Hettterscheid et al. 2012). Furthermore, a novel species of mistletoe (*Lepeostegeres cebuensis* Barcelona, Nickrent & Pelser) and three new species of *Begonia* were described from the limestone areas in Southern Cebu and Panay Island, respectively (Pelser et al. 2016, Peng et al. 2017). Similar to its diverse flora, many terrestrial faunas have been discovered for the first time in limestone karst forests. For instance, twelve species of Murinae rodents were first encountered from the limestone karsts in Thailand (Latinne et al. 2013). Species of a bird were newly discovered and found rare in the Sino-Vietnam limestone forest (Jiang et al. 2019). New and endemic species of amphibians and reptiles were also noted from the karst forests in Malaysia, Indonesia, Thailand, and Myanmar (Grismer et al. 2014, 2016, 2018). In the Philippines, a series of herpetological studies reported new species of forest frogs in different karst habitats in Visayas and Luzon (Siler et al. 2007, 2009, 2010, Brown et al. 2015). These reports provided robust evidence that karst habitats are tremendously promising habitats and reflect the high potential of discovering rare and novel taxa of flora and fauna. Can this be also true for the fungus-like eukaryotic protists such as myxomycetes?

Myxomycetes are widely distributed in forested habitats in temperate and tropical ecoregions. Several studies revealed that the community of myxomycetes is also abundant and diverse in areas with limestone topography, but only a handful of surveys are available so far. Earlier reports on myxomycetes in forests that lie over limestone were reported in Augusta County, Virginia (Carr 1939), Turkey (Ing 2000), Bohemian Karst (Dvořakova 2002), and in few sites in El Elden Reserve in Mexico (Lado et al. 2003). Interestingly, some of these studies suggested that myxomycetes under the Physarales group with calcareous fruiting bodies (due to lime deposits) were observed frequently in the limestone forests than the other group of myxomycetes which are non-calcareous (no lime deposits). In the Philippines, forests over limestone are extensive. To date, there have been four major studies on myxomycetes in the limestone forests in the country. This includes a survey in Hundred Island National Park in Pangasinan with 30 species in 10 genera (dela Cruz et al. 2011) and followed by a study in Quezon National Park which reported 35 species under 16 genera (Dagamac et al. 2015). Recently, Macabago et al. (2020) reported 25 taxa from Coron, Palawan as an addition to the previous reports of 33 species in Puerto Princesa Subterranean River National Park in mainland Palawan (Pecundo et al. 2017). Both study areas were of limestone forests. One interesting karst area in Luzon is found in Nueva Ecija, the Minalungao National Park (MNP). MNP covers a total land area of about 2,000 has with intricate terrain on limestone. It has a dense limestone rock formation with a lowland secondary-growth forest. The area has been categorized as a national park gaining profound efforts of protection from different environmental organizations. Several research articles have been published that documented macrofungi (Paraguas et al. 2013), chiroptera (Pader et al. 2017, Judan Cruz & Pader 2018), trees (dela Cruz et al. 2018), vascular plants (Alfonso et al. 2018), and gobies (Judan Cruz et al. 2018) in MNP. To date, no microbial diversity, e.g., myxomycetes, has been reported in the area. With this, the primary goal of the present study is to assess whether the karst habitat of Minalungao National Park hosts unique

assemblages of myxomycetes. Our study also assessed the status of limestone-inhabiting myxomycetes in the Philippines.

Materials & Methods

Site description and collecting protocol

The Minalungao National Park (15°18'00" N, 121°07'20" E; elevation at 106-247 masl) is located in the municipality of General Tinio, Nueva Ecija in Central Luzon, Northern Philippines (Fig. 1). It has a total land area of 2000 hectares (ha) bordering the sides of Peñaranda River by 16 ft.-high limestone walls. The sampling area has a Type I climate with two pronounced seasons: rainy from May to November and dry for the rest of the year. The Minalungao natural landscape primarily consists of a dense, lowland secondary growth forest, mostly with dipterocarp trees, bamboo shrubs, and is home to several tree species endemic in the area (Whitford 1911, Co et al. 2006, dela Cruz et al. 2018). The general collection site is characterized by the presence of a limestone wall and has an open to the slightly close forest canopy. Within this limestone forest, six collecting plots (25 m² per plot) were randomly chosen.

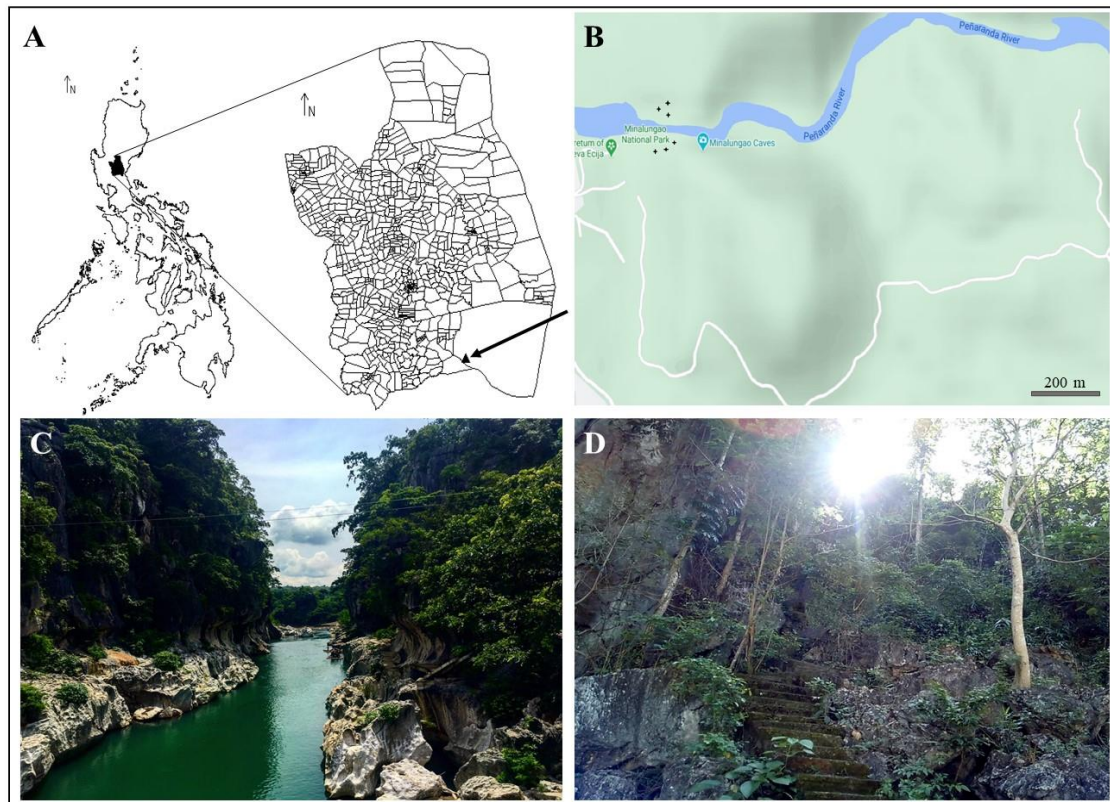


Fig. 1 – A. General map showing the location of Minalungao National Park (MNP) in General Tinio in the province of Nueva Ecija, Philippines. B. The six collecting points in the forest site of MNP. C-D. The unique natural limestone landscape of the study site bordering the Peñaranda river (C) and the actual photo of one of the collection points in MNP limestone forest (D). (Photos by RLG Carillaga).

Myxomycete fruiting bodies that were observed in the field were carefully collected and glued inside herbarium boxes for examination in the laboratory. In addition, dried or decayed plant materials, i.e., aerial leaf litter (AL), ground leaf litter (GL), twigs (TW), and woody vines (VN), were randomly collected within the collecting plots and represented by 10 samples/substratum/plot (n = 240). Leaves attached to tree branches or those that are detached but still hanging on to something and not touching the ground were considered as the aerial leaf litter. Also, woody vine

samples in this study were considered as aerial plant materials since they were located above the surface of the ground. Furthermore, decayed plant materials that fell on the ground, in between stones and rocks, and/or fallen branches on soil were considered as ground plant materials and represented by ground leaf litter and twigs. During collection, the plant materials were placed in a medium-sized brown paper bag and brought to the laboratory for setting up of moist chambers. Note that wet samples in this study were air-dried for five to seven days prior to the preparation of moist chambers.

Moist Chamber Cultivation

Using the collected four substrate types (microhabitats), the moist chamber cultures were prepared following the standard method of Stephenson & Stempen (1994). The substrate in each bag was split evenly to fill two 90-mm Petri dishes lined with three layers of tissue paper. A total of 480 moist chamber cultures were produced from the 240 collected plant litters. The duplication of moist chambers in this study aims to extract all possible records of myxomycetes per sample since dela Cruz et al. (2014) reported that replications of MC yielded a three-fold increase in the number of myxomycete collections and a higher number of recorded taxa. In that same study, about 25% of the total recorded species would have not been accounted if only one moist chamber per sample was used. Substrata in each Petri dish was then soaked in distilled water and left close overnight for 24 hours. The excess water was poured off from the moist chambers after the pH value has been recorded. The average pH of the water in each substrate culture was determined by getting the mean of the three readings of pH obtained from different sides of each moist chamber culture. All MC cultures were stacked in a well-lighted room with a temperature of 22-25°C following the standard incubation of at least eight weeks. These were then checked twice to thrice a week for the presence of myxomycete plasmodia and fruiting bodies. Dried moist chamber cultures were sprayed with water and further incubated for continuous myxomycetes observation. Only the Petri dishes with plasmodial growth and/or fruiting bodies were noted as a positive collection for myxomycetes. Finally, each fruiting body was carefully removed from the moist chamber and kept in herbarium box for identification. All fruiting bodies collected from this study were kept in the Herbarium Section of Far Eastern University, Manila, Philippines.

Myxomycete Characterization and Identification

Identification of myxomycetes was done up to species level to the best that we can. As the characterization and identification can only be done through fruiting bodies observation, the recovered specimens from the field and moist chambers were carefully handled for clear visual examination of their gross morphology such as size, color, shape, structure of stalk and sporotheca, and presence of calcium carbonate. In addition, further characterization was carried out using microscopic examination to see the internal structures of each fruiting body, i.e., presence of peridium, structure, and appearance of columella and capillitium, and ornamentation of spores (Stephenson & Stempen 1994). Lactophenol and/or potassium hydroxide (KOH) were used as mounting media. Preparation of slide was done by obtaining a piece of fruiting body and slightly crushing it on a clean glass slide which was viewed under a microscope (Optika). Finally, the identification of the specimens was done using keys from published literature (Mitchell 1980) and other available references such as identification keys (SYNKey, Mitchell 2008) and online databases (Eumycetozoa Project [<http://slimemold.uark.edu/>]). We further checked the current valid names of the identified myxomycetes through an online nomenclatural information system (<http://eumycetozoa.com>) (Lado 2005-2020).

Data processing and evaluation

We calculated the percentage of moist chamber productivity for the general sampling area and for each substratum by counting the number of moist chambers positive for plasmodium/sclerotia and/or fruiting bodies of myxomycetes. Therefore, the positive moist chambers were divided by the total number of prepared MC (which is 480) and multiplied by 100.

To quantify our sampling effort, a series of extrapolation was done to plot species accumulation curves using the free-downloaded program EstimateS (Version 9.0 with 100 randomizations). The cumulative number of species recorded in the sampling area was also plotted as similarly done in the study of Pecundo et al. (2017). The relative abundance (RA) for each species of myxomycetes in our sampling site and each substrate was calculated wherein the total number of collections for each species was divided by the total number of collections. After which, the resulting value was used to rank each species based on the modified grade given by Stephenson et al. (1993) and as used in other myxomycete study (Redeña-Santos et al. 2017). Therefore, each myxomycete species was categorized as R = rare if the resulting RA value is less than 0.5%, O = occasional if the RA value is greater than 0.5% to 1.5%, C = common if the RA value is greater than 1.5% up to 3%, and A = abundant if the RA value is more than 3%.

The taxonomic and species diversity in the study site and microhabitats (substrates) were also computed using different diversity indices. Initially, the taxonomic diversity index (TDI) was computed by getting the ratio of the number of species with the number of genera (S/G ratio). In this index, the lower the ratio, the more diverse it is as the value of the S/G ratio is inversely proportional to its taxonomic diversity. To determine species diversity, we used Shannon-Weiner (HS) Index and Fishers' Alpha Index (FAI) to account for the abundance and evenness in our samples. Furthermore, we used Gleason Index (HG) to determine the species richness and Pielou's Index (E) to express the similarity in species relative abundance in a community. The lower the value of the Pielou's index the less the evenness in communities between species. These measures were included to provide additional information about the ecological patterns of limestone-inhabiting myxomycetes. In this study, the FAI was computed using the downloadable software SPADE (Species Prediction and Diversity Estimation) (Chao & Shen 2003) while the rest of the species diversity indices were calculated using Microsoft Excel based on the formula of Magurran (2004) as similarly used in the study of Rea-Maminta et al. (2015). All diversity values resulted from the computation hereafter were statistically compared using the modified t-test (Magurran 2004). In analyzing the beta diversity, we used the Bray-Curtis (BC) dissimilarity index to statistically determine the compositional dissimilarity between substrates based on the presence or absence of species at each substrate. The resulting values in a similarity index, take from 0 to 1, which means that closer to 1 share high similarity while a value of 0 means the communities are different. The raw data file was inputted in the software PAST (Hammer et al. 2001; <http://folk.uio.no/ohammer/past>) and the resulting values were used to generate the clustering graph.

Results

Moist chamber productivity and species accumulation curve

Of the 480 moist chamber cultures, 72.7% or equivalent to 349 MC were recorded positive for either plasmodium/sclerotia and/or fruiting bodies of myxomycetes. Among the microhabitats examined, both AL and VN had the greatest number of positive moist chambers (78%), followed by TW (72%) and GL (63%). Upon checking all positive moist chambers, 30% (104 MC) of the moist chamber with plasmodia failed to fructify while 70% (245 MC) yielded 295 determinable records of fruiting bodies (Fig. 2A). All 318 collections (295 records from moist chambers, 23 records from field collection) were later grouped and classified as belonging to 28 species of myxomycetes under 14 genera. The extent of the sampling effort done in the limestone forests of Minalungao National Park showed a cumulative sampling effort of 80%. As the value of Chao 2 mean (= 35) was compared with the number of morphospecies recorded (= 28), it reflects that the field and moist chamber collection was good enough to recover the majority of the myxomycetes present in the sampling area. This also indicates that a saturation can be observed in the SAC, thereby implying that intuitively the present sampling area has been extensively sampled, and that rare species are expected to be discovered by increasing effort on field survey and/or perhaps by including a survey of special substrata such as inflorescences, grass leaf litter, and other specialized microhabitats.

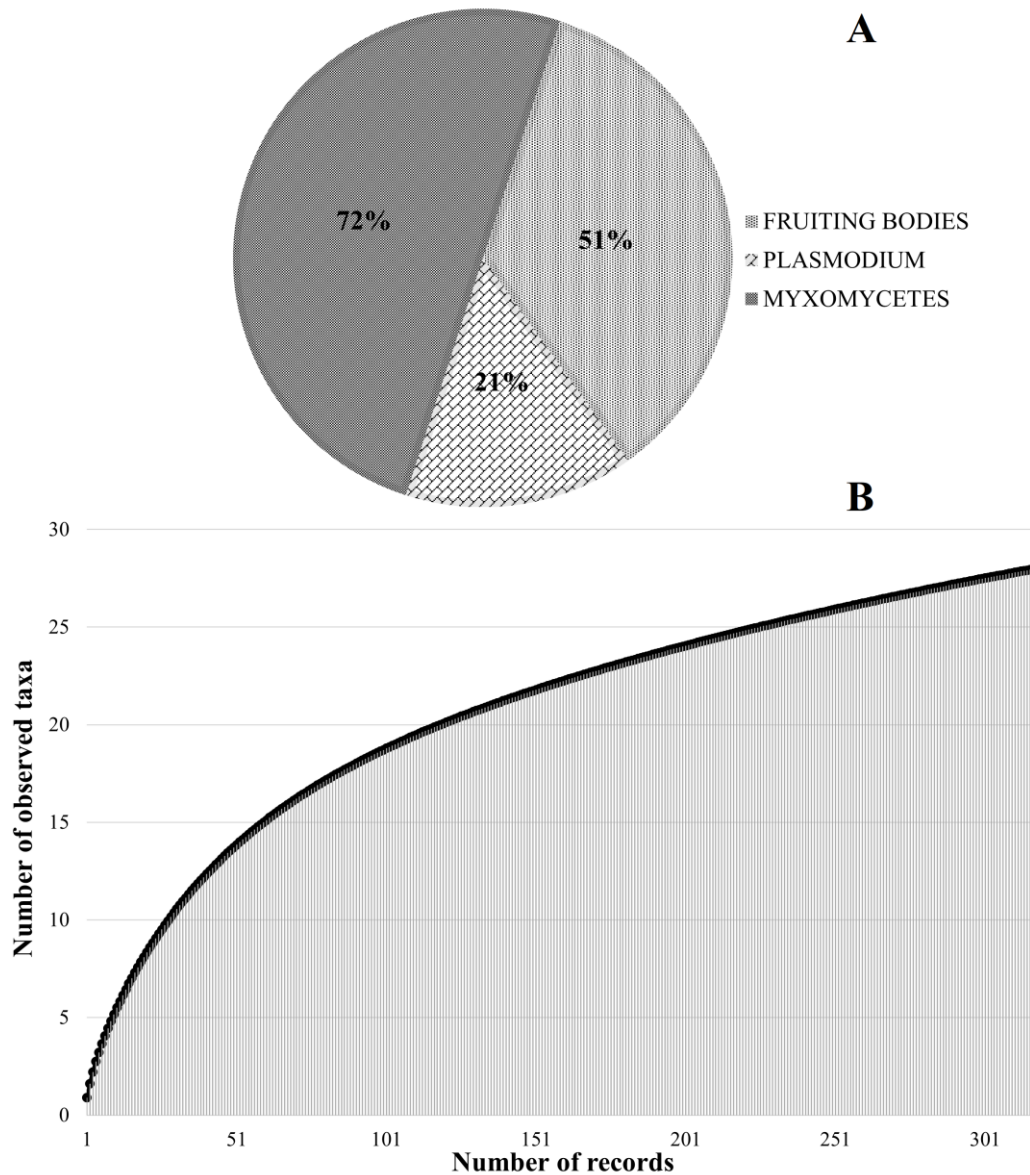


Fig. 2 – A. Proportion of plasmodia and fruiting bodies recorded from moist chamber cultures. B. The species accumulation curve (SAC) generated using EstimateS to show the sampling effort carried out in Minalungao National Park.

Species composition and abundance

From the 318 collections of myxomycetes recorded herein, nine species belonging to seven genera were found directly from 23 collections in the field. This includes *Didymium nigripes* with the highest number of collections (5) followed by *Arcyria cinerea*, *A. denudata*, and *Comatricha tenerrima* with three collections each. One to two collections were recorded for *Diachea leucopodia*, *Diderma effusum*, *D. hemisphaericum*, *Hemitrichia serpula*, and *Stemonitis fusca*. A total of 295 records were recovered from the duplicate moist chamber cultures which resulted in the identification of 27 species under 14 genera. Interestingly, all of the species found during the field survey with the exemption of *D. nigripes* also appeared in the moist chamber cultures, while 20 species were uniquely recorded from the moist chambers. The relative abundance of all collected myxomycetes was also determined. Our results showed that of the 27 species recovered from the moist chambers, 9 species ranked as rare, 7 species as occasionally occurring, while 6 species were recorded as common, and another 5 species as abundant (Fig. 3). The collated list of species presented in this study represents the first formal inventory of myxomycetes for the Minalungao National Park.

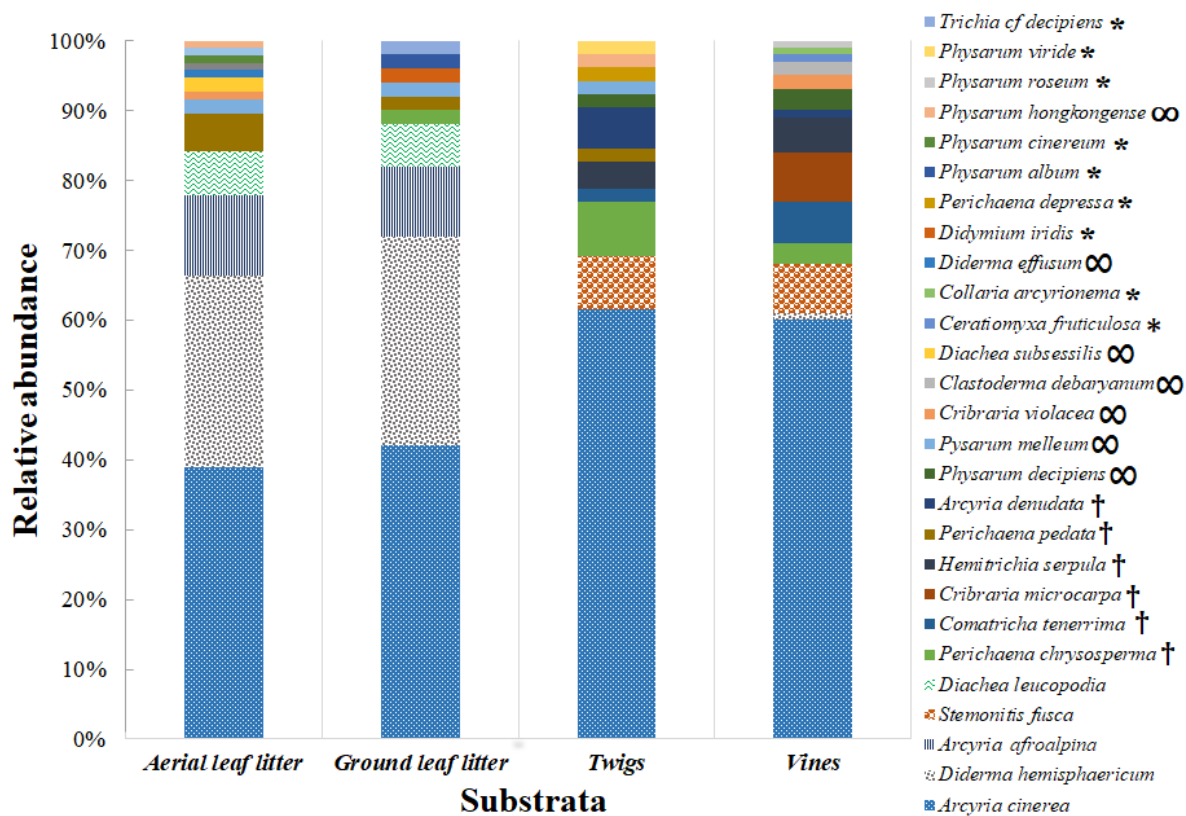


Fig. 3 – List of myxomycetes and their abundances based on the number of records from moist chamber cultures. Symbols after species names indicate the abundance index for each species: common (dagger †), occasional (infinity ∞), or rare (asterisk *). Abundant species are represented by pattern-filled boxes with no symbols added after species name.

Diversity and community analyses

The overall diversity of myxomycetes in the karst forest of Minalungao National Park was computed based on the combined number of records from the field and the moist chamber collections. The taxonomic diversity index of 2.07 for the whole study area with the five diversity indices is presented in Table 1. However, the diversity indices between microhabitats (substrata) were calculated using the records obtained from the moist chambers only. The lowest S/G ratio was noted on vines (TDI = 1.36) with the highest recorded from twigs (TDI = 2.00). The taxonomic diversity of aerial and ground leaf litter was 1.83 and 1.43, respectively. This implied that woody vines are more intuitively diverse than other substrates. For the species diversity, the highest species richness was noted on woody substrates (VN = 2.82, TW = 2.78) than leaf litter (AL = 2.21, GL = 2.30). Fifteen species under 11 genera were noted on woody vines while 12 species under six genera were found on twigs. Meanwhile, 11 species under six genera were noted on aerial leaf litter while 10 species and seven genera were recorded on ground leaf litter. Considering the abundance and distribution of myxomycete species between substrates, the Shannon indices among substrates did not show any significant difference ($p \geq 0.05$). Interestingly, the use of another intuitive diversity index such as FAI showed that twigs (FAI = 4.89) and woody vines (FAI = 4.43) have higher diversity than leaf litter (GL = 3.76, AL = 3.25). The difference, however, was still insignificant at a 95% confidence level. Furthermore, Pielou's index signified that the evenness of myxomycetes is relatively similar across all substrates (Table 1). Low values of Pielou's index obtained in this study likewise showed that the community between the species was also not evenly distributed.

The similarities in myxomycete assemblages between the different microhabitats were analyzed using the Bray-Curtis index. Results showed that aerial and ground leaf litter clustered together while the woody vines and twigs were separated and formed another group (Fig. 4A). In addition, clustering analysis showed that species composition in aerial and ground leaf litter was

more similar (BC = 0.57) than woody vines and twigs (BC = 0.51). In contrast, relatively low index values were computed between woody vines and aerial leaf litter (BC = 0.30) and between ground plant materials (TW and GL, BC = 0.36), indicating a high dissimilarity in terms of species composition between these substrates. This can also verify upon looking at the number of shared species between substrates. Venn diagram (Fig. 4B) showed that two species were found common in leaf litter while five were found between woody vines and twigs. Interestingly, the exclusive or unique species was also noted in each substrate. The term exclusive here applies to species of myxomycetes seen in one substrate but did not appear in other substrates. The substrate with the highest number of species exclusive in them were woody vines (5), followed by aerial litter (3), while ground leaf litter and twigs both had three and two exclusive species, respectively.

Table 1 Diversity of myxomycetes in Minalungao National Park. This table shows the number of records, taxa, taxonomic diversity (S/G ratio), species diversity as computed by SPADE, and the average pH values recorded for each substrate

	Number of observations ^c			TDI (S/G) ^d	Diversity indices ^e				Average pH ± STD
	R	S	G		HG	HS	FAI	E	
MNP ^a	318	29	14	2.07	4.86	0.91	7.76	0.37	-
AL ^b	93	11	6	1.83	2.21	0.73	3.25	0.35	6.10±0.35
GL	50	10	7	1.43	2.30	0.69	3.76	0.35	5.91±0.42
TW	52	12	6	2.00	2.78	0.66	4.89	0.34	5.82±0.38
VN	100	15	11	1.36	2.82	0.69	4.43	0.35	6.04±0.41

^aMNP – Minalungao National Park (general sampling site). The values were computed from field and moist chamber collections.

^bSubstrates: aerial leaf litter (AL), ground leaf litter (GL), twigs (TW), woody vines (VN)

^cNumber of observations: individuals or records (R), species (S), genera (G)

^dS/G: Species/Genera ratio (taxonomic diversity)

^eDiversity indices: HG = Gleason's Richness, HS = Shannon Diversity, FAI = Fisher's Alpha Index, E = Pielou's Evenness

Discussion

The islands of the Philippines are gifted with exceptionally rich biodiversity, yet poorly documented. For instance, the limestone landscape of Minalungao National Park in Nueva Ecija is home to a lowland dipterocarp forest with the reported taxonomic assessment of plants, chiropterans, gobies, and pteridophytes (Pader et al. 2017, Judan Cruz et al. 2018, Judan Cruz & Pader 2018, Alfonso et al. 2018). Microbial diversity is often lacking in many of these biodiversity surveys. This study is the first assessment of cryptogamic protists, particularly plasmodial slime molds or myxomycetes, in the area.

Extent of sampling effort and substrate culture productivity

Our one-day field excursion to look for fruiting bodies and collect substrata for MC preparation recovered about 80% of myxomycetes present in the area as revealed by the species accumulation curve (Fig. 2B). The amount of sampling effort in this study was likely the same as the other rapid biodiversity surveys conducted in the country, e.g., by Dagamac et al. (2015), Pecundo et al. (2017), and Macabago et al. (2017). The collection of suitable microhabitats to prepare a moist chamber has been a well-recognized classical procedure to recover myxomycetes (Dagamac et al. 2017b). However, additional field sampling is needed to recover other myxomycetes since the plasmodia of many species of myxomycetes failed to fructify in moist chambers. Therefore, similar to other surveys, we recorded these instances as myxomycete presence but did not attempt to identify them as plasmodial morphology is a poor indicator of species identity. Apparently, this circumstance may have a significant effect on the acquisition of its diversity since the diversity and abundance can only be conducted when myxomycetes produce fruiting bodies (Pecundo et al. 2017), therefore assessing diversity of myxomycetes in a particular habitat has always been a case-to-case basis (Nguyen et al. 2020). Nevertheless, the authors' best effort has been put in the present study to fairly gather and assess the myxomycetes that can be

recovered in the sampling area. Interestingly, one possible way to gather the missed species (as predicted by SAC) in the present study is to examine other special substrates, i.e., succulent plants, decayed inflorescence, dung, herbaceous plants, epiphyllous liverworts, and grasses that may be present in the area. These special microhabitats support unique assemblages of myxomycetes, even rare taxa (Lado et al. 1999, Schnittler 2001, Schnittler & Stephenson 2002).

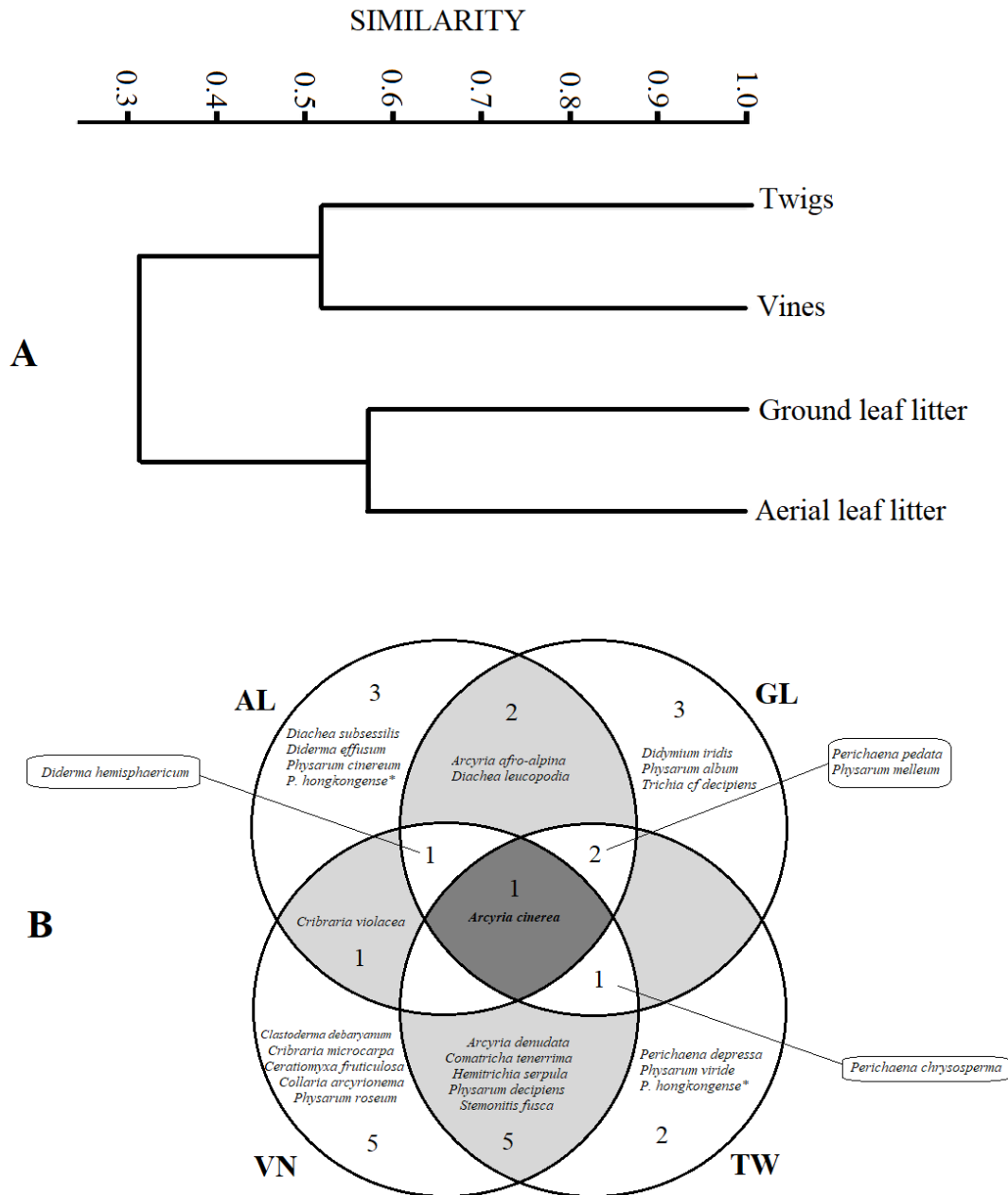


Fig. 4 – A. Bray-Curtis analysis showed the clustering of substrates with high similarity based on species composition. B. The Venn diagram also displayed the number of unique and shared myxomycete species between substrates. *P. hongkongense* (*) appeared in the MCs of AL and TW.

As the best laboratory cultivation technique to imitate ideal natural conditions, a moist chamber has been used in this study to provide a suitable condition in expanding the number of total collections. Our study yielded a comparable result of myxomycete productivity (72%) with other national parks in the country, i.e., Mt. Makulot National Park (84%, Cheng et al. 2013), Quezon National Park (82%, Dagamac et al. 2015), and Puerto Princesa Subterranean River

National Park (70%, Pecundo et al. 2017). Looking at the percent yield among substrates, we found that the highest productivity was noted in substrates located above the ground than on substrates collected on the forest floor. This is in congruence with the previous studies that in tropical moist forests, myxomycete tends to be high in microhabitats in the aerial strata than those at the ground level (Schnittler & Stephenson 2002, Rojas et al. 2014, Dagamac et al. 2015). Moreover, myxomycete spores are somehow components of the biological portion of particulate matter in the air like fungal spores. The large quantities of light-weight minute spores produced by myxomycetes can easily be carried away and stay uplifted in air, dispersed over significant spatial scales, and adapted to highly fluctuating environmental moisture, all of which may contribute to the high productivity of aerial substrates (Black et al. 2004, Rojas et al. 2014). Furthermore, the slightly high pH conditions of AL and VN, also found in this study, may also provide myxomycetes a better condition for them to thrive as also demonstrated in the studies of Alfaro et al. (2015) and Rea-Maminta et al. (2015). In addition, the aerial substrates used in our study such as AL and VN were reported to be excellent microhabitats to trap myxomycete spores and support their growth and development (Rojas & Stephenson 2008).

Identified myxomycete taxa and their abundances in the karst forest of MNP

The number of records presented in this study clearly shows a rapidly increasing propensity towards the depiction of rare myxomycete taxa based on a single collection. Such observation was also described by Schnittler & Mitchell (2000). This first survey of myxomycetes in the karst forest of MNP resulted in the recovery of 28 species belonging to 14 genera, namely *Arcyria* (3), *Ceratiomyxa* (1), *Clastoderma* (1), *Collaria* (1), *Comatricha* (1), *Cribraria* (2), *Diachea* (2), *Diderma* (2), *Didymium* (2), *Hemitrichia* (1), *Perichaena* (3), *Physarum* (7), *Stemonitis* (1), and *Trichia* (1). Among these, the widely distributed species *Arcyria cinerea* was recorded as the most abundant species in the karst forest with a total of 153 collections. *A. cinerea* had been reported as a cosmopolitan generalist and a high occurrence species which is known to thrive on a wide range of habitats. Thus, this species showing high records in our study site is not surprising as other studies also recorded this species abundantly even in the temperate region, i.e., Altay Mountains in Russia (Novozhilov et al. 2010), and in the tropical region, i.e., in the Neotropics such as the Luquillo Experimental Forest in Puerto Rico, (Stephenson et al. 1999) and the Paleotropics as shown in the various habitats in the Philippines (Alfaro et al. 2015, Macabago et al. 2017), Vietnam (Tran et al. 2014, Nguyen et al. 2020), and Thailand (Tran et al. 2008, Dagamac et al. 2017b). Employing the modified abundance ranking, other species noted abundantly in the present study are *Diderma hemisphaericum* (43 collections), *A. afroalpina* (16), *Diachea leucopodia* (12 collections), *Stemonitis fusca* (12 collections), and *Comatricha tenerrima* (10 collections). Relatively high records of *Diderma hemisphaericum* were also noted across different habitats in the Philippines (Rea-Maminta et al. 2015, Dagamac et al. 2017a, Macabago et al. 2017, Pecundo et al. 2020). Perhaps this species is widely distributed in the country. This is supported by the model analysis of Almadrones-Reyes & Dagamac (2018) which showed that the Philippines offers suitable habitat for this species. Moreover, *A. afroalpina* is surprisingly found abundant in our study while it was commonly documented as a rare taxon in previous studies in the country (Macabago et al. 2012, Dagamac et al. 2014). Interestingly, this species has also been recorded several times in leaf litter in Neotropics such as Ecuador and Costa Rica (Schnittler 2001). The rest of the abundant species belong to order Stemonitales which form long, solitary to gregarious, erect large sporocarps producing a large mass of spores which can easily disperse by wind (Stephenson 1989).

In this study, species were collected mostly at different levels of frequencies in different substrates. Decomposing materials with high organic matter provide a more suitable environment for the myxomycetes (Macabago et al. 2010, Kuhn et al. 2013), thus, explaining variations in species richness and abundance of myxomycetes in different substrata. For instance, although ranked as abundant across all substrates, *A. cinerea* was more common on twigs and woody vines than on aerial and ground leaf litter as is the case of the present study. Similarly, *P. chrysosperma* was more common on woody substrates than leaf litter. On the contrary, other species of

myxomycetes prefer to grow on leaf-based substrates than woody substrates. For example, high records of *D. hemisphaericum* and *A. afroalpina* were evident on leaf litter than the other two substrates. These taxa also occurred in different studies as follicolous myxomycetes in tropical Asia (Schnittler 2001, Macabago et al. 2012, Carascal et al. 2017). *Perichaena pedata* preferred more aerial leaf litter than any other substrate. Interestingly, the following species, e.g., *Cribraria microcarpa*, *Stemonitis fusca*, *Comatricha tenerrima*, and *Hemitrichia serpula*, have been known as common inhabitants of woody substrates on the forest floor (Everhart & Keller 2008) but our records showed they were more common on aerial vines than on twigs. The rest of the species were noted as either singletons or doubletons and appeared in one substrate only. For instance, two collections of *Clastoderma debaryanum* and singleton species such as *C. fruticulosa*, *C. arcyriionema*, and *P. roseum*, were recovered from woody vines only. *Diderma effusum* and *P. cinereum* were noted on aerial leaf litter only. *C. fruticulosa* which rarely occurred in moist chamber culture, appeared on woody vines in this study and in a previous study of Pecundo et al. (2017). Of interest, one collection of *Trichia* cf. *decipiens* has been collected from moist chamber culture with GL sample in the present study but the fruiting body of the specimen was not well-developed, and thus the determination of this species is not certain.

Species diversity and community structure among different microhabitats

The species diversity between the different microhabitats (substrata) was also computed. Interestingly, the highest number of species were recorded for woody vines (15 species) followed by twigs (12 species), aerial leaf litter (11 species), and ground leaf litter (10 species). Twigs had the highest S/G ratio (TDI = 2.00) while woody vines had the lowest (S/G = 1.36) and thus, was more taxonomically diverse among other substrates. It can be noted that several species of myxomycetes tend to thrive more on woody materials (Stephenson et al. 2008). The higher species richness and diversity values of myxomycetes obtained for woody vines may be also attributed to the high presence of bacteria, fungal spores, and other smaller protozoans on this decaying plant material. These microscopic organisms are ingested as food by the myxomycete amoeboflagellates and plasmodium (Stephenson 1989). In addition, the quality of the substrate is also another critical factor that can perhaps influence the diversity of myxomycetes, particularly if coupled with other environmental factors such as pH, moisture, and decay rate (Everhart & Keller 2008). As stated by Takahashi (2015), nutrient supply from decomposing plant material is an important factor when investigating the diversity of myxomycetes. It has also been reported that wood decomposition goes through different changes than leaf litter resulting in high water content, accumulation of nutrients, and decrease in pH (Cornwell et al. 2009, Fukasawa et al. 2015). Although the rate of decay of the substrates used in this study was not determined, this may be a factor as to why a higher richness was observed with woody vines and twigs.

Substrates overlap by the assemblages of myxomycete were also assessed in this study. A high similarity index was noted between AL and GL and between VN and TW. Apart from the mechanism of spore dispersal from the ground to air, the similarity in myxomycete composition between substrates may be associated with the composition and structure of the substrate material. The wide-diameter size of both aerial and ground leaf litter and the rough texture, water-absorbing capacities like a sponge of woody vines and twigs may be the attributable factors why a high similarity of myxomycete assemblages was noted between these microhabitats. For instance, most of the members of Stemonitales were consistently recovered from substrates with high water-holding capacities such as canopy logs, bark, twigs, and vines. Woody substrates especially vines have been reportedly present as a special microhabitat for some myxomycete species (Rojas et al. 2014). On the contrary, overlapping taxa, e.g., *Diachea leucopodia*, *Arcyria afroalpina* between leaf litter probably showed the preference of these species to these substrata, perhaps due to its wide surface area which was favorable for their gregarious or closely crowded formation of sporangia (Lado 2005-2020). However, this observation of substrate preference of myxomycetes certainly needs further exploration and validation especially in tropical forests.

Forest with extensive karst landscape is a myxomycete hotspot in the country

About 10% of the Philippine archipelago is covered with the karst landscape (Wagner 2013). Surprisingly, forests over limestone offer unique habitat as it differs from other ecological niches in terms of geomorphology and hydrology (Restificar et al. 2006). Previous claims stated that karst microhabitats supported high floral diversity and endemism due to unique vegetation structure and extreme microclimate conditions (Clements et al. 2006, Tolentino et al. 2020). In fact, restoration of vegetation in limestone forests was reported to be driven by high deposition of calcium and other minerals on bedrocks and soil (Wei et al. 2018). This could also be true for myxomycetes. The collected myxomycetes from limestone forests were said to be members of the Physarales which requires calcium carbonate deposits on their sporangia. Earlier comparative study of Carr (1939) between limestone and sandstone regions revealed a pattern where species of myxomycetes containing lime were recovered abundantly in the limestone region than in sandstone region where non-lime-bearing species were higher. A similar result was obtained from the study of Dvořakova (2002) that a limestone habitat, e.g., Bohemian Karst, harbored a higher number of Physarales (12 species) than a non-limestone forest, e.g., Hřebeny Mts., with only 5 species recorded and the sporangia appeared less. Interestingly, surveys on the limestone forests in the Philippines tend to follow a similar pattern revealing that many, which is about half of the myxomycetes recovered from every study, were members of the lime-bearing Physarales. A comparative species list based on several different studies was compiled and shown in Table 2. The previous study of dela Cruz et al. (2011) in the limestone forest sites in Hundred Island showed that 50% of the identified taxa were Physarales. The taxonomic survey of Dagamac et al. (2015) reported 35 species under 16 genera from the karst forest of Quezon National Park where 15 (46.87%) of these were also lime-containing. A similar observation was obtained from the reports of Pecundo et al. (2017) and Macabago et al. (2020) from the geological limestone forests in Puerto Princesa Subterranean River National Park and Coron in Palawan Island. The former study recorded 33 taxa, and the latter has 25 taxa with 16 (48.5% of the total records) and 11 (48%) species under Physarales, respectively. In the present study, of the 28 taxa, 46% of the recorded species (= 13) were also lime-containing. When compared with the earlier studies, the observed trend reflects the influence of the unique conditions, e.g., basic pH, abundant availability of calcium carbonate, in the limestone forests, and favoring the growth of lime-bearing myxomycetes. This also corroborates with the ecological observation that the demands of order Physarales with higher elemental calcium than to other mycetozoa orders were probably supported (Carr 1939), thus explaining their recurring pattern of abundance in limestone forest habitats.

Interestingly, the species that were recorded in these former studies and the present work were the lime families Didymiaceae and Physaraceae. Specifically, the species *Diderma hemisphaericum* with entire bodies blanketed by whole white-chalk lime, and the two species with peridial lime *Didymium nigripes* and *D. iridis* were found in all the investigated limestone forests. Moreover, the species *Diachea leucopodia* with dense deposits of lime crystals on the stalk and hypothallus was also found common and found in the limestone forests of Palawan Island (Pecundo et al. 2017, Macabago et al. 2020) and in the present study. Limestone forests also harbor several taxa which are rare and reported for the first time for the Philippines. For instance, the lime-containing species *Lepidoderma tigrinum* and *Badhamia macrocarpa* recorded for the first time in the country were recovered from the limestone forests in Hundred Island and Coron Island, respectively (dela Cruz et al. 2011, Macabago et al. 2020). After the first report of Reynolds three decades ago, the limy species *Physarum pezizoideum* was recorded again in the karst landscape in Atimonan, Quezon (Dagamac et al. 2015) but found nowhere else in the country. This implies that many rare taxa and potentially new species of myxomycetes are hidden in the forests over limestone and can be discovered with upcoming research. Furthermore, our data suggest that the Philippine limestone forests possibly holds a unique assemblage of myxomycetes specifically those taxa that have calcium carbonate as the major component of their sporangial structure. Our data contributed to the existing surveys carried out in karst forests in the Philippines and helps to better understand the diversity of myxomycetes in the limestone habitat. Although endemism is true for

the flora and fauna in limestone landscapes, this cannot be directly said for myxomycetes, but our study as an additional baseline work provided evidence that myxomycetes with calcareous structure were in greater abundance in karst habitats like Minalungao National Park.

Table 2 List of myxomycetes on limestone forests in the Philippines. Note that only taxa assigned with species epithet were included in this comparative list.

	HINP ^a	QNP ^b	PPSRNP ^c	CIP ^d	MNP ^e
Protosteliales					
<i>Ceratiomyxa fruticulosa</i> (Müll.) T. Macbr.	-	+	-	-	+
Clastodermatales					
<i>Clastoderma debaryanum</i> A. Blytt	-	-	+	+	+
Echinosteliales					
<i>Echinostelium minutum</i> de Bary	-	-	+	-	-
Cribrariales					
<i>Cribraria microcarpa</i> (Schrad.) Pers.	-	-	+	-	+
<i>Cribraria violacea</i> Rex	-	+	-	+	+
Reticulariales					
<i>Lycogala exiguum</i> Morgan	-	+	-	-	-
Physarales					
<i>Badhamia macrocarpa</i> (Ces.) Rostaf.	-	-	-	+	-
<i>Craterium minutum</i> (Leers) Fr.	-	+	-	-	-
<i>Diachea leucopodia</i> (Bull.) Rostaf.	-	-	+	+	+
<i>Diachea subsessilis</i> Peck	-	-	+	-	+
<i>Diderma effusum</i> (Schwein.) Morgan	-	+	+	+	+
<i>Diderma hemisphaericum</i> (Bull.) Hornem.	+	+	+	+	+
<i>Didymium iridis</i> (Ditmar) Fr.	+	+	+	+	+
<i>Didymium minus</i> (Lister) Morgan	+	-	-	-	-
<i>Didymium nigripes</i> (Link) Fr.	+	+	+	+	+
<i>Didymium ochroideum</i> G. Lister	-	+	-	+	-
<i>Didymium squamulosum</i> (Alb. & Schwein.) Fr. & Palmquist	+	+	+	+	-
<i>Lepidoderma tigrinum</i> (Scrad.) Rostaf.	+	-	-	-	-
<i>Physarella oblonga</i> (Berk. & M.A. Curtis) Morgan	-	+	-	-	-
<i>Physarum album</i> (Bull.) Chevall.	-	+	+	-	+
<i>Physarum bivalve</i> Pers.	-	-	+	-	-
<i>Physarum bogoriense</i> Racib	+	-	-	-	-
<i>Physarum cinereum</i> (Batsch) Pers.	+	+	+	-	+
<i>Physarum compressum</i> Alb. & Schwein.	+	+	+	-	-
<i>Physarum decipiens</i> M.A.Curtis	-	+	+	-	+
<i>Physarum echinosporum</i> Lister	+	-	-	+	-
<i>Physarum hongkongense</i> H.C. Chao	-	-	-	-	+
<i>Physarum leucophaeum</i> Fr.	-	-	+	-	-
<i>Physarum melleum</i> (Berk. & Broome) Masee	-	+	+	-	+
<i>Physarum notabile</i> T. Macbr.	-	+	-	-	-
<i>Physarum oblatum</i> T. Macbr.	-	-	-	+	-
<i>Physarum pezizoideum</i> (Jungh.)	-	+	-	-	-
<i>Physarum pusillum</i> (Berk. & M.A. Curtis) G. Lister	-	-	-	+	-
<i>Physarum roseum</i> Berk & Broome	-	-	-	-	+
<i>Physarum viride</i> (Bull.) Pers.	-	-	+	-	+
<i>Willkommllangea reticulata</i> (Alb. & Schwein.) Kuntze	-	-	+	-	-
Trichiales					
<i>Arcyria afroalpina</i> Rammeloo	-	+	-	-	+
<i>Arcyria cinerea</i> (Bull.) Pers.	+	+	+	+	+
<i>Arcyria denudata</i> (L) Wettst.	+	+	+	+	+
<i>Calomyxa metallica</i> (Berk.)	+	-	-	-	-

Table 2 Continuation.

	HINP ^a	QNP ^b	PPSRNP ^c	CIP ^d	MNP ^e
<i>Hemitrichia serpula</i> (Scop.) Rostaf. ex Lister	-	+	+	-	+
<i>Hemitrichia pardina</i> (Minakata) Ing	-	-	+	-	-
<i>Perichaena chrysosperma</i> (Curr.) Lister	+	+	+	-	+
<i>Perichaena depressa</i> Lib.	+	+	+	+	+
<i>Perichaena dictyonema</i> Rammeloo	-	+	-	+	-
<i>Perichaena minor</i> (G. Lister) Hagelst.	+	-	-	-	-
<i>Perichaena pedata</i> (Lister & G. Lister) G. Lister	+	+	-	-	+
<i>Perichaena vermicularis</i> (Schwein.) Rostaf	-	-	-	+	-
<i>Trichia cf. decipiens</i> (Pers.) T. Macbr.	-	-	-	-	+
Stemonitidales					
<i>Collaria arcyronema</i> (Rostaf.) Nann. -Bremek. ex Lado.	+	+	+	-	+
<i>Comatricha laxa</i> Rostaf.	-	+	-	-	-
<i>Comatricha nigra</i> (Pers. ex J.F. Gmel.) J. Schröt.	+	+	+	-	-
<i>Comatricha pulchella</i> (C. Bab. & Berk.) Rostaf.	-	-	+	+	-
<i>Comatricha tenerrima</i> (M.A. Curtis) G. Lister.	-	-	+	+	+
<i>Lamproderma scintillans</i> (Berk. & Broome) Morgan	-	+	+	+	-
<i>Stemonitis axifera</i> (Bull.) T. Macbr.	-	-	+	-	-
<i>Stemonitis fusca</i> Roth	+	+	+	+	+
<i>Stemonitis pallida</i> Wingate	-	+	-	-	-
<i>Stemonitis splendens</i> Rostaf.	-	-	+	-	-
Total number of species	20	32	33	22	28
Number of species with lime	10	15	16	11	13
Number of species without lime	10	17	17	11	15

^aHINP: Hundred Island National Park, Pangasinan (dela Cruz et al. 2011)

^bQNP: Atimonan trail, Quezon National Park (Dagamac et al. 2015)

^cPPSRNP: Puerto Princesa Subterranean River National Park, Palawan (Pecundo et al. 2017)

^dCIP: Coron Island, Palawan (Macabago et al. 2020)

^eMNP: Minalungao National Park, Nueva Ecija (present study)

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