THE OCCURRENCE
OF MYXOMYCETES
FROM A LOWLAND
MONTANE FOREST
AND AGRICULTURAL
PLANTATIONS OF
NEGROS OCCIDENTAL,
WESTERN VISAYAS,
PHILIPPINES

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- abundance
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- diversity

ABSTRACT

Higher floral and faunal biodiversity is expected in multi-species-covered mountainous forests than in mono-typic agricultural plantations. To verify this supposition for cryptogamic species like the plasmodial slime molds, a rapid field survey was conducted for myxomycetes and substrates in forest floor litter and agricultural plantation were collected in Negros Occidental, Philippines. Morphological characterization identified a total of 28 species belonging to the genera Arcyria, Ceratiomyxa, Collaria, Comatricha, Craterium, Cribraria, Diderma, Didymium, Hemitrichia, Lamproderma, Physarum, Stemonitis, Trichia and Tubifera. The myxomycete species Arcyria cinerea was the only abundant species found both in the agricultural and forested areas. The majority of collected species were rarely occurring. In terms of species composition, more myxomycetes were recorded in the mountainous forest (27) compared to agricultural sites. Furthermore, aerial leaf litter collected in the forests had the highest number of records for fruiting bodies but in terms of species diversity, twigs yielded higher value based on Shannon index. Findings in this study verify that a habitat with more heterogenous plant communities yields higher species of myxomycete assemblages. This research is the first study to report myxomycetes from Negros Occidental.

INTRODUCTION

Myxomycetes, commonly known as true slime molds, are acellular, phagotrophic, eukaryotic organisms under the Kingdom Protista (9). These organisms have been known to exhibit both fungal and protozoan characteristics but through an amoeboid phase, they feed on other microorganisms, including bacteria and yeast (9). These microorganisms are distributed worldwide and usually occur on dead substrata such as bark, twigs, and dried leaves of plants (29). Several studies regarding the taxonomy and ecology of myxomycetes have been conducted but most of this research was carried out in temperate regions, such as North America (35, 36), South America (16), and Europe (8,10). Numerous studies have also been completed in the tropics, such as Costa Rica (30), Puerto Rico (23) and Mexico (17). Despite the number of the studies that were executed and the high potential of biodiversity in tropical systems, little is known about them particularly in the tropical Southeast Asia such as the Philippines.

Thus, myxomycete profiling in the Philippines is still considered incomplete. As a result, there is limited knowledge on the ecology and taxonomy of myxomycetes found in such tropical areas where there are abundant forested areas serving as excellent habitats for myxomycetes (25). Previous studies on Philippines myxomycetes in the late 1970s and early 1980s encompassed the most comprehensive listing for the country (24). However, most of these publications during that time were merely extensive annotated lists. But in recent years, myxomycete diversity and ecology studies in the Philippines has progressed, as several reports have accounted on myxomycete distribution and occurrences in several habitat types, e.g. in selected forest parks (7, 19), in coastal forests (12, 18), and lowland mountain forests (3,4). These papers surveyed different forest habitats in the Luzon main island, which is just a small portion of the large archipelagic geography of the

Philippines. No previous reports had ever documented myxomycetes on the scatter islands of the Visayas region, or reported myxomycete occurrence in large agricultural plantations in the country. As such, the findings from this research paper on myxomycete composition in lowland forests will serve as a baseline reference for the profiles of myxomycetes in a local scale of Negros Occidental, but will also contribute to understanding their distribution in the whole of the Philippines.

Thus, the objectives of this paper are to (1) collect myxomycetes using opportunistic sampling methods in the field, and moist chamber cultures; (2) determine the collected species of myxomycetes; (3) measure the sampling strategy used in the survey; (4) assess the occurrence of each myxomycete species; (5) calculate the species diversity of the myxomycetes from the different substrates; and (6) compare the similarities of myxomycete assemblages between mountainous forests and agricultural plantations.

MATERIALS AND METHODS

STUDY SITES AND ITS COLLECTING LOCALITIES

Field survey and substrate collection were carried out during May 2013 in Negros Occidental, Western Visayas. The province is part of the whole Negros Island, the third largest island in the Philippines. It is estimated that the province is approximately 375 kilometers long from north to south with basically volcanic vegetation, making its arable land ideal for cultivation of economically important crops, especially sugarcanes (http://www.negros-occ.gov.ph). Based on the Philippine Atmospheric, Geophysical and Astronomical Services Administration — Department of Science and

Technology (PAG-ASA DOST) climatological data, the whole area is characterized as having two distinct seasons: dry from December to May and wet from June to November. Along the study area, two different habitat types, namely, a lowland forested area and an agricultural plantation were chosen. The descriptions of each habitat types and its collecting localities (Fig. 1) are further described below.

A. Forested Areas (Mt. Kanlaon National Park, 10° 24.787N, 123° 07.982E). This area rises to a height of 2,465 m (7987 ft.) and is located in the province of Negros Occidental, Western Visayas. It is characterized by low serrated mountain ranges. The forest can be

described as a moist tropical disturbed rainforest dominated with large dipterocarp trees and non-vascular plants. Within this forest area, six sites were randomly selected along an established 1000 m accessible forest trail that serves as a transect.

B. Agricultural Area (Sugarcane Plantations, Saccharum officinarum). Only sugarcane plantations along the road in the northwest part of the province were selected for this study. Three collecting localities characterized as dry and with extensive light exposure were selected to collect substrates that were subjected for moist chamber cultures. Dried substrata are ideal for the preparation of moist chambers, as these substrates retain spores better. The collecting localities are: Silay City (SC1, 10°46'46.18"N 123°0'24.80"E), Bacolod City (SC2, 10°42'29.30"N 122°58'56.72"E), and Bago City (SC3, 10°32'37.29"N 122°51'47.51"E).

FIELD COLLECTION OF MYXOMYCETE SPECIMENS

Fruiting bodies of myxomycetes directly observed in the field were immediately placed in clean compartmentalized plastic collecting boxes. These specimens were brought back to the laboratory and after several days of air drying, the specimens was glued in herbarium trays and placed inside matchbox–sized herbarium boxes for permanent storage.

MOIST CHAMBER PREPA-RATION FROM SUBSTRATE COLLECTED IN THE TWO HABITAT TYPES

Ninety samples each of ground leaf litter (GL), aerial leaf litter (AL), twigs (TW), 60 samples each of ferns (F), and 30 samples of vines (V) from mountain forests, and 90 sugarcane leaf litter (SC) from agricultural plantations were collected, accounting

for a total of 450 substrates used for this study. The collected substrates were placed inside dry paper bags, labeled, and transported back to the laboratory. Collection of samples was done following the methods described by Stephenson (36). Samples of ground floor litter were gathered at 3-5 m regular intervals. These samples consisted of mixtures of leaves. Twigs < 1.0 cm in diameter in size were also collected. Samples of aerial litter were collected from dead twigs or leaves still attached to branches of plants and trees. The specimens were wrapped gently in paper before being transported to the laboratory, where they were placed in small boxes for storage to prevent insects from getting into the samples. The samples were air dried for one week to prevent the growth of molds. To avoid pseudoreplication, a single moist chamber (MC) was prepared for each substrate collected. The moist chambers used consisted of disposable plastic Petri dishes, 10 cm in diameter and 4 cm deep, lined with filter paper. Samples were moistened with sterile distilled water. After 24 h, excess water was removed up to the point adequate enough for the chamber to be moist, and the pH of each of the substrate was checked using a pH meter (Sartorius PB-11). Following the incubation conditions of Dagamac et al. (3), moist chambers were kept at room temperature (22-25°C) in diffuse daylight. When necessary, a small amount of water was added to each culture to maintain moist conditions.

DETERMINATION OF MYXOMYCETE SPECIES

The specimens obtained from the moist chamber cultures were identified using a dissecting microscope three times per week (two day intervals) for a period of up to three months, by comparing the color, size, and structure of the myxomycete plasmodia, types of fruiting bodies (e.g. sporangium, aethalium, pseudoaethalium, and plasmodiocarp), and spores of myxomycetes in the descriptions stated in the standard monographs of myxomycetes (21, 32). Web-based electronic databases, e.g. Eumycetozoan Project (http://slimemold.uark.edu), were also utilized for verification of some morphological features. Nomenclature used for the identified myxomycetes follows the names used in Nomenyx (http://nomen.eumycetozoa. com). For specimens that could not be fully identified with strong certainty due to some malformed specimens but with distinguishing character enough to separate as a species, the abbreviation "cf" was used in the taxon name. All specimens listed herein are deposited in the myxomycete herbarium of the Fungal Biodiversity and Systematics Group of the Research Center for the Natural and Applied Sciences at the University of Santo Tomas in Manila, Philippines.

EVALUATION OF DATA

To evaluate the sample effort of the myxomycete survey in this study, an individual-based rarefaction curve was established. Using the rarefaction formula that computes for a number of estimators for species richness of the free downloadable program EstimateS (Version 9, Colwell 2013, 100 randomizations), species accumulation curves were initially constructed. In accordance with Unterseher et al. (39), the Chao2 estimator was then chosen as the best estimator to use and was calculated using the classical settings of EstimateS. The estimated value for the sampling effort in the study area was then determined using the formula of Ndiritu et al. (22) by dividing the actual number of species recorded by the mean number of species expected as estimated by the Chao 2 estimator. Additionally, a hyperbolic regression in the form of Coleman rarefaction curve according to the Michaelis-Menten formula y = ax/(b+x), where x represents

the number of samples, y is the number of species recorded and the parameter a giving an estimate for the maximum number of species to be expected at this kind of substrate resulting in a very close curve shape (Magurran 20) was applied to the dataset.

For the assessment of species occurrence of myxomycetes, species composition was initially determined for the collection site. Occurrence refers to the frequency of the presence of a particular species of myxomycetes in a positive MC. A moist chamber positive for having a fruiting body of a particular species was considered as one positive collection. A collection was then considered as a single unit. The number of collections reflects the abundance of myxomycetes in Negros Occidental and was expressed as relative abundance. The relative abundance for every species of myxomycetes were then calculated and reported as Abundance Index (AI) by Stephenson et al. (1993). Each species were then categorized as: (1) abundant if their relative abundance (RA) is>3% of the total collections, (2) common if RA is >1.5% but <3% of the total collections, (3) occasionally occurring if RA is >0.5% but <1.5% of the total collections, and (4) rare if the myxomycetes had an RA of < 0.5% of the total collections.

To further determine the myxomycetes diversity for the different substrates, species diversity was also calculated using three different diversity indices provided in Magurran (20). Shannon diversity index (HS) measures species diversity with respect to both species evenness and richness. This index assumes that individuals are randomly sampled from an infinitely large community and that all species are represented in the sample (14). The Gleason Index (HG) measures the species diversity in relation to species richness. Richness is defined as the number of different species

found in a biota. Pielou's species evenness index (E), on the other hand, quantifies how equal communities are in a given sampling area. These indices are computed as follows:

Equation 1: Shannon Index of Diversity (HS) = -_i(pi ln pi), where pi = the total number of individuals in the ith species.

Equation 2: Gleason Index (HG) = Np - 1/ln Ni, where Np = the total number of species and Ni = the total number of individuals in the ith species.

Equation 3: Pielou's index of species evenness (E) E = HS/Hmax where HS = Shannon Index of Diversity and Hmax= the maximum value of HS.

The similarities of myxomycete assemblages between the mountainous forest and agricultural plantation were also compared by Sorensen's coefficient of community index and the Percentage Similarity index. The equation for Sorensen's coefficient is based on the presence or absences of species.

Equation 4: Coefficient of Community (CC = 2c/(a+b)), where a = total number of species in the first habitat, b = total number of species in the second habitat, and c = no. of species common to both habitat.

The value of CC ranges from 0-1 where 0 is if there are no species present in both habitat and 1 when all species are present in both habitat. On the other hand, the Percentage Similarity (PS) index considers not only the presence or absence of species but their relative abundance. The PS value was computed as follows:

Equation 5: PS = Σ min (A, B, ... X) where min = the lesser of the two percentage compositions of species A, B, C, ... X in the two communities.

RESULTS

Using the combined opportunistic sampling in the field and moist chamber culture preparation, a total of 193 records of myxomycetes were noted for this survey. From these 193 records, 42 were fruiting body records in the field and 151 were recovered either as plasmodia or fruiting body records in the moist chamber cultures. In terms of the field survey, there were no field specimens that were observed in the agricultural plantations. Moreover, a higher yield of myxomycetes was noted among moist chambers in forest litter than agricultural litter. Only two bright-spored myxomycetes species (Arcyria cinerea and Tubifera ferruginosa) were recorded in the sugarcane litter.

A total of 32 morphospecies were identified from the 193 myxomycete records. However, four of these species were only determined to the genus level (Arcyria, Comatricha, Didymium, and Stemonitis) because they were recovered from moist chambers wherein most of the fruiting bodies were already withered. The list of species presented hereafter has a total of 28 species belonging to 14 genera. To evaluate the sampling effort used in this study, an individual based species accumulation curve was constructed using the software estimates and showed that the mean Chao 2 estimator reached a constant value of 59 (Fig. 2). Using the formula of Ndiritu et al. (22) to calculate the exhaustiveness of the sampling effort for the whole study, our results gave us a computed sampling effort of 54.2% for the present study.

Assessing the occurrence of the 28 determinable myxomycete species, two species were reported to be abundant,

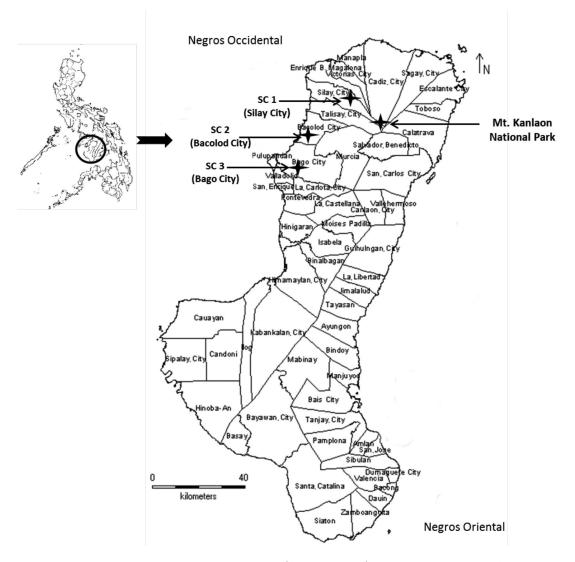


Fig. 1. Study sites: Mt. Kanlaon National Park (forested areas) and the agricultural plantation (SC) in Negros Occidental, Western Visayas, Philippines, May 2013.

namely Arcyria cinerea and Didymium nigripes. Nine species were common, four were occasional and 13 were reported to be occurring as rare (Table 1). Comparing the composition of myxomycetes from the different substrates collected from two habitat types, 27 species were found in forested areas that were characterized to have heterogenous plant litter and only two species were accounted in the

sugarcane plantations.

In terms of productivity of the microhabitats tested in this study by using the moist chambers, 121 of the 450 MCs (27%) were positive for growth of myxomycetes either as plasmodia or as fruiting body. All of the moist chambers prepared had a relatively acidic mean pH condition. Highest percent yield

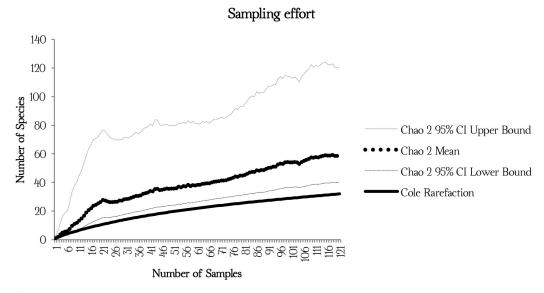


Fig. 2. Individual based species accumulation curve smoothed by Cole rarefaction for the myxomycetes collection in Negros Occidental.

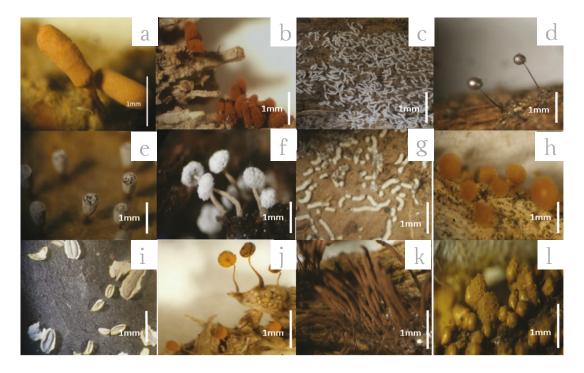


Fig. 3. Some representative myxomycetes collected in Negros Occidental: (a) Arcyria cinerea, (b) Arcyria denudata, (c) Ceratiomyxa fruticulosa, (d) Collaria arcyrionema, (e) Craterium leucocephalum var. cylindricum, (f) Didymium squmolosum, (g) Diderma effusum, (h) Hemitricha calyculata, (i) Physarum bogoriense, (k) Stemonitis fusca, and (l) Trichia decepiens.

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Table 1. Occurrence of myxomycetes in Negros Occidental showing the number of records accounted from the rapid field survey and the use of moist chamber cultures

	Number of Records							
Species	Field	Moist Chamber Cultures						Total
	Collection	AL	GL	F	TW	VN	S	
Abundant								
Arcyria cinerea (Bulls.) Pers.	1	25	10	10	5		2	53
Didymium nigripes (Link) Fr.	4	2						6
Common								
Arcyria denudata (L.) Wetts.	4							4
Ceratiomyxa fruticulosa (O.F. Mull.) Macbr.	3				2			5
Craterium leucophaeum var. cylindricum (Pers ex J. F. Gmel)								
Ditmar	3							3
Diderma effusum (Schwein.) Morgan	2	2				1		5
Hemitrichia calyculata (Speg.) M.L. Farr.	4							4
Lamproderma scintillans (Berk& Broome.) Morgan.	1	1			2			4
Physarum album (Bull.) Chevall.	3	1						4
Physarum viride (Bull.) Pers.	2		2		1			5
Stemonitis fusca Roth	1	1			1			3
Occassional								
Cribraria cancellata (Batsch) Nann. – Bremek.	2							2
Cribraria microcarpa (Schrad) Pers.	2							2
Didymium iridis (Ditmar) Fr.	1	1						2
Physarum melleum (Berk. & Broome.) Massee.	2							2
Rare								
Arcyria afroalpina Rammeloo		1						1
Collaria arcyrionema (Rostaf.) Nann. – Bremek. Ex Lado.					1			1
Comatricha nigra (Pers. Ex J. F. Gmel) J. Schroet	1							1
Didymium squamolosum (Alb. & Schwein.) Fr.	1							1
Hemitrichia serpula (Scop.) Rostaf. Ex Lister.	1							1
Physarum bogoriense Racib.	1							1
Physarum cinereum (Batsch) Pers.						1		1
Physarum compressum Alb. & Schwein.	1							1
Physarum echinosporum Lister				1				1
Physarum nucleatum Rex	1							1
Stemonitis axifera (Bull.) T. Macbr.	-				1			1
Trichia decepiens (Pers.) T. Macbr.	1				-			1
Tubifera ferruginosa (Batsch) J.F.Gmel.	-						1	1

was observed from the aerial litter (72%), and consequently had the most number of records of myxomycetes (Table 2). The other substrates, such as the twigs and vines had the next highest number of percent yield. However, these substrates had a relatively low number of determinable records due to the fact that most of the substrates were recorded as positive culture because of the appearance of plasmo-

dium during the incubation period. These MCs were unsuccessful in developing into fruiting bodies (Table 2). Lowest percentage yield (18%) was observed in sugarcane litter randomly collected in the agricultural plantations of the study area. Moreover, among the six substrates collected in the two types of habitats (forest and agricultural), twigs had the highest species diversity as calculated using Gleason index (Hg

=2.12) and ferns had the highest species evenness based from Pielou's Evenness index (E =1.00). However, using the Shannon index that considers species diversity and species evenness, twigs gave the highest value (Hs = 0.75). Comparing the assemblages of myxomycetes between the two habitat types, a CC value

of 0.08 and PS value of 0.48 were computed in the study (Table 3). These results show that species similarities between the two sites were only 8.3%, which is relatively low since the only species of myxomycetes that was present in both sites was Arcyria cinerea.

Table 2. Statistics of the different substrate types used in the moist chamber

Substrate Type	Cultures prepared	Positive for myxo	Percent Yield	Number of determinable records	mean pH value	Hg	E	Hs
Mountainous forest								
Aerial litter	90	65	72	34	5,82	1.99	0.31	0.47
Ground litter	90	19	21	12	5,99	0.40	0.18	0.13
Twigs	90	35	38	13	5,86	2.12	0.61	0.75
Vines	30	11	37	2	5,68	1.44	0.10	0.30
Ferns	60	13	22	11	6,20	0.42	1.00	0.13
Agricultural plantation								
Sugarcane	90	16	18	3	5,91	1.44	0.50	0.15

DISCUSSION

In terms of biodiversity, the Philippines is considered to be one of the most diverse countries in the Asia Pacific basin. However, microbial diversity assessments in the country are under-investigated. This particularly holds true among the less explored fungus-like protists like the myxomycetes where a generally tropical condition would seem to be favorable for their growth and development (15). In fact, in recent years, most investigations on myxomycetes in the Philippines were concentrated only among the forest vegetation and coastal habitats of the Luzon main island (3,18). Thus, findings in this research paper are the first intensive diversity report for the Visayas group of islands of the Philippine archipelago.

PRODUCTIVITY OF MYXOMYCETES IN MOIST CHAMBER CULTURES

The use of a moist chamber culture in assessing the occurrences of myxomycetes was a vital component for this study. Current studies from arid environments in China (28) and submerged plant materials in the Big Thicket National Preserve (40) employed the usage of this technique to recover species of myxomycetes not easily seen on the field. In our study, a total of 450 moist chambers were prepared wherein only 27% were positive for myxomycete growth. After almost 15 weeks of incubation, 11% showed positive results for fruiting bodies, while 16% were positive for plasmodial growth. This now shows that the majority of

the plasmodium specimens were not able to develop to fruiting bodies. In comparison to related local studies, a similarly low yield was observed by Dagamac et al. (5), wherein 17.5% yielded plasmodia and only 5.1% yielded fruit– ing bodies from different bark samples collected from the Luzon Islands. However, Kuhn et al. (13) had a percentage yield of 51%, or 214 out of 420 moist chamber cultures containing 40% positive for plasmodia and 23% positive for fruiting bodies in six highland areas in Luzon. Substrates collected in a protected ecopark by Macabago et al. (19) had a percentage yield of 51%, or 121 out of 240 moist chambers. It seems now that most studies of myxomycetes in the Philippines that used moist chambers always supported a higher level of plasmodium yield than recovering fruiting body phenologies. Perhaps the fast dessication of most of the moist chambers during the incubation time can be a factor here, since a suitable moist environment is needed to allow for the plasmodium to successfully develop into fruiting bodies. It is important to note that in doing myxomycetes biodiversity and distribution studies by means of the moist chamber technique, fruiting bodies are more important as compared to plasmodium or sclerotia, since most species identification is based on the determinable morphologies of the fruiting body (11, 31). Moreover, among the six substrates collected in our study, aerial leaf litter yielded the highest level of success. The highest productivity yield from aerial leaf litter was also recorded from other studies in the tropics, including Rojas & Stephenson (27) who reported 93% in the Coco's Island, Costa Rica, and in a more recent comparative species listing of dela Cruz et al. (6) between substrates collected in the tropics and the temperate ecoregions. This may be attributed to specimen exposure to open air where aerial litter has a higher potential in catching spores. This supposition was already demonstrated by the studies from Schnittler & Stephenson (30) where the authors noted that slight breeze can cause the myxomycetes spores to be dispersed

more than one kilometer from the starting point. Perhaps aerial litter from our study has a higher probability to trap spores dispersed by wind.

SPECIES COMPOSITION OF MYXOMYCETES IN NEGROS OCCIDENTAL

In this study, 28 morphospecies of myxomycetes were collected from lowland montane forests and sugarcane plantations in the northern part of Negros Occidental. This number is similar in comparison to other lowland montane vegetation area studies conducted in the Luzon main islands, including Mt. Arayat National Park (3) and in Mt. Makulot (1), which reported 30 and 28 morphospecies of myxomycetes, respectively. Albeit this number of morphospecies is not yet reflective of the overall number of myxomycetes that can be accounted in Negros Occidental as was suggested by the 54.2% sampling effort for this study, findings from this research paper serve as a good starting basis for future directives in understanding the distribution of myxomycetes in a local setting. To expand the sampling effort, it is recommended to increase the distance covered during intensive surveys and to add other substrates, i.e. barks of living deciduous trees, dung of herviborous animals, and inflorescences where myxomycetes can also thrive.

In terms of species composition in the whole study area, Arcyria cinerea was noted to be the only abundant species found in both the agricultural and forest habitats, with the other species occurring relatively rarely. Stephenson (35) had the highest percentage yield of A. cinerea in moist chambers (85% in the upland temperate forest of Southwestern Virginia, USA). Similar results in terms of occurrence were also obtained from the studies conducted by Rojas et al. (26) in the northern Neotropics and

Kuhn *et al.* (12) in Anda island in Pangasinan, Philippines. Our findings now support other previous results that also showed *Arcyria cinerea* to be of cosmopolitan distribution worldwide, since it is widely known to be tolerant to many environments.

MYXOMYCETE DISTRIBU-TION IN AGRICULTURAL LIT-TER IS MORE LIMITED THAN IN FOREST LITTER

Most of the related studies on myxomycetes in the Philippines always used litter from the forest floor. To the best of our knowledge, the findings in this paper are the first report for the Philippines attempting to evaluate myxomycetes in a sugarcane plantation where the decaying litter and vegetation is generally specific and to compare it to the myxomycete communities in forest litter where decaying litter is more heterogenous. In contrast to related studies in the Paleotropics, our findings seems to contradict the observations of Tran et al. (38), which intensively evaluated distribution of myxomycete assemblages in agricultural ground litter and the forest floor. Their results showed a relatively higher productivity among agricultural litter than the forest floor litter during both the rainy and dry seasons. Perhaps the smooth surface of the sugarcane leaf is not

a favorable spore trap for other myxomycetes species in contrast to the pubescent surfaces of the three agricultural litters used in Thailand (banana, mango and corn plantations). Nonetheless, findings from our study supports the theory that diversities of plant communities and litter heterogeneity (37) in a study area influence the composition of myxomycete assemblages, as evident from a higher number of myxomycete occurrences in the forest floor litters in Negros Occidental.

MYXOMYCETES FROM NEGROS OCCIDENTAL AS BASELINE INFORMATION

An understanding of the distribution for myxomycetes in the Philippines is still far from complete. Many ecological factors and/ or unexplored landscapes in the country still need to be investigated. Despite the findings presented in this study, it is still significant to note the limitations of a descriptive study like this are associated with the sampling efforts in collecting the substrata used in the study. The most noteworthy contribution of this paper relates to the fact that it increases the knowledge about the local ecology of myxomycetes in an ecoregion of the world where investigations about myxomycete diversity is still considered to be in its infancy.

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