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Plasmodial slime molds of a tropical karst forest, Quezon National Park, the Philippines

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Abstract

Karst forest represents a distinct landscape with highly alkaline soil and limestone rocks. This specialized topography supports many unique species of plants and animals. Thus, documenting species in this area is important for any biodiversity research. In this study, a field survey was conducted to assess the abundance, diversity and distribution of myxomycetes in a karst forest within Quezon National Park, Philippines. Fruiting bodies were collected in addition to decaying substrates, e.g. aerial leaves and ground leaf litter, and twigs for culture in moist chambers. A total of 35 species from 16 genera were identified. The majority of these species occurred only rarely.. Myxomycete communities between aerial and ground litter had the highest level of similarity based on their species composition and corresponding relative abundance. This study documented the diversity of myxomycetes from the lowland Karst landscape in the Philippines and now serves as baseline information for investigating plasmodial slime molds in Quezon National Park.

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Introduction

Plasmodial slime molds or myxomycetes are phagotrophic eukaryotes that have bewildered many taxonomists and ecologists all over the world. In previous years, myxomycetes were classified under the Kingdom Animalia (Class Mycetozoa) since they are recognized as having an animal-like characteristic of feeding on microorganisms by means of engulfing (Stephenson and Stempen 1994). In addition, these organisms are also capable of moving by using microfilament rearrangements and cytoplasmic streaming across any substrate (Nagai et al. 1978). Since myxomycetes are usually seen in habitats where fungi are typically found and since they exhibit a fungus-like reproductive phase (Keller and Braun 1999), they were also previously treated as taxa within the Kingdom Fungi (Class Myxomycetes). However, advances in molecular phylogenetic analysis of highly conserved elongation factor 1-alpha (EF-1α) gene sequences had already revealed that myxomycetes are not fungi (Baldauf and Doolittle 1997) and that their physiology, morphology, life history, and genetic analysis supported the classification of myxomycetes in the Kingdom Protista along with other amoeboid, eukaryotic microorganisms (Spiegel et al. 2004, Fiore – Donno et al. 2010).

But besides this baseline information regarding myxomycetes, relatively little is known about their distribution and diversity in the Paleotropical Asia Pacific ecoregion of the world, particularly in the Philippines. Recent studies on Philippine myxomycete biodiversity assessed their occurrence in conservation ecoparks (dela Cruz et al. 2010, Macabago et al. 2010), coastal habitats (Macabago et al. 2012, Kuhn et al. 2013) and lowland mountain vegetation (Cheng et al. 2013, Dagamac et al. 2014) of the main island of Luzon. With these intensive diversity assessments, 127 records of myxomycetes were documented for in the country (dela Cruz et al. 2013), a significant increase of records since they were last comprehensively listed by Reynolds (1981). An additional 19 species were recently found to be new records in the comparative species listing of dela Cruz et al. (2014). Though these recent studies are a good indication that

more attention is now being paid to myxomycete research in the Philippines, this information is still limited in comparison to the biodiversity studies on myxomycetes that have been carried out in other ecoregions world-wide. For a tropical country like the Philippines, gifted with a vast and rich diversity, other unexplored sites in the country with unique landscapes or vegetation such as karst forest (having alkaline, limestone substrate) in Quezon National Park are very promising for further myxomycete diversity studies. Thus, besides contributing to the local inventory for the Philippines, the primary goal of this investigation was to increase knowledge on myxomycete species in a region of the world where there is still a large gap to fill. This research specifically aims to (1) identify myxomycete species from field and moist chamber collections and assess the occurrence of each of the recorded myxomycetes, and (2) analyze the diversity of myxomycetes on different substrates collected along the karst forest floor of Quezon National Park, Atimonan, Quezon, Philippines.

Materials and Methods

General Study Area.

Quezon Protected Landscape or Quezon National Park (N13°59'35.4" E121°49'25.0") is located in the southern Sierra Madre mountain range on Luzon Island spanning the municipalities of Pagbilao, Padre Burgos, and Atimonan in Quezon Province. This landscape of 938 hectares is a lowland rainforest with karst landscape and vegetation. The underlying bedrock is mainly limestone with karstic sinkholes. Several animal species endemic to the Philippines can be found within the park area, e.g. *Buceros hydrocorax, Penelopides panini*, and *Varanus olivaceus* (Bird Life International 2014). Among the most common endemic trees are *Diospyros blancoi*, *Shorea contorta, Shorea negrosensis* and *Canarium ovatum* (DENR 2014). The province has two pronounced seasons, i.e. dry from November to April and wet during the rest of the year with an

annual average temperature range between 23.3-30.2°C and a mean annual precipitation of 2,751.4 mm rainfall (World Weather Online 2013).

Collection of field specimens and substrate sampling.

Three types of dead or decaying substrates, namely detached leaves that are not yet on contact to the ground or aerial litter (AL), leaves found on the forest floor or ground litter (GL) and pieces of twigs (TW) along the forest trail, were haphazardly collected along the west and east trail of the study area. At each trail, three accessible sampling points that are approximately 200m apart were assigned making a total of six collecting points for the whole study (Fig. 1). GPS coordinates of each sampling points were determined by using Garmin eTrex. Ten samples each of AL, GL and TW were collected at each sampling point and were then placed immediately into brown paper bags. This sampling effort resulted in a total of 60 samples for each substrate group and 180 samples in total. Samples were then air-dried in the laboratory for three to four days before being placed in moist chambers. Determinable field specimens of plasmodial slime molds that were observed during the survey were also collected and placed on the same day in clean matchboxes for permanent storage. All of the samples were collected during May 2013.

Preparation of moist chamber cultures and voucher specimens for the herbarium

To set-up moist chamber cultures, air-dried samples of twigs and leaf litter were cut in postage stamp-sized pieces (ca. 2.5 cm square) and placed in standard petri dishes lined with filter paper (Stephenson and Stempen 1994). Then, distilled water was poured onto the moist chambers and the substrates were soaked overnight. After soaking, the pH of each substrate was checked with a pH meter (Sartorius PB-11) and excess water was drained. All moist chambers were maintained under diffused light at room temperature (22-25^{o}C) for up to 12 weeks. The moist chambers were checked every week for the presence of plasmodia and/or fruiting bodies. Dried substrates with myxomycetes were then transferred and glued to herbarium boxes for voucher specimens. All

voucher specimens were labeled with specimen number, collection site, date of collection, collector's name, substrate, identity of the species, and other relevant information. All collected specimens were deposited at the Pure and Applied Microbiology Laboratory, Research Center for the Natural and Applied Sciences, University of Santo Tomas in Manila, Philippines.

Characterization and identification of myxomycetes.

Collected specimens were observed under a dissecting microscope to note the following characters: type, size, shape, and color of fruiting bodies, appearance of stalk, and presence of lime. Slides were also prepared to show internal structures like spore, capillitium, columella and calcareal details of the myxomycetes. To prepare the slides, a myxomycete fruiting body was obtained from the moist chamber culture and placed at the center of a slide with a drop of mounting medium. Lactophenol for non-calcareous-bearing myxomycetes or Hoyer's medium for calcareous-bearing myxomycetes was used as a mounting medium. The slides were then checked under a compound microscope (Olympus CX21) at 400X to 1,000X magnification. Identification of the species was done up to the species level using web-based identification keys, e.g. SYNKey (Mitchell 2008) and the Eumycetozoan Project (http://slimemold.uark.edu/), and published literature (Liu et al. 2007, Poulain et al. 2011). Valid names were based on the online nomenclatural information database for eumycetozoans (http://nomen.eumycetozoa.com).

Data evaluation

To estimate the extent to which the survey was exhaustive in terms of species that were recorded in the study area, a species accumulation curve from the records obtained from the collection in the field and moist chambers was constructed according to the rarefaction formula using the default settings of the program EstimateS (Version 9.0, Colwell 2013, with 100 randomizations). The Chao2 estimator was then chosen as the best estimator in accordance with the findings of Unterseher et al. (2008). The estimated value for the percentage of completeness for the study area and for each microhabitat was then determined following the formula of Ndiritu et al.

(2009) by dividing the actual number of species recorded by the mean number of species expected as estimated by the Chao 2 estimator. In addition, a hyperbolic regression for each microhabitat according to the Michaelis-Menten formula, $y = ax/(b+x)$, was applied to the data, with x representing the number of samples, y the number of species recorded and the parameter *a* giving an estimate of the maximum number of species to be expected on this kind of substrate, and resulting in a very close curve shape (Magurran 2004).

Moist chamber cultures (MC) that showed either plasmodia or fruiting bodies were recorded as one positive culture. This was used to calculate the percent yield from MC in the study area. Percent yield was then calculated as the number of a moist chamber cultures positive for myxomycetes divided by the total number of moist chamber cultures prepared (Dagamac et al. 2012). The composition of species was then initially determined by creating a list of all species noted both in the field and in the MCs. The occurrence (the presence or absence of a particular species of myxomycete) of each single myxomycete species recorded was then calculated by using the formula of relative abundance as described by Cheng et al. (2013) and Dagamac et al. (2012). From the computed relative abundance, an abundance index (AI) value from Stephenson et al. (1993) was given for each species, namely, rare (R) for species less than 0.5% of the total number of collections, occasional (O) for species more than 0.5% but less than 1.5% of the total number of collections, common (C) for species more than 1.5% but less than 3% of the total number of collections, and abundant (A) for species more than 3% of the total number of collections.

The α diversity of myxomycetes from the study area and the three microhabitats was then computed using the software SPADE (Chao and Shen 2010) by generating the bias corrected maximum likelihood estimator, the maximum likelihood estimator and the classic formula for Shannon (SHA), Simpson (SIM) and Fisher (FIS) indices, respectively. Although the Shannon Index is the most commonly used for ecological research, the addition of more intuitive indices

such as the Simpson and Fisher indices can be useful for smaller sample sizes, as is the case in our study. Thus, these indices can help in the interpretation of species diversity because similar to the Shannon index, both take into consideration species richness and evenness. The statistical comparison of these indices by simple T-test was calculated using XLStat Version 2014.1. Furthermore, the Taxonomic Diversity Index (TDI) was also calculated by simply dividing the ratio of the number of species by the number of genera. Consequently, a lower ratio indicates a higher overall taxonomic diversity. This particular ecological concept was supported by Magurran (2004) who stated that if two communities have identical numbers of species and equivalent patterns of species abundance, but differ in the diversity of the taxa to which the species belong, it seems intuitively appropriate that the most taxonomically varied assemblage is considered to be more diverse. For β diversity, the communities of myxomycetes associated with the different substrates were further analyzed using Sorensen's Coefficient of Community (CC) and the Percentage Similarity (PS) indices as described previously by Stephenson (1989). The Coefficient of Community (CC) index is based solely on the presence or absence of a species in the two communities being compared, whereas Percentage Similarity (PS) considers both the presence and absence of a species and its relative abundance (Stephenson et al. 1993).

Results

Percent yield of the moist chambers and species accumulation curve

In this study, a total of 205 records were compiled with 68 plasmodial records and 137 identifiable fruiting bodies. From the 137 records of fruiting bodies, 35 species of myxomycetes were identified. The expected number of myxomycetes species (Chao2) in the area is around 45.9 (Fig.2a), suggesting that our sampling in the study area identified 76% of the expected species. The hyperbolic regression via the rarefaction curve of the three microhabitats used for the moist chamber showed that species number collected from the twigs is still limited (Fig. 2b).

The rarefaction curves for the three substrates (aerial litter, ground litter and twigs) are still progressing, suggesting that more species of myxomycetes are still to be found (Fig. 2b). From the 180 moist chamber cultures, 148 (82%) yielded positive growth for myxomycetes either as plasmodium or sclerotia and fruiting bodies.

Species composition and occurrence

A total of 35 species belonging to 16 different genera were identified from the rapid field survey and moist chamber cultures. From these 35 species, four species, namely *Arcyria denudata, Ceratiomyxa fruticulosa, Lycogala exiguum* and *Physarum pezizoideum,* were found only from samples collected in the field survey and 31 species were recorded from the moist chamber cultures. Myxomycetes collected from the moist chambers included one species each of *Collaria, Craterium, Echinostelium, Hemitrichia, Lamproderma,* and *Physarella,* two species each of *Arcyria*, *Comatricha, Cribraria, Diderma,* and *Stemonitis,* four species of *Didymium* and *Perichaena*, and seven species of *Physarum.* Three species recorded in this study could only be identified to the genus level since the fruiting bodies did not develop in a normal fashion. Among the collected species, *Arcyria cinerea, Lamproderma scintillans, Perichaena depressa,* and *Stemonitis fusca* were abundant (Table 1). The majority of the collected myxomycetes were considered to be rare, since 15 species had a relative abundance of less than 0.5%. Furthermore, nine myxomycete species were occasional, while seven myxomycete species were common (Table 1).

Taxonomic and species diversity

Considering only the myxomycetes species from the moist chamber collections, our results showed that aerial litter harbored 19 species belonging to 10 genera, twigs had 19 species belonging to 12 genera, while ground leaf litter had 10 species belonging to 8 genera (Table 1). From this, the highest TDI was calculated in aerial litter (1.90), followed by twigs (1.58) and

then by ground litter (1.25), indicating that the ground litter substrates had the most taxonomically diverse myxomycete assemblages of the three microhabitats used in this study. Considering species diversity, the highest SHA and FIS values were observed from twigs (SHA = 2.86; FIS = 10.98), followed by aerial litter (SHA = 2.73; FIS = 10.13) and ground litter (SHA = 2.32; FIS = 6.73). The computation of the SIM values generated higher values in ground litter (SIM = 0.20) in comparison to twigs (SIM =0.15) and aerial litter (SIM = 0.13). There were no statistically significant differences between the species diversities among the three microhabitats (p value = 0.843, $\alpha = 0.05$).

Community analysis

The similarities of myxomycete assemblages that were recorded for the different substrates were further evaluated. Based on our result, the number of myxomycete assemblages that are exclusive for ground litter, aerial litter and twigs was one, seven, and 10, respectively (Fig. 3). When comparing the myxomycete communities found on the different substrates, ground litter and twigs have one common species*, i.e. Cribraria violacea*. Four myxomycetes species were found on all three substrates, namely, *Arcyria cinerea, Diderma effusum, Lamproderma scintillans* and *Perichaena depressa* (Fig. 3). Computing the two different similarity indices showed that the highest values were between myxomycete communities in aerial and ground litter (CC = 0.55; PS = 0.60), followed by aerial litter and twigs (CC = 0.42; PS = 0.51) and twigs and ground litter (CC = 0.34; PS = 0.54).

Discussion

Information regarding microbial diversity of plasmodial slime molds in the Philippines is limited. The present study reports a rapid classical diversity assessment of the myxomycete assemblages recorded within the karst landscape of Atimonan trail in Quezon National Park. In

spite of the fact that there have been numerous studies on the plant and animal communities in this popular forest park, none have ever recorded the myxomycetes in this site.

Moist chamber productivity

The high percent yield (82%) reported in this paper is comparable to the yield found in moist chambers from other tropical and temperate forests (Rojas and Stephenson 2007, Ndiritu et al. 2009). However, Macabago et al. (2010) and Kuhn et al. (2013) reported a lower percent yield of myxomycetes (51%) from moist chamber cultures prepared from substrata obtained from Cavite, Laguna, Benguet, and Manila in the Philippines. Nonetheless, the moist chamber technique had already been demonstrated to be effective for assessing the diversity of myxomycetes (Stephenson and Stempen 1994, Novozhilov et al. 2000).

Completeness of the survey

With the 35 species identified in the study area, the number is comparably greater than that found in previous surveys conducted in other mountain forests in the Philippines. For example, only a total of 21 species was reported from the two accessible trails in Mt. Arayat (Dagamac et al. 2012) and the northern slope of Mt. Makulot (Cheng et al. 2013), and 28 species were reported from the protected La Mesa Ecopark (Macabago et al. 2010). The sampling effort (76.3%) suggests not all myxomycete species in the locality were discovered. This value is in congruence with the rarefaction curves of the three substrates used for the moist chamber experiments, which show that the curves are not yet at their saturation points. Similar results were reported in other rapid diversity assessments of myxomycetes in the neotropical Amazons (Rojas and Stephenson 2012a), in which the aerial litter, ground litter and twigs were also the only substrate types collected, since they harbor the most common myxomycetes species. It is possible that other myxomycetes can grow from other microhabitats such as dead bark of living trees, dung of herbivorous animals in the forest and decaying inflorescence. Even if our sampling effort does not reflect the overall myxomycete communities in the study area, it is significant to

note that our sampling and the number of species recovered were comparable to a number of studies carried out in other areas of the world that employed the same sampling methods (Novozhilov and Schnittler 2008, Ndiritu et al. 2009, Schnittler et al. 2013) and thus our data establish the baseline information that can be used for future investigations on myxomycetes in the same study area.

Myxomycete diversity and community dynamics among microhabitats

All of the substrates used in the present study were decaying organic matter randomly collected along the accessible trail of Quezon National Park. This supports the general assumption that myxomycetes, regardless of the type of substrate being considered, are common inhabitants of many kinds of decaying plant material in a forest ecosystem (Schnittler and Stephenson 2002) and that forest structures play an influential factor in the various occurrences of myxomycetes in tropical forests (Rojas and Stephenson 2012a). Moreover, it is clear from our results that myxomycetes are not found with equal abundance on all substrates potentially available to them. For example, *Stemonitis pallida,* which is reported in this study as an occasional occurrence in aerial litter and twig, was previously reported in the Philippines to be a rare corticolous myxomycete (Dagamac et al. 2010). As noted from previous studies, several factors can affect the occurrence of myxomycetes. For instance, temperature and moisture are considered to be the primary factors limiting the occurrence of myxomycetes in nature (Alexopolous 1963). More so, recent global studies of tropical myxomycetes suggest that forest disturbances (Rojas and Stephenson 2013), leaf preferences (Takahashi 2013), elevation (Dagamac et al. 2014) and seasonality can also influence their diversity (Ko Ko et al. 2011, Dagamac et al. 2012).

In terms of community similarity among myxomycete assemblages, both CC and PS values clearly showed a considerable similarity of myxomycete composition between aerial and ground litter. Results from Dagamac et al. (2012) also revealed the same pattern among myxomycetes assemblages, the myxomycete species *Diderma hemisphaericum, D. effusum* and *Physarum*

compressum being found on both substrate types. Although specific substrate species were not identified in our study, and in spite of the fact that the explanations made here are vastly speculative, one possible reason for differences in distribution of myxomycetes among the substrates may be resource partitioning. For example, the microbial biota, which serves as food sources, might differ in abundance and/or in the composition necessary to support similar species of myxomycetes. Vascular plants may also vary in their microenvironmental conditions, e.g. water retention or even chemical composition, thus affecting the heterogeneity of myxomycete distribution. Moreover, intensive studies by Rojas et al. (2011) on the distribution of myxomycetes at higher elevations in the neotropics revealed that macroclimatic parameters influence the distributional patterns of myxomycetes in general. Unfortunately, these aspects of myxomycete ecology in the Paleotropics are still not fully understood and thus merit further exploration.

Implications of myxomycete occurrence for Philippine biodiversity

The high endemicity among our plant and animal communities make the Philippines a hotspot of global biodiversity. But this supposition is not applicable to microbial biota such as the myxomycetes. Although, all of the myxomycetes species were already previously accounted for in the country, the myxomycete assemblages reported in this paper are the first for this unique tropical karst forest landscape. Of the 35 myxomycete species reported in this paper, it is of particular interest to note that this is the first report of the species *Physarum pezizoideum* since its last annotation by Reynolds in 1981. Furthermore, *Perichaena dictyonema,* recently reported as a new record for the Philippines (Dela Cruz et al. 2014), seems to be restricted to the tropics. In comparison to other tropical countries in Southeast Asia that have been surveyed for myxomycetes, e.g. Thailand (Ko Ko et al. 2011), Singapore (Rosing et al. 2011) or Myanmar (Ko Ko et al. 2013), the number of myxomycetes accounted for here may still be low. However,

considering our generally smaller study area and the abundance of unique landscapes in the Philippines, we can assume that many plasmodial slime molds are still awaiting discovery.

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Table 1: Occurrence of myxomycetes in the entire study area and their abundance (based on the number of records) from the three collected substrates, including the sampling effort and species diversity indices generated from SPADE.

SPECIES	mean pH value of the MC	AI	Frequency (Pooled Data)	Records			
				Field	AL	GL	TW
Arcyria afro-alpina Rammeloo	5.80	R	1		1		
Arcyria cinerea (Bull.) Pers.	5.61	A	43	1	17	9	16
Arcyria denudata (L.) Wettst.		R	1	1			
Ceratiomyxa fruticulosa (Müll.) T. Macbr.		R	1	1			
Collaria arcyrionema (Rostaf) Nann - Bremek. ex. Lado	5.73	C	4		3		1
Comatricha laxa Rostaf.	3.50	R	1				1
Comatricha nigra (Pers. ex J.F. Gmel.) Schroet.	5.60	R	1		1		
Craterium minutum (Leers) Fr.	5.85	O	2			2	
Cribraria sp.	5.30	R	1				1
Cribraria violacea Rex	5.82	C	5			2	3
Didymium iridis (Ditmar) Fr.	5.57	O	3		2	1	
Didymium nigripes (Link) Fr.	3.50	O	2	1	1		
Didymium cf. *ochroideum* G. Lister	6.00	R	1				1
Didymium squamulosum (Alb. & Schwein.) Fr.	6.20	O	2		2		
D*iderma effusum* (Schwein.) Morgan	5.87	C	6		3	2	1
Diderma hemisphaericum (Bull.) Hornem.	5.38	C	4		3	1	
Echinostelium sp.	6.20	R	1		1		
Hemitrichia serpula (Scop.) Rostaf.	5.70	R	1				1
Lamproderma scintillans (Berk. & Broome) Morgan	5.99	A	9		4	2	3
Lycogala exiguum Morgan		O	2	2			
Perichaena chrysosperma (Currey) Lister	5.88	C	4		3		1
Perichaena depressa Libert	5.67	A	9		2	2	5
Perichaena dictyonema Rameloo	4.85	O	2		2		
Perichaena pedata (Lister & G. Lister) G. Lister	6.07	C	6		4		2
Physarella oblonga (Berk. & M.A. Curtis) Morgan	4.90	R	1				1
Physarum sp.	5.70	R	1				1
Physarum album (Nees) Fr.	6.30	R	1				1
Physarum cinereum (Batsch) Pers.	5.70	R	1				1
Physarum compressum Alb. & Schwein.	5.65	O	2		1	1	
Physarum decipiens M.A. Curtis	5.60	O	2				2
Physarum melleum (Berk. & Broome) Massee	5.88	C	5		4	1	
Physarum cf. *notabile* T. Macbr.	6.20	R	1		1		
Physarum pezizoideum (Jungh.) Pavill & Lagerh.		R	1	1			
Stemonitis fusca Roth	4.88	A	8				8
Stemonitis pallida Wingate	4.35	O	2		1		1
Total Number of Records			**137**	**7**	**56**	**23**	**51**
Number of Species			**35**		**19**	**10**	**19**
Number of Myxomycete Genera			**16**		**10**	**8**	**12**
Chao 2 Estimate Number of Species			**45.9**		**23.1**	**11.5**	**40.6**
% Sampling Effort			**76.3**		**82.3**	**87.0**	**46.8**
Shannon's Index of Diversity (SHA)			**3.02**		**2.73**	**2.32**	**2.86**
Simpson's Index of Diversity (SIM)			**0.12**		**0.13**	**0.20**	**0.15**
Fischer's Index of Diversity (FIS)			**15.19**		**10.13**	**6.73**	**10.98**
Taxonomic Diversity Index (TDI)			**2.19**		**1.90**	**1.25**	**1.58**

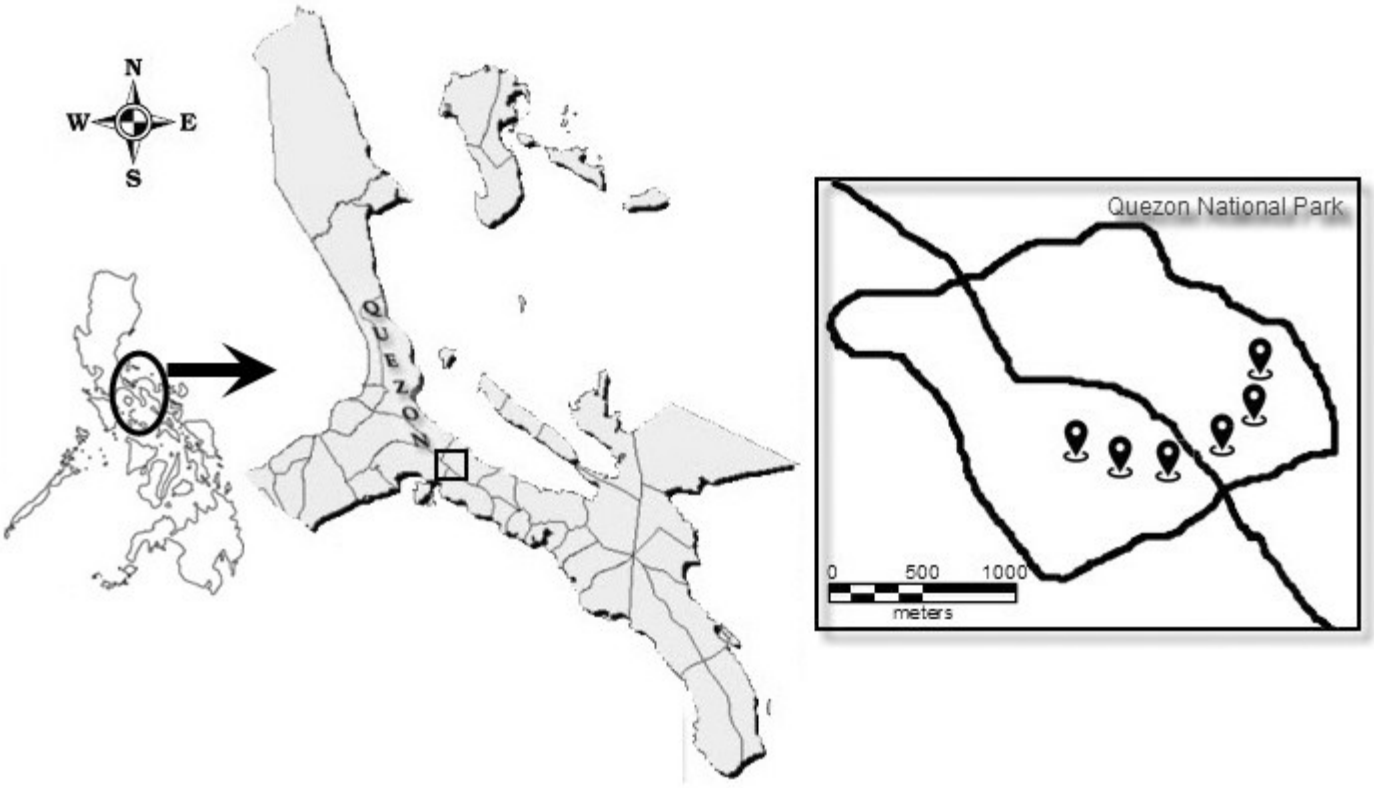

Fig. 1: Map of the general study area showing the west and east trails of Quezon National Park with three sampling points each.

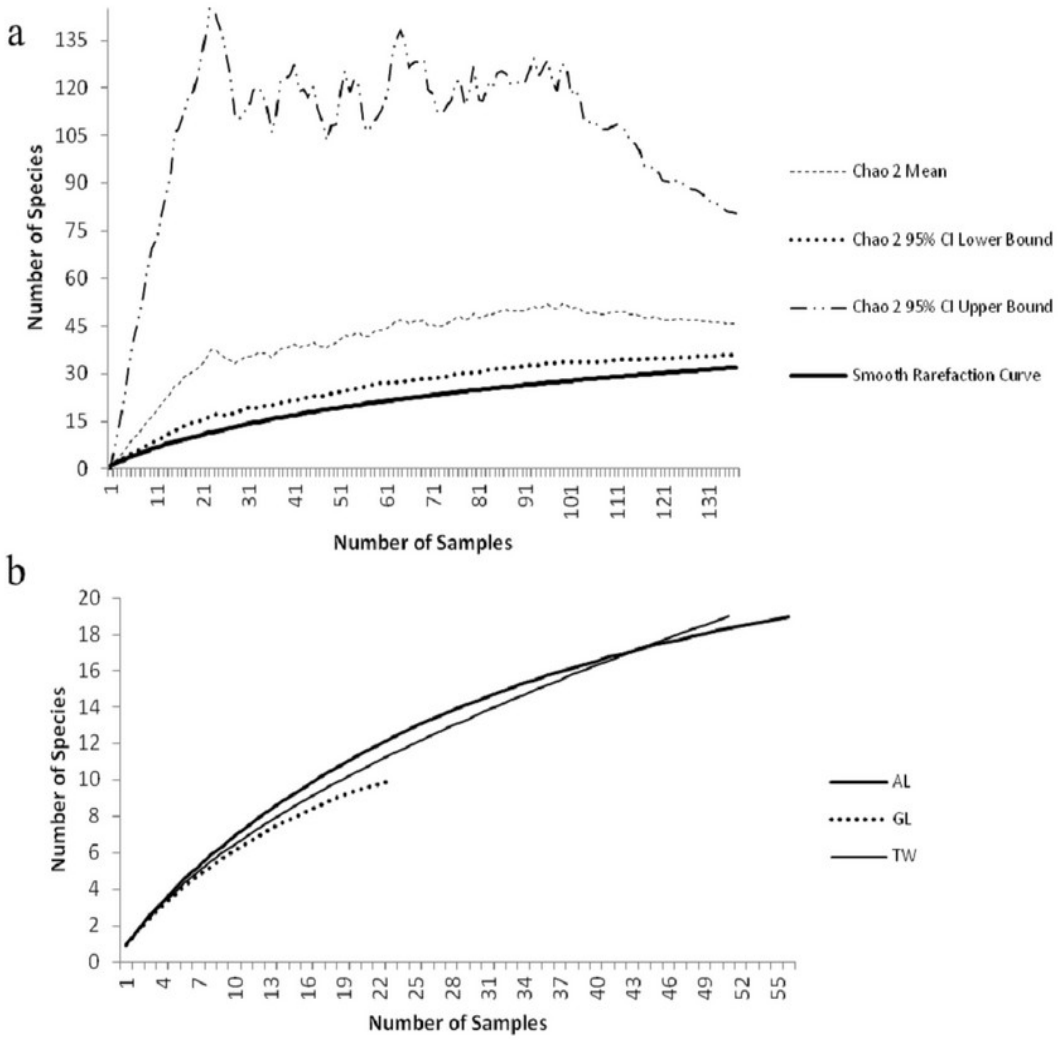

Fig. 2: Species accumulation curve of myxomycetes sampled from the Atimonan trail of Quezon National Park and generated using EstimateS and Chao 2 Estimator (Figure 2a). Coleman rarefaction curve for the three different microhabitat used in the moist chamber set-up (Figure 2b)

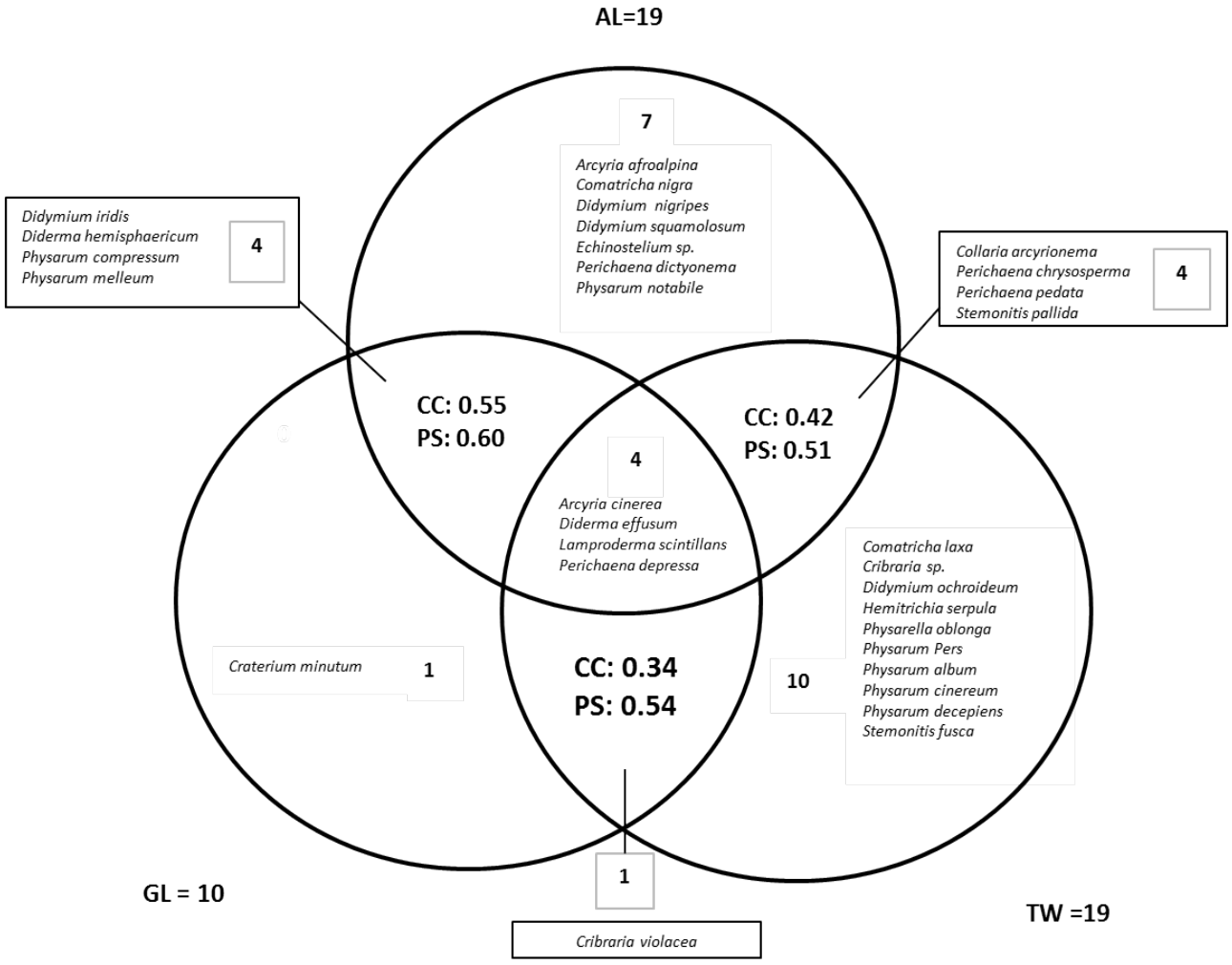

Fig. 3: Venn diagram showing the distribution of myxomycete assemblages collected from three different substrates (AL, GL, TW) and the β diversity values (CC and PS) between the communities.

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