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Myxomycetes of Coron Island and additions to the Myxomycetes of Palawan group of islands in the Philippines

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Abstract

The main objective of the research reported herein is to present an annotated checklist generated from the first survey of myxomycetes in the limestone forests of Coron Island in the province of Palawan, Philippines. A total of 25 morphotaxa were identified from specimens isolated in the laboratory from samples of ground leaf litter, twigs, and vines (lianas) collected from five sites along the coasts and inland forests of Coron Island. Among the identified taxa one (*Badhamia macrocarpa*) was a new record for the country, while another was temporarily assigned to the genus *Perichaena* (Trichiida: Trichiidae) until the proper classification of the specimen could be determined. In addition, the present study brings the updated total number of records of myxomycetes for the Palawan group of islands to 56 morphospecies.

Introduction

There have been a number of previous studies of the myxomycetes, a diverse group of eukaryotic amoebozoans that are capable of producing fruiting bodies containing microscopic spores, of tropical and subtropical islands outside the Philippines (e.g., Eliasson & Nannenga-Bremekamp 1983, Eliasson 1991, Pando 1997; Nieves-Rivera & Santos-Flores 1998; Novozhilov et al. 2001; Rojas & Stephenson 2008; Stephenson 2009; Kryvomaz et al. 2017; Stephenson & Stephenson 2019). In the Philippines, the study of myxomycetes from insular habitats has increased substantially over the last decade, such as examples as the Hundred islands in Pangasinan (dela Cruz et al. 2011), Lubang island in Occidental Mindoro (Macabago et al. 2012, 2016), Anda island in Pangasinan (Kuhn et al. 2013), Polilio island in Quezon (Viray et al. 2014), Bohol island (Macabago et al. 2017), and in Puerto Princesa in mainland Palawan (Pecundo et al. 2017). Before the study reported herein no other myxomycete exploration has been documented for Coron Island, Palawan.

Coron Island is a triangular shaped landmass that is situated on the northeastern part of the Palawan group of islands in the Philippines (Fig. 1A). It is about 20 km long and less than 9 km wide, and is located on the southeastern corner of the larger island of Busuanga (Stasolla & Innocenti 2014). Kiessling and Flugel (2000) characterized Coron Island as being a carbonate platform entirely covered by limestone. The island topography includes various karstic depressions and lakes, having elevations ranging from 200 to 500 masl, and some coasts and inner portions that are inaccessible (Stasolla & Innocenti 2014). Moreover, the island covers 22, 284 hectares of ancestral land and water belonging to the Tagbanua tribe (Samonte-Tan et al. 2008), a local ethnic minority.

Previous studies of the flora and fauna of the Palawan group of islands, including the disconnected island of Coron, have revealed the presence of notable and novel organisms. The purpose of the present study was to conduct the first survey of myxomycetes on Coron Island, and to update the collective records of myxomycetes in the province of Palawan, Philippines.

Materials and Methods

Study area, collecting protocols, laboratory isolation techniques, and determination of myxomycetes: The collecting effort for this study was conducted in 2013 in randomly selected, accessible sites on Coron island (Fig. 1A), in the Palawan group of islands in the Philippines (Fig. 1B), which is located on the western part of the archipelago on the West Philippine Sea (a.k.a. South China Sea). Coron island is characterized by rolling and steep hills with 5,954 hectares of forest areas dominated by *Pterocymbium tinctorium* (Malvaceae), *Instia bijuga* (Fabaceae), *Koordersiodendron pinnatum* (Anacardiaceae), and *Heritiera sylvatica* (Sterculiaceae) (PCSDS 2006). Samples of mostly dry ground leaf litter (GL) and twigs (TW), and a combination of relatively fresh and mostly dry woody vines (V) were collected in a haphazard manner along the coasts and within areas that were characterized by limestone forests that had a mixture of tall shrubs and mostly dipterocarp and evergreen trees with the common presence of woody vines (lianas). Some palms were spotted in the island, but were not collected in the largely carbonatic forest sites that were fairly adjacent to other ecosystems such as inland lakes, brushlands, and mangrove forests to name a few. Field specimens that developed under natural conditions were excluded from the study.

The samples were kept on Coron Island until they were cleared by the local government unit (LGU) of Coron for transport to the Research Center for the Natural and Applied Sciences of the University of Santo Tomas in Manila, Philippines, a protocol that is enforced by the LGU for ancestral domains. All samples were air-dried for several weeks and then were sent to the University of Arkansas Department of Biological Sciences for processing. A total of 180 feasible samples (72 GL, 65 TW, and 43 V) from the five sites (A = 39, B = 21, C = 40, D = 32, E = 48) were used (**Table 1**) to prepare moist chamber cultures, following the protocol described by Stephenson and Stempen (1994). More than half of the samples were discarded due to the following: visible fungal contamination of substrates, torn collecting bags, and/or destroyed labels, a decision made by the coauthors in order to preserve the reliability/validity of the results. The moist chambers were observed weekly up to 12 weeks for the presence of

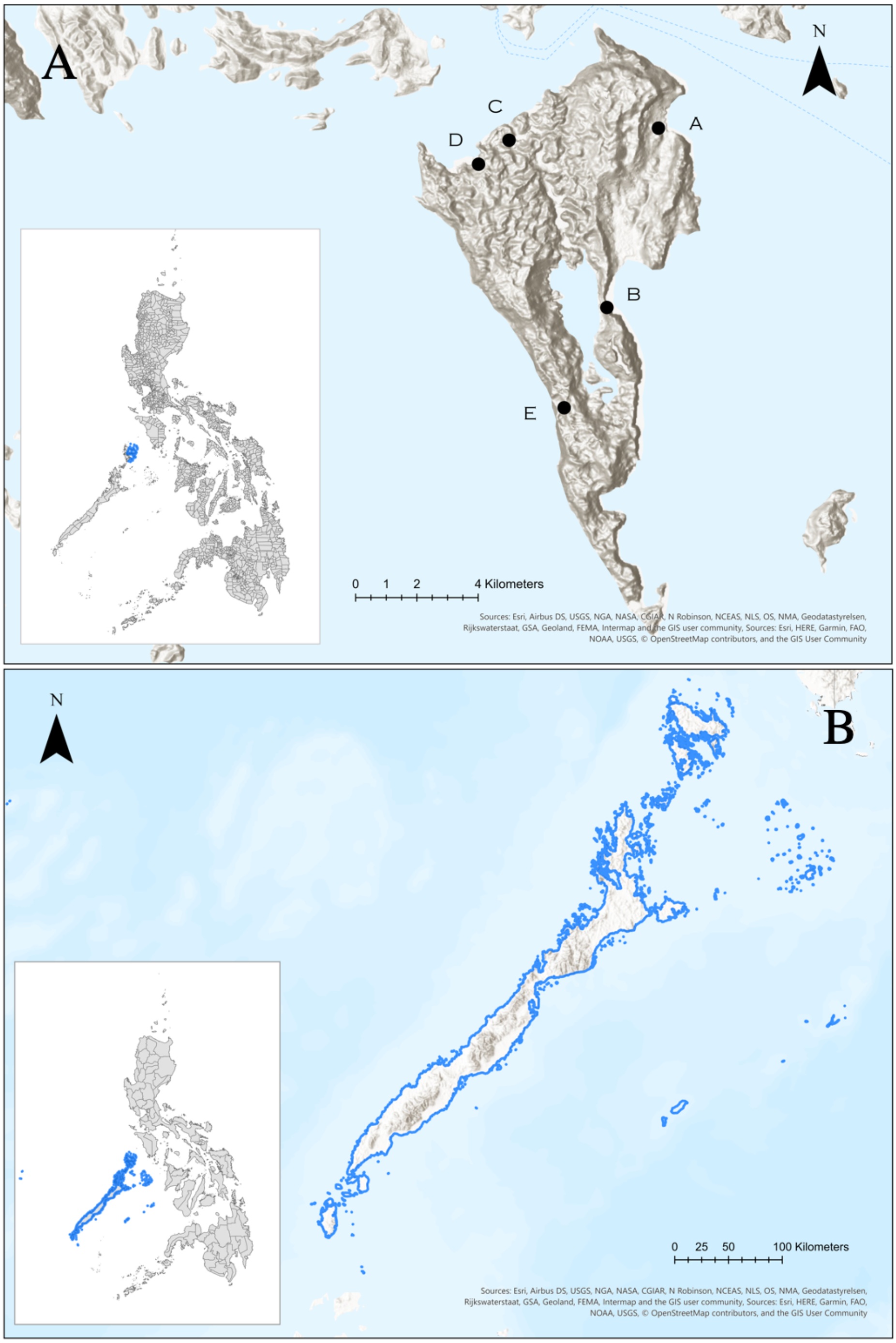

Figure 1. (A) Map of Coron Island with the collecting sites [A-E] marked by black dots (·). The inset on the lower left shows a map of the Philippines, with Coron delimited in blue. (B) Map of the Palawan group of islands including Coron Island. The inset on the lower left shows a map of the Philippines with the Palawan group of islands demarcated by a blue line. This map is generated using ArcGIS Pro.

myxomycetes. The morphological species concept was used to identify and classify the myxomycetes. Nomenclature followed Lado (2005-2020). The Percent Yield was then calculated by dividing the number of positive moist chambers (those that have shown the presence of determinable myxomycetes) over the total number of samples (Dagamac et al. 2012).

Exhaustiveness of the survey: The software program EstimateS (Version 9.1, Colwell 2013, 100 randomizations) was used to construct a species accumulation curve (SAC) as described by Unterseher et al. (2008) and Ndiritu et al. (2009) to evaluate the completeness of our survey for (1) each of the study sites located around the island, and (2) the pooled dataset from all five study sites for Coron Island. A species abundance input file was generated and eventually loaded in the program. The results of the Chao 1 estimator, an estimator for species richness for individual-based data as such that one record of a species in a moist chamber culture is considered as one individual (*sensu* Stephenson 1988), were then used to calculate the percentage exhaustiveness by dividing the actual number of species recorded by the mean number of species expected.

Analysis of the myxomycete assemblages: To examine the dataset obtained from the records of myxomycetes appearing in the moist chamber cultures, the online software SpadeR and abundance-based species data were used for the evaluation of species richness and diversity.

Initially, the relative abundance of each species was obtained by dividing the total number of collections for each species of myxomycetes by the total number of myxomycetes collected (Stephenson et al. 1993). The computed values were then translated to an abundance index described by Stephenson et al. (1993), for which species < 0.5% of the total number of collections are defined as rare; species > 0.5% but < 1.5% of the total number of collections defined as occasional; species > 1.5% but < 3% of the total number of collections defined as common; and species > 3% of the total number of collections defined as abundant. The actual number of occurrences of each species were used to create an annotated list noting their abundance indices in the island and on which substrates they were found.

The Taxonomic Diversity Index (TDI), also known as the S/G ratio, was calculated by obtaining the ratio of the number of species to the number of genera. The value of this index is inversely proportional to the taxonomic diversity (Stephenson et al. 1993), where a higher ratio indicates a less diverse biota.

The online software Species-Richness Prediction and Diversity Estimation with R

(SpadeR) (Chao et al. 2015) was used to generate Chao 1-bc, which is the bias-corrected form of the Chao 1 species richness estimator (Chao & Chiu 2016), and the maximum likelihood estimator for the more intuitive Inverse Simpson index, which is a heterogeneous measure of species diversity that accounts for both the species richness and evenness.

Table 1. Summary data for the microhabitat types. TDI = Taxonomic diversity index.

Microhabitat type	Number of samples	Percent yield	Determinable records	Recorded genera	Recorded taxa	TDI
Ground litter	72	65	71	9	14	1.56
Twigs	65	72	71	8	15	1.88
Vines	43	100	61	8	9	1.13
Total	180	76	203	13	25	1.92

Results

One hundred thirty-seven samples (a yield of 76%) produced some evidence of the presence of myxomycetes. As shown in Table 1, woody vines (lianas), albeit with the lowest number of collected substrates, produced the highest myxomycete yield (100%), followed by twigs (72%), and then ground leaf litter (65%). Vines also recorded the most taxonomically diverse assemblage, which included nine taxa in eight genera (Table 1), followed by TW and GL. A total of 25 morphotaxa were identified among the 203 records of myxomycetes from 180 samples, which resulted in a relatively low survey completeness of 53% for the entire island. Despite this fact, some of the sites still recorded notable survey exhaustiveness, such as site B with 100% along with sites C, D, and E with 71%, 62%, and 71%, respectively. Site A recorded the lowest value, which is 34%.

An annotated list of species also was also generated from these data, containing the respective abundance index (A = abundant, O = occasional, C = common, and R = rare) of each taxon, the total number of collections, and the substrates on which the taxa were found. After the name and authority, the abundance index and total number of collections were placed in brackets [ ]. This was followed by the number of records per microhabitat/substrate, where GL stands for ground leaf litter, TW stands for twigs, and V stands for woody vines. An asterisk after the name and authority indicates that the species is a new record of myxomycetes for the Palawan group of islands. No specimens that developed under natural conditions in the field were included in this list.

List of Species

Arcyria cinerea (Bull.) Pers. [A, 32] GL: 5, TW: 13, V: 14
Arcyria denudata (L.) Wettst. [R, 1] V: 1
Badhamia macrocarpa (Ces.) Rostaf. * [O, 2] TW: 2
Clastoderma debaryanum A. Blytt [A, 7] TW: 1, V:6
Comatricha cf. *pulchella* (C. Bab.) Rostaf. [O, 2] V: 2
Comatricha tenerrima (M.A. Curtis) G. Lister [R, 1] TW: 1
Cribraria violacea Rex * [O, 2] GL: 2
Diachea leucopodia (Bull.) Rostaf. [C, 4] GL: 4
Diderma effusum (Schwein.) Morgan [C, 4] GL: 4
Diderma hemisphaericum (Bull.) Hornem. [A, 9] GL: 9
Didymium ochroideum G. Lister * [R, 1] GL: 1
Didymium iridis (Ditmar) Fr. [A, 7] GL: 2, TW: 5
Didymium nigripes (Link) Fr. [C, 5] GL: 3, TW: 2
Didymium squamulosum (Alb. & Schwein.) Fr. & Palmquist [C, 5] GL: 2, TW: 1, V: 2
Lamproderma scintillans (Berk. & Broome) Morgan [A, 9] GL: 9
Perichaena depressa Lib. [A, 58] GL: 23, TW: 14, V: 21
Perichaena dictyonema Rammeloo * [O, 3] GL: 1, TW:2
Perichaena vermicularis (Schwein.) Rostaf. * [R, 1] TW: 1
Perichaena sp. (Trichiida: Trichiidae) [O, 3] GL: 2, V: 1
Physarum echinosporum Lister * [O, 6] GL: 4, V: 2
Physarum oblatum T. Macbr. * [R, 1] TW: 1
Physarum pusillum (Berk. & M.A. Curtis) G. Lister * [R, 1] TW: 1
Physarum sp. (Physarales: Physaraceae) [R, 1] TW: 1
Stemonitis fusca Roth [A, 37] TW: 25, V: 12
Stemonitis sp. (Stemonitidales: Stemonitidaceae) [R, 1] TW: 1

Although the original purpose of this study was to analyze the data as a whole and not compare the individual collecting sites, **Table 2** shows the individual statistics and diversity indices of each site in order to provide additional information. Site E, which had the most records and identified morphospecies, demonstrated a species diversity close to that of Site C (second highest number of morphospecies) and a species richness closest to Site A (second highest number of records).

Before this study, there were 48 species of myxomycetes (Pecundo et al. 2017; Reynolds 1981) reported for the Palawan group of islands. The study added eight morphospecies (**Table 3**), excluding the one as yet unidentified taxon that presumably belongs to the genus *Perichaena* (Trichiida: Trichiidae).

Discussion

Coron Island is a disjointed landmass from the main island and other islets of the Palawan group of islands (Fig. 1) on the West Philippine Sea (a.k.a. South China Sea) just above the equator. It has a Type I climate like most of Palawan, which is characterized by two pronounced seasons—dry from November to April and wet during the rest of the year with an average rainfall of 170 mm, average temperature of 27°C, and average relative humidity of 76% (PCSDS 2006). This survey collected substrates in July and obtained a 76% yield from the moist chamber cultures used to isolate the myxomycetes already present in the microhabitats examined. This number is comparable to the study carried out by Pecundo et al. (2017) in several areas on the main island of Palawan, which produced a yield of 73%, but this is less than what has been reported from other studies such as for Christmas island, where samples were characterized by a yield of 95% for plasmodia and/or fruiting bodies (Stephenson and Stephenson 2019). However, it is noteworthy that the percent yield obtained in the present study included only samples that generated fruiting bodies, which was comparably higher than the 46% yield of bodies from the mainland Palawan study by Pecundo et al. (2017).

Among the microhabitats examined, the woody vines (V) produced the highest myxomycete yield, followed by twigs (TW), and lastly ground leaf litter. Comparing the results to the recent mainland Palawan study (Pecundo et al. 2017), twigs (86% vs 72% in this study) also yielded more myxomycetes than ground litter (57% vs 65% in this study). However, it is not surprising that woody vines recorded the highest taxonomic diversity index (TDI), as other studies also have indicated their high level of productivity as a substrate (e.g., Kryvomaz et al. 2017,

Table 2. Statistics and indices of individual-based species accumulation for the different collection sites (A-E) on Coron Island, showing numbers of taxa, records, and rarefied species, the values for expected species according to Chao1-bc estimator, and species diversity according to Simpson's inverse index computed in SpadeR, and the values for the measure of taxonomic diversity (TDI) of each site.

Collecting sites	Records	Genera	Species	TDI	Species richness	Species rarefaction	Species diversity
Site A	44	8	12	1.50	22.23	7.34	5.61
Site B	18	4	4	1.00	1.00	4.00	1.00
Site C	41	9	14	1.56	17.64	9.41	8.94
Site D	42	9	10	1.11	13.24	6.42	3.60
Site E	58	11	15	1.36	19.12	7.75	5.99
Pooled (Coron)	203	13	25	1.92	47.40	7.58	5.85

Dagamac et al. 2015, Wrigley de Basanta et al. 2008).

The 203 records from 137 productive moist chambers indicated a relatively low value of survey exhaustiveness for the entire island. This is probably due the large number of discarded samples due to fungal contamination of substrates prior to preparation of moist chambers or "compromised" substrata resulting from mixing of samples due to damaged collecting bags and/or the lack of legible labels, as mentioned in the methods section. However, among all of the sites, it was only site A that had a survey completeness value lower than 60%. All four others ranged from 62 to 100%. These results suggest the strong possibility of recovering more species of myxomycete from the areas considered in the present study.

Following the protocol of Stephenson et al. (1993), the relative abundances of each of the 25 morphotaxa were determined, where A was abundant, O was occasional, C was common, and R was rare. As evident from the list generated, seven morphotaxa were found to be abundant, four were common, six were occasional, and eight were rare. *Perichaena depressa* was found to be the most abundant taxon, accounting for almost 29% of all collections, followed by *S. fusca* and *A. cinerea*. A study by Rojas and Stephenson (2008) on Cocos Island, which is another tropical insular site, also found *A. cinerea* and *P. depressa* to be abundant species. As was the case for a study of Mahe Island in Seychelles (Kryvomaz et al. 2017), taxa recorded as rare were singletons in one of the three microhabitats considered, including the two that were not assigned to a particular species but were designated as *Physarum* sp. and *Stemonitis* sp., both of which were associated with TW. In the present study, the collection that was temporarily assigned to *Perichaena* (Trichiida: Trichiidae) was associated with GL and V.

As mentioned in the results section, the objective of this paper was to examine the data for the island as a whole. However, since the substrates were independently collected from different sites, it would seem likely to consider the values obtained for each site. In terms of species richness, all four sites except for site B (no. of species = 4) had relatively similar numbers of morphospecies collected (Table 2). The expected number of species for each site as generated by the Chao 1-bc estimator indicated the highest value (22.23) associated with site A, meaning that approximately 22 species would be expected for A, around 19 for E, around 18 for C, and around 13 for D. The value of 1 in site B could very well be because of the low numbers of species found from 18 records in 21 samples, which is about half the number as compared to other sites. These data could be correlated to the survey completeness outcomes for which sites E and C showed similar values (71%), followed by C, and then A, which appears to signify that if there were more samples collected in A (reflected by 34% exhaustiveness) there could have been more species found (12 actual vs. 22 expected). Looking at species diversity, which takes into consideration both richness and evenness, site C had the highest value, which means that the species of myxomycete in C are more evenly distributed among the genera than in either E (2nd) or A (3rd). The TDI, however, tells a slightly different story, since site B was found to be the most taxonomically diverse site, followed by D, E, A, and C. This means that the morphospecies found in A are relatively more evenly distributed among a number of genera, that even though site C was found to be the most species diverse, it had the least taxonomically diverse assemblage of myxomycetes.

There have been two major papers (Reynolds 1981; Pecundo et al. 2017) that considered myxomycetes from the province of Palawan prior to the present study. Reynolds (1981) noted 26 species, while Pecundo et al (2017) listed 33 species, with 22 of these new to the Palawan group of islands. With the exception of the one unassigned species [*Perichaena* (Trichiida: Trichiidae)], this study adds eight new morphospecies for the collective islands of the province of Palawan, updating the total known to 56 species. *Badhamia macrocarpa* also represents as a new record for the country, which brings the new total for the Philippines to 159. Dagamac and dela Cruz (2019) already tallied 159 for the country after eight additions by Macabago et al. (2017) and one by Bernardo et al. (2018) to the previous summary of 150 by Dagamac and dela Cruz (2015). In the latter, however, the species *Didymium anellus* Morgan was counted twice; therefore, the corrected total then was 149. This now amended the total number of myxomycetes for the Philippines to 159.

Table 3. The updated list of myxomycetes reported from the Palawan group of islands.

Myxomycetes of the Palawan group of islands	**Synonym (as reported)**
Arcyria cinerea (Bull.) Pers. +	
Arcyria denudata (L) Wettst.+	
Arcyria incarnata (Pers.) Pers. +	
Arcyria obvelata (Oeder) Onsberg +-	*Arcyria nutans*
Badhamia macrocarpa (Ces.) Rostaf. *	
Ceratiomyxa fruticulosa var. *fruticulosa* (Müll.) T. Macbr. +	
Clastoderma debaryanum A.Blytt ++	
Collaria arcyrionema (Rostaf.) Nann.-Bremek. ex Lado.++	
Comatricha nigra (Pers. ex J.F.Gmel.) J.Schröt. ++	
Comatricha pulchella (C.Bab. & Berk.) Rostaf. ++	
Comatricha tenerrima (M.A.Curtis) G.Lister. ++	
Cribraria atrofusca G.W. Martin & Lovejoy +	
Cribraria cancellata (Batsch) Nann.-Bremek. +-	*Dictydium cancellatum*
Cribraria microcarpa (Schrad.) Pers. +	
Cribraria violacea Rex *	
Diachea leucopodia (Bull.) Rostaf. +	
Diachea subsessilis Peck ++	
Diderma effusum (Schwein.) Morgan ++	
Diderma hemisphaericum (Bull.) Hornem. +	
Didymium iridis (Ditmar) Fr. ++	
Didymium nigripes (Link) Fr. +	
Didymium ochroideum G. Lister *	
Didymium squamulosum (Alb. & Schwein.) Fr. & Palmquist +	
Echinostelium minutum de Bary ++	
Fuligo aurea (Penz.) Y. Yamam. +-	*Erionema aurea*
Hemitrichia calyculata (Speg.) M.L. Farr +-	*Hemitrichia stipitata*
Hemitrichia pardina (Minakata) Ing ++	
Hemitrichia serpula (Scop.) Rostaf. ex Lister ++	
Lamproderma scintillans (Berk. & Broome) Morgan ++	
Lycogala epidendrum (L.) Fr. +	
Perichaena chrysosperma (Curr.) Lister +	

Myxomycetes of the Palawan group of islands	Synonym (as reported)
Perichaena depressa Lib. +	
Perichaena dictyonema Rammeloo *	
Perichaena vermicularis (Schwein.) Rostaf. *	
Physarum album (Bull.) Chevall. ++	
Physarum bivalve Pers. ++	
Physarum cinereum (Batsch) Pers. ++	
Physarum compressum Alb. & Schwein. ++	
Physarum decipiens M.A.Curtis ++	
Physarum echinosporum Lister *	
Physarum leucophaeum Fr. ++	
Physarum melleum (Berk. & Broome) Massee ++	
Physarum nicaraguense T. Macbr. +	
Physarum notabile T. Macbr. +	
Physarum oblatum T. Macbr. *	
Physarum pezizoideum (Jungh.) Pavill. & Lagerh. +	
Physarum pusillum (Berk. & M.A. Curtis) G. Lister *	
Physarum stellatum (Massee) G.W. Martin +	
Physarum viride (Bull.) Pers. ++	
Reticularia lycoperdon (Bull.) +	
Stemonitis axifera (Bull.) T.Macbr. + -	*Stemonitis smithii*
Stemonitis fusca Roth +	
Stemonitis herbatica Peck +	
Stemonitis splendens Rostaf. ++	
Stemonitopsis typhina (F.H. Wigg.) Nann.-Bremek. +-	*Comatricha typhoides*
Willkommlangea reticulata (Alb. & Schwein.) Kuntze ++	

Legend:
* = new records for Palawan from this study
+ = records from Reynold's (1981) annotated list
++ = new records from Pecundo et al. (2017)
 - = taxon listed as its synonym (as shown on the next column)

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