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Myxomycetes associated with grassland litter in the Philippines

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Abstract

Myxomycetes are homogenous group of eukaryotic fungus-like protists that are commonly associated with any decaying plant materials. Since no report of myxomycete association in grass litters were reported for the Philippines, a myxomycete survey employing moist chamber technique was conducted from 300 grass litter samples collected from ten different grassland areas in the country. Our results showed that 81% of the moist chambers yielded myxomycetes either as fruiting bodies or plasmodia. Species of myxomycetes identified in this study included the genera *Arcyria*, *Badhamia*, *Collaria*, *Comatricha*, *Cribraria*, *Diderma*, *Didymium*, *Lamproderma*, *Perichaena*, *Physarum* and *Stemonitis*. These species can easily be dispersed by wind and hence, their presence in the collected substrata. The cosmopolitan species *Arcyria cinerea* and the common grass myxomycetes *Physarum cinereum* were reported to be abundant among the myxomycetes recorded. Overall, the study presented the myxobiota present in tropical grasslands and grass patches found in the Philippines.

Key words – microhabitats – leaf litter – slime molds – species distribution

Introduction

Myxomycetes, also referred to as myxogastrids or plasmodial slime molds, are known scavengers in decaying plant materials where they engulf microorganisms as a primary source of nutrients. Through various environmental cues, its motile plasmodium phase sporulates to produce a fruiting body which describes their fungus-like character. Myxomycetes spores are widely distributed by air in various substrata such as aerial and ground leaf litter (Stephenson 1989) and twigs (Stephenson et al. 2008) substrates collected from areas with high metal content (Rea-Maminta et al. 2015) and even herbivore dung (Stephenson & Stempen 1994, Novozhilov & Schnittler 2008). Moreover, myxomycetes are known to thrive in places with extreme conditions such as Arctic tundra (Novozhilov et al. 1999) and desert (Lado et al. 2011). This clearly shows the high distribution of myxomycetes in the biosphere.

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The Philippines has approximately 1.5 million hectares of grasslands used primarily as a feed resource for the ruminant industries (Moog 2006). In addition, grasses, particularly cogon (*Imperata cylindrica*) cover approximately one to five million hectares of degraded land areas in the Philippines (Lasco et al. 2001). However, despite the dominance of grasses in most parts of the Philippines, its role in habitat rehabilitation and association with other organisms remain largely underestimated (Lasco et al. 2001). This is particularly true about the state of myxomycete research in grasslands in the country.

Despite numerous studies on the ecology and distribution of myxomycetes, relatively little is known about its association in grasslands. Only few studies utilized the actual grass litter as a substrate in myxomycetes isolation. Ing (1994) described several species of myxomycetes from various grass substrates but the extensive study on grassland myxomycetes was done by Rollins (2007) by documenting 18 myxomycetes species in a local temperate area in Thunderbasin National Grassland. A recent study on myxobiota of grasslands of Western Central United States reported a total of 59 myxomycetes species (Rollins & Stephenson 2013). Abundance of *Didymium difforme* in various grasslands of United States (Winsett & Stephenson 2011) and field sightings of the fruiting bodies of *Mucilago crustacea* (Mitchel 2007) in the grassland of the Vice County of West Cork in Ireland further highlights the importance of documenting myxomycetes from different ecoregions. Although previous studies presented extensive data regarding the diversity of myxomycetes in temperate grasslands, only a few to no study has been reported on the occurrence of myxomycetes in other grassland habitats, particularly in a paleotropical country like the Philippines. Thus, this study aims to (1) assess myxomycete diversity in the understudied grasslands and grassland patches in the Philippines, (2) determine the association of myxomycetes in a particular microhabitat such as grass litter, and (3) provide baseline information as a first report of myxomycete diversity and occurrence in tropical grasslands, particularly in the Philippines.

Materials & Methods

Study Sites

A total of ten sites in the Philippines were selected for this study. Two sites are from Central Luzon, six from Eastern Luzon, one from Eastern Visayas, and one from Southern Visayas (Table 1). The collection sites were about 1 km in diameter across the grasslands and grassland patches, with the actual samples coming from the random distant areas covered by the site.

Collection and Preparation of Substrates

From each of the ten study sites, 30 sets of ground grass litter samples were collected. The grass litter that were randomly gathered are dried, detached leaves and stalk of “talahib” (grass of the Genus *Saccharum*, particularly *S. spontaneum*) or “cogon” (grass of the Genus *Imperata*). These samples were placed in labelled paper bags with codes corresponding to the collection sites, the substrate type and the sample number. The collected substrates were further air-dried for 3–5 days before processing as moist chamber cultures.

Preparation of Moist Chamber and Identification of Myxomycetes

A total of 300 moist chambers (MC) were prepared by lining plastic Petri-dishes with clean filter paper. About one to two grams of the dried grass litter were placed in the MC and soaked with distilled water for 24 hours. The pH of the substrate in each plate was then measured using a standardized pH meter (Sartorius PB–11). Afterwards, excess water from each plate were drained. The moist chamber set-ups were maintained and observed for the growth of myxomycetes for 8–12 weeks under diffused light conditions at room temperature (24–28 °C). Growth of myxomycetes was recorded based on life stage present on the moist chamber (i.e., plasmodium, fruiting body, sclerotia). When fruiting bodies were present in MC, it was immediately preserved in a herbarium box as voucher specimen. Identification was done through morphological characters of the fruiting body as described using online database [i.e.: the Eumycetozoan Project (http://slimemold.uark.edu/)] and through spore characterization. Gross morphology was observed using a dissecting microscope, and characters such as size, shape, colour, arrangement and additional features of the fruiting bodies were noted. For the spore characters, a single fruiting body was teased in a lactophenol solution for dark spores or potassium hydroxide (KOH) for spores containing lime, and was observed using light microscope (Olympus CX21).

Table 1 Collection coordinates and habitat description of the ten sampling sites

Study Sites [Codes]	Coordinates	Grassland Description
Porac, Pampanga [PA1]	N15°07'10" E120°54'01" 87 m.a.s.l. (meters above sea level)	Lahar-covered grassland
Bacolor, Pampanga [PA2]	N15°00'00" E120°65'00" 30 m.a.s.l.	Roadside slope grassland patches
Caleruega Road, Batangas [BA1]	N14°19'01" E120°07'67" 960 m.a.s.l.	Hillside grassland patches
Fernando Airbase, Mataas na Kahoy, Batangas [BA2]	N13°57'37" E121°06'57" 500 m.a.s.l.	Open field/ Pastureland
Volcano Island, Taal, Batangas [BA3]	N14°07'00" E120°59'34" 311 m.a.s.l.	Taal Volcano area grassland
Pantabangan, Quezon Province [QU1]	N15°81'00" E121°14'00" 253 m.a.s.l.	Roadside grassland patches
Makiling, Quezon Province [QU2]	N13°15'63" E121°19'44" 520 m.a.s.l.	Slope grassland patches
Spillway view, Angat Dam, Bulacan [BU]	N14°43'20" E121°93'02" 367 m.a.s.l.	Angat Dam cliff grassland patches
Sto Tomas Road, Guimaras [GU]	S10°91'58" W122°30'48" 833 m.a.s.l.	Roadside grassland patches
Chocolate Hills, Bohol [BO]	S09°47'89" E124°01'04" 391 m.a.s.l.	Hilltop grassland

Ecological Analysis

The productivity of the moist chamber cultures was calculated to assess the ability of the chosen substrate or microhabitat (grass litter) to support growth of myxomycetes. Taxonomic diversity was computed by dividing the number of myxomycetes species with the number of genera present in the given site. Relative abundance of each myxomycetes species were also determined by dividing the number of records of a particular species with the total collection (total number of records of all species). The values obtained were assigned with Abundance Index (A. I.) indicator based from Stephenson et al. (1993). Particular species were indicated as rare if it has a value of < 0.5% of the total collection, occasional if the value is > 0.5% but not exceeding 1.5% of the total collection, common if the value is > 1.5% but not exceeding 3% of the total collection, and abundant if the value is > 3% of the total collection. Based from the relative abundance, species diversity (Shannon Index of Diversity [HS], Gleason Index of Species Richness [HG], Pielou's Index of Species Evenness [E]) were calculated using the computer software Estimate S and SPADE.

Results

A total of 244 MC yielded positive for myxomycetes. This represents 81% of the all the moist chamber set-up (Fig.1). There are 312 records obtained from the study which represented a total of 26 myxomycetes species from 12 different genera, namely, *Arcyria, Badhamia, Collaria, Comatricha, Cribraria, Diderma, Didymium, Hemitrichia, Lamproderma, Perichaena, Physarum,* and *Stemonitis*. Of the recorded taxa, *Arcyria cinerea, Arcyria globosa, Comatricha tenerrima, Diderma hemisphaericum, Perichaena chrysosperma, Perichaena depressa,* and *Physarum cinereum* were found to be abundant in the overall study each comprising of greater than 3% of the total collection. *Arcyria cinerea* and *Physarum cinereum* were the species present in all of the study sites, with the exception of BA1 for *P. cinereum*. Some myxomycetes species were collected only in a particular study site such as *Stemonitis fusca* in GU, *Badhamia affinis* in PA2, *Cribraria microcarpa* in BA1, *Cribraria violaceum* in BO and *Didymium minus, Perichaena corticalis, Physarum compressum, Perichaena pedata* and *Physarum leucophaeum* in BU (Table 2).

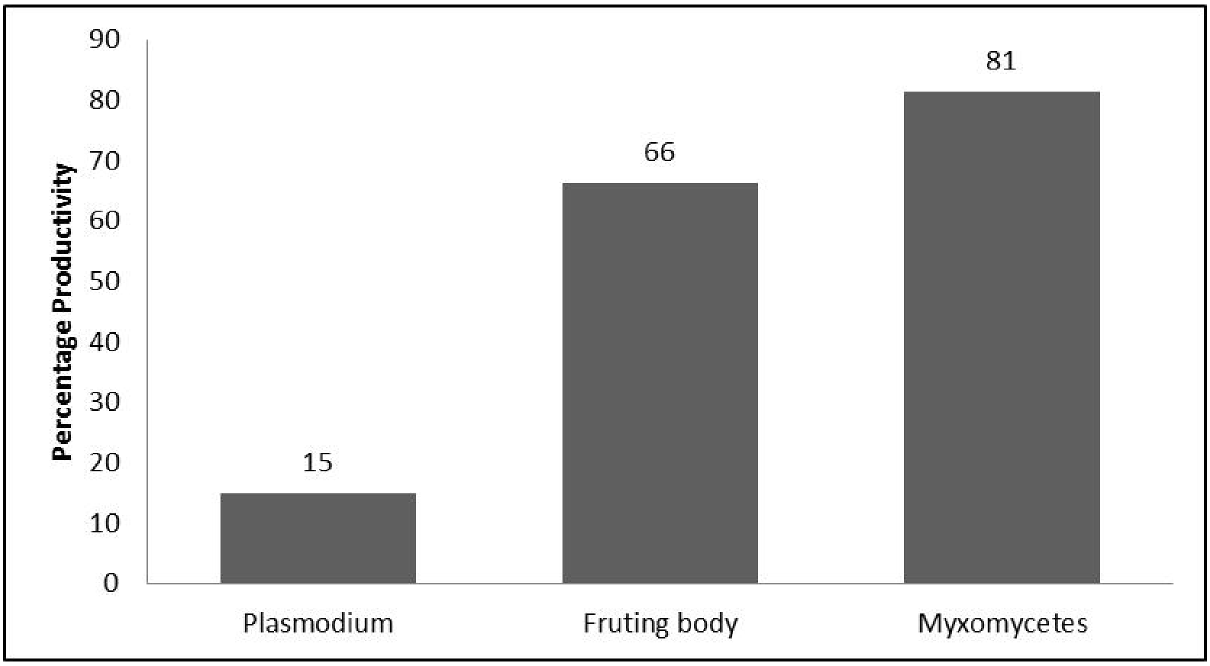

Fig. 1 – Percentage productivity of myxomycetes in moist chamber cultures of grass substrate.

In terms of diversity in each study site, BA1 and GU was taxonomically diverse among the other study sites (S/G=1.17). On the other hand, a comparison of the species diversity for each study sites showed that BA2 had the highest species diversity (Hs=0.98) and GU had the highest species evenness (E=0.65) (Table 3).

Discussion

The study yielded a relatively high percentage (81%) of moist chambers positive for myxomycetes either as plasmodium (15%) or fruiting body (66%). This may indicate that the grass substrates are utilized as microhabitat for myxomycetes especially in grasslands and other habitats dominated by these plant species. This is supported by previous studies that reported grass litter as a possible substrate for myxomycetes growth (Flentje & Jeffery 1952, Agnihothrudu 1955, Muchovej & Muchovej 1987, Ing 1994, Rollins, 2007).

The abundant myxomycetes recorded in this study were previously reported as cosmopolitan in distribution such as *Arcyria cinerea* (Stephenson et al. 2004, Ndritu et al. 2009, Dagamac et al. 2012), *Perichaena depressa* and *Physarum cinereum* (Stephenson et al. 1989, Ndiritu et al. 2009). In relation to grassland habitats, *Didymium squamulosum, P. depressa, P. cinereum* and *Stemonitis fusca*, all of which are reported in the study, were noted by Rollins & Stephenson (2013) as included in the core myxomycetes biota of temperate grasslands. Similarly, *D. squamulosum* and *S. fusca* were reported to be associated to grass habitats (Rollins 2007). *P. cinereum* was previously reported as the most common grass lawn species (Ing 1994) and a common myxomycetes in *Papsalum* sp. grass in Brazil (Muchovej & Muchovej 1987). This was corroborated by the result of this study by reporting *P. cinereum* as an abundant myxomycete in grass litter in almost all of the study sites (except BA1). *Hemitrichia minor* (syn. *Perichaena minor*) was reported to be the most abundant species in Black Hills, South Dakota which used grass substrates in moist chamber culture (Gabel et al. 2010). In this study, *H. minor* was an occasionally occurring species in grass substrates and is only found in two sites (QU1 and BO).

Table 2 Relative abundance of the different myxomycetes species per study site.

Taxon	PA1	PA2	BA1	BA2	BA3	QU1	QU2	BU	GU	BO	ALL
Arcyria cinerea (Bull.) Pers.	A	A	A	A	A	A	A	A	A	A	A
A. denudata (L.) Wettst.									A		R
A. globosa Schwein.				A	A	A				A	A
Badhamia affinis Rostaf.		A									R
Collaria arcyrionema (Rostaf.) Nann.-Bremek.				C				A	A	C	C
Comatricha nigra (Pers. ex J.F. Gmel.) Schroet.		A	A	A							C
C. tenerrima (M.A. Curtis) G.Lister	A	A	A	A						C	A
Cribraria microcarpa (Schrad.) Pers.			A								R
C. violacea (Schrad.) Pers.										A	O
Diderma effusum (Schwein.) Morgan	A							A			C
D. hemisphaericum (Bull.) Hornem.					A	A		A			A
Didymium minus (Lister) Morgan								A			O
D. nigripes (Link) Fr.	A		A								O
D. squamolosum (Alb. & Schwein.) Fr.				C	A			A		C	C
Hemitrichia minor Minakata						A				C	O
Lamproderma scintillans (Berk. & Broome) Morgan			A	C				A	A	C	C
Perichaena chrysosperma (Currey) Lister	A			A	A	A	A	A		A	A
P. corticalis (Batsch) Rostaf.						A					R
P. depressa Libert	A	A		A	A			C			A
P. pedata (Lister & G. Lister)G. Lister								A			O
Physarum cinereum (Batsch)Per	A	A		A	A	A	A	A	A	A	A
P. compressum Alb. & Schwein.								A			O
P. leucophaeum Fr.								C			R
P. melleum (Berk. & Broome) Massee	C	A						C		C	O
Stemonitis fusca Roth	C			C	A		A		A	C	C
S. herbatica Peck			A				A				O

Mucilago crustacea, regarded to be the most common myxomycetes in grass habitats (Ing 1994, Novozhilov et al. 1999, Mitchel 2007), and *D. difforme*, reported to be a common myxomyetes in grass litter (Rollins 2007, Winsett & Stephenson 2011), were not reported in this study which may be attributable to specificity of thriving myxomycetes based on ecoregions, e.g. temperate, tropical, and arid regions. However, further sampling is required to support this claim.

Some of the species reported for the first time in grass substrates are *Badhamia affinis*, *Perichaena pedata*, *Cribraria microcarpa* and *C. violaceum*. Several *Badhamia* sp. were reported in grass litter such as *B. gracilis* (Rollins 2007) and *B. melanospora* (Rollins & Stephenson 2013). In this study, *B. affinis* was reported to be abundant in PA2. This may indicate that the genus *Badhamia* could be a common component of grassland myxobiota both in temperate and tropical countries. The same status could be true for *Pericahena pedata*. This species was first recorded in the Philippines on leaf litter in Luzon (dela Cruz et al. 2011). This implies that *P. pedata* has the ability to occasionally thrive on grass litters in tropical environments. Species of the genus *Cribraria* such as *C. microcarpa* and *C. violaceum*, which are common myxomycetes of forest litters (Lado et al. 2005), were only recorded rare in the study, albeit only on a certain study sites

(BA and BO). This may indicate that these myxomycetes can also thrive on grassland litter. In addition, many of the species reported in this study were also reported in varied substrata in the Philippines, e.g. in aerial and ground leaf litter, twigs, barks, and woody substrates from different inland (Kuhn et al. 2013a, Dagamac et al. 2014, 2015, 2017, dela Cruz et al. 2014, Alfaro et al. 2015, Rea–Maminta et al. 2015, Pecundo et al. 2017) and island (Kuhn et al. 2013b, Viray et al. 2014, Macabago et al. 2012, 2016, 2017) forest habitats.

Table 3 Taxonomic and species diversity of myxomycetes in the ten study sites.

Study Site	**Number of Species**	**Number of Genera**	**S/G ratio[a]**	**Hs[b]**	**E[c]**
Porac, Pampanga [PA1]	9	7	1.29	0.79	0.49
Bacolor, Pampanga [PA2]	7	5	1.4	0.74	0.60
Caleruega Road, Batangas [BA1]	7	6	1.17	0.61	0.47
Fernando Airbase, Mataas na Kahoy, Batangas [BA2]	11	8	1.38	0.98	0.56
Volcano Island, Taal, Batangas [BA3]	8	6	1.33	0.71	0.51
Pantabangan, Quezon Province [QU1]	5	4	1.25	0.16	0.11
Makiling, Quezon Province [QU2]	7	4	1.75	0.64	0.46
Spillway view, Angat Dam, Bulacan [BU]	14	7	2.00	0.92	0.51
Sto Tomas Road, Guimaras [GU]	7	6	1.17	0.80	0.65
Chocolate Hills, Bohol [BO]	13	9	1.44	0.87	0.54

Acquired by dividing the number of species with the number of genera per study site
Shannon Index of species diversity
Pielou's Index of species evenness

As described, the hillside grass patch area in Batangas (BA1) and the roadside forest patches in Guimaras (GU) are the most taxonomically diverse areas (Table 3). This would indicate a higher number of myxomycetes species of a particular genera found in this sites. High Hs value (Hs=0.98) for the open field pastureland in Batangas (BA2) may be attributable to the wider area covered by the grass patches. This enables a wider spore dispersal pattern by wind to various substrates which could facilitate in the higher number of species recorded. The same factor can be true for the sites with still relatively high HS such as Angat dam cliff grass patches in Bulacan (BU) and hilltop grassland in Bohol (BO) (Table 3).

Overall, this study suggests that tropical grasslands and grassland patches in the Philippines harbour diverse species of myxomycetes. Previously devastated areas, e.g., lahar-covered areas in PA1 and PA2, now dominated by grasses can support microbial life, thus allowing growth of these myxomycetes. Hence, the ecological rehabilitation of this area can be pioneered by both microorganisms and myxomycetes by playing a critical part both as decomposers and as food for other organisms. Results of this study serves as baseline information on the species of myxomycetes in tropical grasslands which adds to our understanding on the distribution and ecology of myxomycetes in tropical countries such as the Philippines. Future studies need to be conducted to look at myxomycetes in highly disturbed areas where grasses are the only plant species present as well as other unexplored microhabitats for myxomycetes.

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