

Myxomycetes Associated with Plant-based Substrata Collected around Bulusan Lake in Sorsogon, Philippines

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Abstract: Ten different types of plant-based substrates [aerial leaf litter (AL), ground leaf litter (GL), twigs (TW), woody vines (WV), tree bark (BK), dried inflorescences (IF), decayed fruits (FR), and dead leaves of *Pandanus* (PD), epiphytic plants (EP), and tree ferns (FN)] collected along the trail leading to and surrounding Bulusan Lake in Sorsogon, Philippines, were assessed for the occurrence and diversity of myxomycetes using moist chamber cultures. The 388 moist chambers prepared in the study yielded 25 species belonging to 12 genera. The greatest number of species was recorded from bark followed by woody vines, aerial leaf litter, twigs, and dried inflorescences. Inflorescences had the highest diversity (FI = 18.60, SID = 9.78) while the lowest was observed for the dead leaves of *Pandanus* (FI = 4.92, SID = 3.99). We also observed high similarities in species composition among the different substrate types.

Keywords: active volcano, biodiversity, microhabitats, moist chamber cultures, slime molds

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Introduction

Myxomycetes, also known as “true slime molds” or “plasmodial slime molds”, have been reported from various plant-based substrata collected in the tropics, e.g., decomposing ground leaf litter (Rojas and Valverde 2022), woody lianas (Wrigley de Basanta et al. 2008), inflorescences (Schnittler and Stephenson 2002), litter of deciduous trees (Lado et al. 2017), and the bark of living trees, other leafy litter and woody twigs including some very specific microhabitats such as aerial leaf litter of herbaceous plants, cacti, and palms (Schnittler et al. 2002; Lado et al. 2003). In the Philippines, microhabitats that were so far explored for myxomycetes through moist chamber culture techniques include the usual substrates of aerial and ground leaf litter and woody twigs (Macabago et al. 2010, 2016, 2017, 2020a; Dagamac et al. 2014, 2015a, 2015b, 2017; dela Cruz et al. 2014; Eloreña et al. 2020; Cabutaje et al. 2021), the dead bark of living trees such as *Samanea saman* (Jacq.) Merr. (Dagamac et al. 2010), *Swietenia macrophylla* King. (Policina and dela Cruz 2020a), and other dipterocarp trees (Policina and dela Cruz 2020b), and woody vines (Rea-Maminta et al. 2015; Isagan et al. 2020; Macabago et al. 2020b; Pecundo et al. 2020, 2021). Other

specialized plant materials used for moist chamber cultures are dead ferns and sugar cane leaf litter (Alfaro et al. 2014), grass leaf litter (Carascal et al. 2017), dead inflorescence (Pecundo et al. 2017; dela Cruz et al. 2021), and mangrove substrates (Lim et al. 2021). However, tropical areas like the Philippines harbour many other substrata for myxomycetes, which could include species of plants endemic to the country. In the Bicol Peninsula where the study was conducted, 23 endemic plants were so far recorded (Buot 2009) and may represent microhabitats where unique myxomycetes may be found.

The Bicol Peninsula is becoming increasingly better known for the documentation of its myxomycetes among the regions in the Philippines. The initial rapid assessment of Dagamac et al. (2017) yielded 746 records of myxomycetes from this region, with eight new records for the Philippines. This study identified 57 species and 18 genera of myxomycetes. The study of Eloreta et al. (2020) also reported 180 records, 24 species, and 11 genera in one of the mountain natural parks in the region. Macabago et al. (2020b) listed 201 records from their study of island forests and identified 38 species and 14 genera including one new record for the country and 16 new records for the region bringing the total number of myxomycetes in the Bicol Peninsula to 73. The present study contributes to this effort with its rapid assessment of myxomycetes from different plant-based substrata that were collected along the walking trails surrounding the touristy Bulusan Lake within the Bulusan Volcano Natural Park in Sorsogon Province. As Mt. Bulusan is an active volcano in the country, the study also provides baseline data for any future research on the possible impact of volcanic activities or eruption to myxomycetes.

Materials and methods

Sampling locality

The sites selected for this study were woodland areas along the cemented road leading to Bulusan Lake and the forested areas adjacent to the walking path surrounding the lake (Fig. 1). The collecting points around the lake had a steep slope and an elevation of 282 to 360 m above sea level. The forest area had 75 to 100% canopy closure but moderately disturbed by local tourists. Bulusan Lake lies at the foot of Mt. Bulusan within the Bulusan Volcano Natural Park (BVNP, N12°45'20.283", E124°05'45.447"). BVNP is within the municipalities of Bulusan, Barcelona, Casiguran, Juban, and Irosin in the province of Sorsogon in the Bicol Region. The natural park, a tropical rainforest, has an area of 3,672 ha and surrounds Mt. Bulusan, an active stratovolcano with an altitude of 1,560 m. Mount Bulusan, considered as the fourth most active volcanoes in the Philippines, had a phreatic eruption in June 2016 and again in December of the same year, generating an ash plume of about 2,000 m high. Collection of substrates for this study occurred in June 2016, 10 days after the first reported major eruption for the year. The study area has a Type 2 climate with a very pronounced rainy period from December to February and no dry season and has an average temperature of 22 - 31°C (Philippine Atmospheric Geophysical and Astronomical Services Administration, PAGASA).

Collection of substrata and preparation of moist chamber cultures

Ten different types of plant-based substrates were used in this study. These were aerial leaf litter (AL, 35 samples), ground leaf litter (GL, 42 samples), twigs (TW, 37 samples), woody vines (WV, 40 samples), tree barks (BK, 43 samples), dead leaves of *Pandanus* (PD, 39 samples), epiphytic plants (EP, 32 samples), and the endemic tree fern *Pronephrium bulusanicum* (Holttum) Holttum (FN, 49 samples), dead inflorescences (IF, 33 samples), and decayed fruits (FR, 38 samples). The collected samples were placed in brown paper bags, air-dried for 16 days, and processed for the moist chamber cultures following

the protocol of Stephenson and Stempen (1994). The moist chambers ($n = 388$) are made up of disposable petri dish lined with three layers of white tissue paper. Each sample was cut with a scissor or twig cutter to fit into the petri plates. Ground and aerial leaf litter, and other leaf samples were cut into postage stamp size while woody vines, twigs, and barks were cut into shorter lengths to fit the petri plates. Decayed fruits and inflorescences were placed enough to fill in the petri plates. One moist chamber per sample was prepared and were soaked with distilled water for 24 hours, after which the excess water was removed. Moist chambers were observed for 12 weeks.

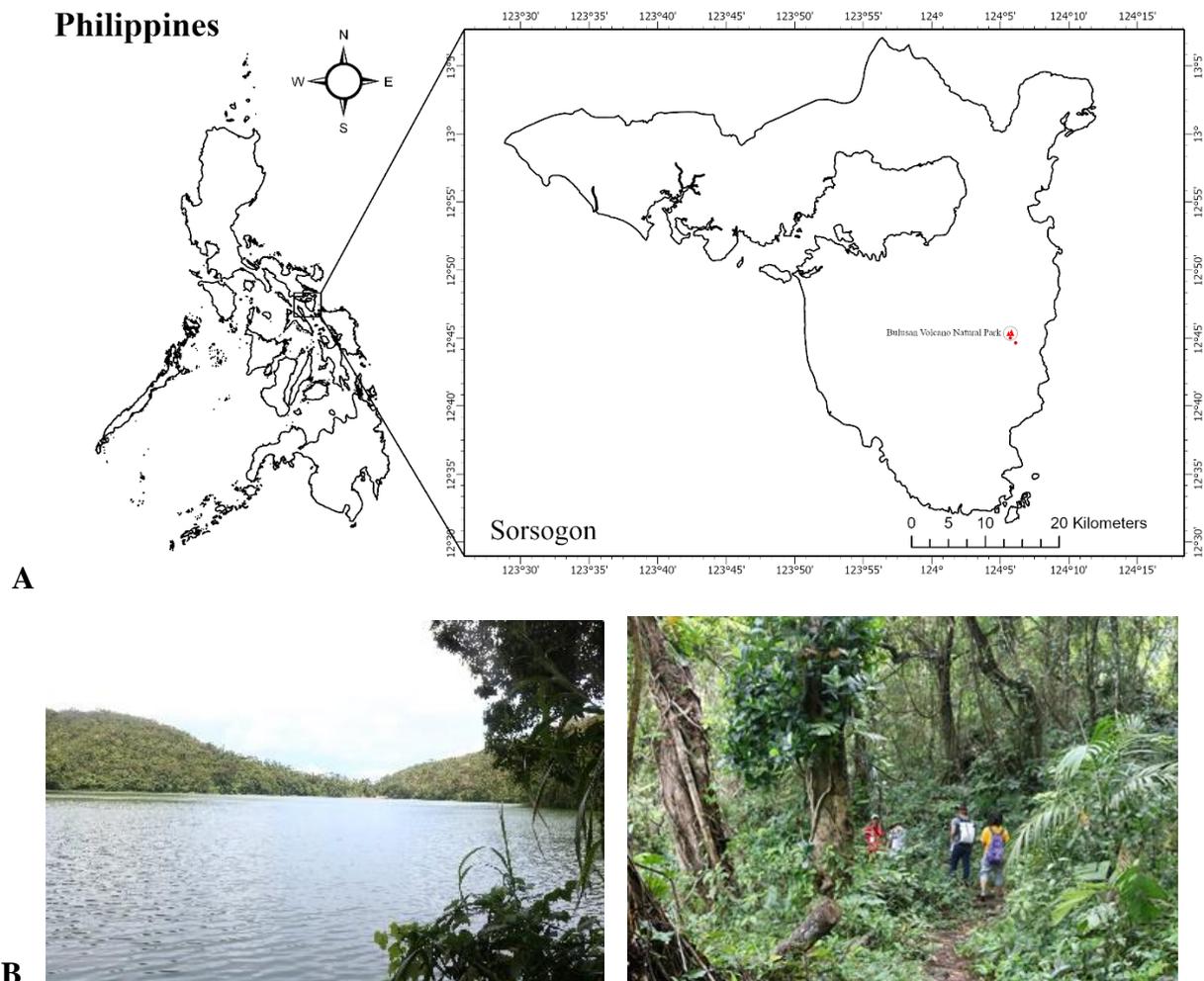


Figure 1. The study area: (A) Map of Bulusan Volcano Natural Park within the province of Sorsogon, and (B) images of the collecting locality.

Identification of myxomycetes and ecological analysis

All identifiable fruiting bodies were viewed using a dissecting microscope (SZ61, Olympus) to observe for morphological traits important for species identification. Spore morphology and other microscopic traits were observed from wet mounts of fruiting bodies with a compound light microscope (CX31, Olympus). Species identification follows comparison of morphologies with published monographs (e.g., Stephenson and Stempen 1994; Keller and Braun 1999) and online identification guides (<http://slimemold.uark.edu/>). Nomenclature follows Lado (2005-2022).

Moist chamber productivity per substrate type was computed as the percentage of positive moist chambers (i.e., moist chambers with fruiting body and/or plasmodium) over the total number of moist chambers as described by Dagamac *et al.* (2012). The estimation of abundance for each species follows Stephenson *et al.* (1993), where the number of records per species is divided by the total number of records of all collected myxomycetes. If a species had a relative abundance (RA) of $\geq 3\%$ of the total collections, this was noted as abundant [A]. If the RA was $\geq 1.5\%$ but $< 3\%$ of the total collections, this was noted as common [C]. RA of $\geq 0.5\%$ but $< 1.5\%$ of the total collections is occasional [O] and RA of $< 0.5\%$ of the total collections is considered as rare [R]. Individual-based rarefaction curve and Chao2 estimates were generated for the collected myxomycetes in this study to determine the exhaustiveness of the sampling. The taxonomic diversity index (TDI) was used as an estimate of taxonomic richness and computed as the ratio between the number of species and the number of genera. For species diversity, the Fisher's alpha and the Simpson's reverse index were computed for each of the substrate types using the classical formula in SPADE (Chao and Shen 2003). These diversity indices are commonly used in the study of myxomycetes, and therefore, this study can be easily contrasted with other published studies. To compare the community of myxomycetes between substrata, a Morisita index for similarity values was computed using SPADE with a range between 0 [= total dissimilarity between the two substrates being compared] to 1 [= identical assemblages for the two substrates being compared]. The similarity of assemblages between the different substrates was also computed using the Jaccard Similarity index with the computer software NTSYSpc v2.21. Data was presented as a cladogram.

Results

A total of 25 species and 12 genera were identified from 210 records of myxomycetes in this study (Table 1). Moist chamber cultures showed an overall productivity of 66% with wood vines (93%) giving the highest MC productivity. The lowest productivity (51%) was recorded with dead leaves of tree ferns. Individual-based rarefaction curve showed a sampling effort of 67%. The highest number of species was recorded from BK (14 species) and is followed by WV (12), AL (11), and IF and TW (10). Other substrates had 7 to 9 recorded species. In terms of relative abundance, seven species are abundant, nine are common, four are occasional, and five are rare. Among the species in this study, *Arcyria cinerea* (62), *Collaria arcyrionema* (34), and *Perichaena depressa* (22) had the highest numbers of records (Table 1).

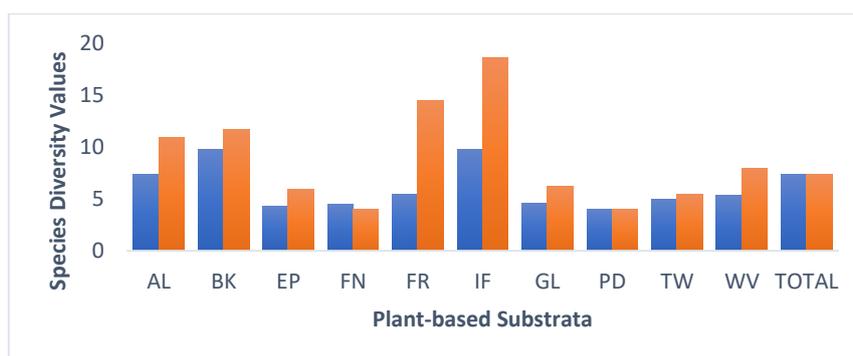


Figure 2. Computed species diversity values [Fisher's Alpha Index = orange, Simpson's Index = blue] per substrate types: aerial leaf litter (AL), dead tree barks (BK), decayed leaves of epiphytic plants (EP) and tree ferns (FN), decayed fruits (FR), ground leaf litter (GL), inflorescence (IF), dead leaves of *Pandanus* (PD), twigs (TW), and woody vines (WV).

Table 1. Frequency, abundance index (AI), and taxonomic diversity of myxomycetes associated with the ten plant-based substrata in Bulusan, Philippines.

Taxa	Frequency										Total	AI ^b
	Woody Substrates				Leafy Substrates				Other Substrates			
	BK ^a	WV	TW	AL	EP	GL	FN	PD	IF	FR		
<i>Arcyria cinerea</i> (Bull.) Pers.	6	9	8	4	5	5	6	15	1	3	62	A
<i>Arcyria globosa</i> Schwein.	1			1		1			2		5	C
<i>Collaria arcyronema</i> (Rostaf.) Nann.-Bremek. ex Lado	2	7	8	4	1		5	5	1	1	34	A
<i>Comatricha alta</i> Preuss		3	1	1							5	C
<i>Comatricha anomala</i> Rammeloo		1									1	R
<i>Comatricha nigra</i> (Pers. ex J.F. Gmel.) J. Schröt.	1	1	1			1					4	C
<i>Cribraria microcarpa</i> (Schröd.) Pers.	2	1		1			1				5	C
<i>Cribraria violacea</i> Rex	2	1	1	1	1				2	1	9	A
<i>Diachea leucopodia</i> (Bull.) Rostaf.	2					1					3	O
<i>Diderma effusum</i> (Schwein.) Morgan	1		1		1	2	1		2	1	9	A
<i>Diderma hemisphaericum</i> (Bull.) Hornem.				1		2	4		2		9	A
<i>Didymium floccosum</i> G.W. Martin, K.S. Thind & Rehill								3			3	O
<i>Didymium iridis</i> (Ditmar) Fr.								1			1	R
<i>Didymium nigripes</i> (Link) Fr.	1			1	1			1			4	C
<i>Hemitrichia serpula</i> (Scop.) Rostaf. ex Lister	1			1			1				3	O
<i>Perichaena chrysoesperma</i> (Curr.) Lister	3	1	1								5	C
<i>Perichaena depressa</i> Lib.	2	1	6	3	6	1	1		1	1	22	A
<i>Perichaena pedata</i> (Lister & G. Lister) G. Lister									1		1	R
<i>Physarum album</i> (Bull.) Chevall.		1		1	1			4			7	A
<i>Physarum compressum</i> Alb. & Schwein.		1			1			1		1	4	C
<i>Physarum decipiens</i> M.A. Curtis			1					1	1	1	4	C
<i>Physarum melleum</i> (Berk. & Broome) Masee			1						1		2	O
<i>Stemonitis fusca</i> Roth	2	1						3			6	C
<i>Stemonitis smithii</i> T. Macbr	1										1	R
<i>Trichia botrytis</i> (J.F. Gmel.) Pers.									1		1	R
Total	27	28	29	19	17	13	19	34	15	9	210	
Taxonomic Diversity Index	1.3	1.7	1.4	1.2	1.1	1.4	1.2	1.8	1.4	1.2		
Number of Species	14	12	10	11	8	7	7	9	10	7		
Number of Genera	10	7	7	9	7	5	6	5	7	6		

^a BK = tree bark, WV = woody vines, TW = twigs, AL = aerial leaf litter, EP = leaf litter of epiphytic plants, GL = ground leaf litter, FN = leaf litter of the tree fern *Pronephrium bulusanicum*, PD = dead leaf samples of *Pandanus*, IF = inflorescences, FR = decayed fruits.

^b Abundance Index: R = rare (<0.5%), O = occasional (>0.5–1.5%), C = common (>1.5–3%), A = abundant (≥ 3%)

^c Names in bold are new records for the Bicol Peninsula.

The Taxonomic Diversity Index (TDI) was recorded lowest from the dead leaf samples of epiphytic plants, and hence, the most taxonomically diverse (Table 1). Here, we recorded eight species belonging to seven genera. We also recorded seven species belonging to six genera for both the dead leaf samples of tree ferns and the decayed fruits and thus, can also be considered as taxonomically diverse. However, for species diversity which accounts for species richness and abundance, inflorescence (IF, SID = 9.78, FAI = 18.60,) had the highest species diversity (Fig. 2), and is followed by decayed fruits (FR, FIA only = 14.49), dead tree barks (BK, SID = 9.72, FAI = 11.71), and aerial leaf litter (AL, SID = 7.37, FAI = 10.90).

Table 2. A similarity matrix derived from Morisita index values (upper right half) between the different substrata. Also indicated are the number of species shared by the substrates (lower left half).

Substrata ^a	AL	BK	EP	FN	FR	GL	IF	PD	TW	WV
AL		0.735	0.758	0.815	0.729	0.872	0.948	0.838	0.899	0.846
BK	8		0.829	0.812	0.714	0.974	0.835	0.791	0.872	0.932
EP	6	6		0.712	0.784	0.884	0.565	0.725	0.973	0.748
FN	6	6	4		0.714	0.991	0.704	0.873	0.968	0.828
FR	4	5	6	4		0.761	0.515	0.783	0.809	0.785
GL	4	6	3	4	3		0.855	0.893	0.791	0.826
IF	6	6	5	5	6	5		0.311	0.604	0.460
PD	4	4	5	2	4	1	3		0.814	0.971
TW	5	7	5	4	6	4	7	3		0.879
WV	7	8	6	4	5	3	4	5	7	

^a BK = tree barks, WV = woody vines, TW = twigs, AL = aerial leaf litter, EP = leaf litter of epiphytic plants, GL = ground leaf litter, FN = leaf litter of the tree fern *P. bulusantum*, PD = dead leaf samples of *Pandanus*, IF = inflorescences, FR = decayed fruits.

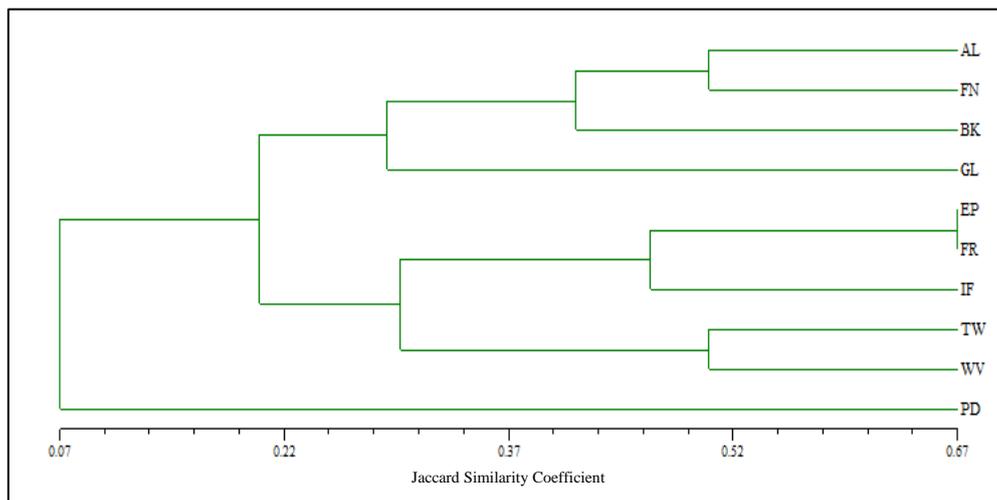


Figure 3. Cluster analysis of the 10 plant-based substrates based on the presence or absence of myxomycetes. Substrates: aerial leaf litter (AL), dead tree bark (BK), decayed leaves of epiphytic plants (EP), decayed leaves of the endemic tree fern *P. bulusantum* (FN), decayed fruit (FR), ground leaf litter (GL), inflorescences (IF), dead leaves of *Pandanus* (PD), twig (TW) and woody vine (WV).

Comparison of the myxomycete assemblages between the different microhabitats showed a high similarity value (= 0.991) between GL and FN based on the Morisita similarity index (Table 2). Other substrata with high similarity values are GL and BK (= 0.974), TW and EP (= 0.973), TW and FN (= 0.968), IF and AL (=0.948), TW and AL (= 0.899), PD and GL (= 0.893), WV and BK (= 0.932), TW and WV (= 0.879), and TW and BK (= 0.872). The highest number of shared species was observed between AL and BK and WV and BK, both with 8 shared species (Table 2). GL and PD had only one shared species, i.e., *Arcyria cinerea*, while FN and PD had two shared species, i.e., *Arcyria cinerea* and *Collaria arcyronema* (Table 1). Cluster analysis based on the Jaccard Similarity Coefficient, which only takes into consideration what species are present and absent in each substrate being compared, showed two clusters (Figure 3). However, the clustering does not reflect the type of materials (i.e., woody vs leafy substrates). Leaf litter of *Pandanus* had a very different myxomycete assemblage from other microhabitats.

Discussion

The last decade has seen a renewed interest on myxomycetes in the Philippines. This is clearly reflected in the number of new records for the country, i.e., additional 52 species (Dagamac and dela Cruz 2015, 2019; Macabago *et al.* 2020a) from the early annotated list of 107 species (Reynolds 1981). Many of these myxomycetes were observed and reported from usual substrates of aerial and ground leaf litter, twigs, and tree barks which are often used for setting moist chamber cultures. Occasionally, woody vines (Rea-Maminta *et al.* 2015; Isagan *et al.* 2020; Macabago *et al.* 2020b; Pecundo *et al.* 2020, 2021), grass leaf litter (Carascal *et al.* 2017), and dead inflorescences (Pecundo *et al.* 2017; dela Cruz *et al.* 2021) are collected and observed for the presence of myxomycetes. Recent studies in the country had also looked at usual leaf litter substrates and woody materials from unique habitats, e.g., mangrove forests (Lim *et al.* 2021) and beach forests (Cabutaje *et al.* 2021). The Philippines with its tropical climate and high plant endemism could offer many other substrata that can be explored for myxomycete diversity.

In addition to the common aerial and ground leaf litter, barks, and twigs, other interesting substrata were surveyed for myxomycetes in this study. These microhabitats included woody vines and dead inflorescence, and some specific substrates such as decayed fruits and leaf litter derived from epiphytic plants, the endemic tree fern *Pronephrium bulusantum*, and the tropical plant *Pandanus*. A total of 25 species belonging to 12 genera were recorded, albeit most of these species were previously observed in other explorations in the Bicol Peninsula and the Philippines (Table 1). Four species, *Comatricha alta*, *C. anomala*, *Stemonitis smithii*, and *Trichia botrytis*, are new additions to the Bicol Peninsula.

Among the recorded myxomycetes, *Arcyria cinerea* was recorded in all microhabitats. *Collaria arcyronema* and *Perichaena depressa* were reported from nine substrates while *Cribraria violacea* and *Diderma effusum* were from seven microhabitats. Interestingly, *Didymium floccosum* and *D. iridis* were observed only on leaf litter of *Pandanus*. Pandans are commonly found in the tropics including the Philippines where 48 *Pandanus* species are so far recorded, many of which are endemic to the country, and therefore would be ideal plant materials to explore for substrate-specific myxomycetes. Seven species were from leaf litter samples of the tree fern *Pronephrium bulusantum*, an endemic plant to the sampling locality, while eight were from leaf litter samples of epiphytic plants. *Perichaena pedata* and *Trichia botrytis* were observed only from inflorescences. While we recorded 14 species from tree bark, common corticolous myxomycetes such as *Echinostelium* and *Licea* were absent from our study, albeit these species were previously reported from the bark of *Swietenia macrophylla* and other dipterocarp trees (Policina and dela Cruz 2020a, 2020b). Nevertheless, our findings illustrate the successful use of moist chamber culture techniques for different plant materials as an excellent tool for myxomycete cultivation. The number of species are expectedly will go higher as the moist chamber productivity and the sampling effort

for this study corresponded only to roughly 66%. The most productive substrate type was woody vine with 93% MC productivity from 40 MCs, but the observed highest taxonomic diversity was for the dead leaves of epiphytic plants (TDI = 1.1).

The highest species diversity in terms of the Fisher's alpha index (FAI = 18.60) and reciprocal of Simpson's index (SDI = 9.78) was noted for the inflorescence. Schnittler and Stephenson (2002) noted inflorescences as an excellent substrate for myxomycetes with their observation of 31 species. They also stated that one of the factors that may affect the richness of myxomycetes in inflorescence is the nectar residuals that may promote the growth of yeasts and bacteria. These microorganisms serve as food for the myxomycetes. Different inflorescences from different plant species would be a worthy of future exploration for myxomycetes, particularly in the tropics, and/or when specificity for microhabitats will be considered.

Decaying fruits are teeming with decomposing bacteria on which myxomycetes can feed, and therefore, would ideally be an excellent candidate as microhabitats for slime molds. However, decayed fruits are seldom studied for myxomycetes and are not often collected during field surveys. Parente and Cavalcanti (2013) did not observe the presence of myxomycetes from their moist chambers with fallen fruits of the palm tree, *Attalea speciosa* Mart, but recorded species from other plant parts. On the contrary, Stephenson et al. (2019) reported myxomycetes from fruits of *Faidherbia albida* and *Argemone ochroleuca* and from fruit pods of *Moringa ovalifolia* and *Rogeria longiflora*. In our study, we observed seven species and six genera from our moist chambers with decayed fruits and was also considered as taxonomically diverse. Our collected decayed fruits also gave the second highest species diversity based on the Fisher's Alpha (FIA = 14.49). Our results also showed a high diversity for tree barks (SID = 9.72, FAI = 11.71) and aerial leaf litter (SID = 7.37, FAI = 10.90). It was previously reported by dela Cruz et al. (2014) that AL harboured high species diversity in the tropics.

Comparing the assemblages of myxomycetes between the different substrata, we computed high similarity values between several microhabitats (e.g., leaf litter and decayed leaves of tree ferns) (Table 2). This is also supported by our cluster analysis (Figure 3). This was expected as these substrates had similar physical properties (e.g., moisture content, and pH) that support growth of myxomycetes. However, we did not observe the full clustering of similar types of substrata based on the presence or absence of myxomycetes. For example, aerial and ground leaf litter and the leaf samples of the tree ferns grouped with barks rather than the other leafy substrates (i.e., leaf samples of epiphytic plants and *Pandanus*). The assemblages of myxomycetes observed from the leaf materials of *Pandanus* were most different from the other substrates leading to its separation from the other clusters. The other two woody substrates, i.e., twigs and woody vines, had high similarity in species composition with seven shared species, but formed a cluster with decayed fruits, inflorescence, and leaf litter of epiphytic plants. Spore dispersal is a major factor that can affect the distribution of myxomycetes among these substrates.

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