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Original article

Diversity of plasmodial slime molds (myxomycetes) in coastal, mountain, and community forests of Puerto Galera, Oriental Mindoro, the Philippines

Nikki Heherson A. Dagamac [a,*], Maria Angelica D. Rea-Maminta [b], Nathan S. Batungbacal [b], Soo Hyun Jung [b], Carmela Rina T. Bulang [b], Akira Gioia R. Cayago [b], Thomas Edison E. dela Cruz [b,c,**]

[a] Institute of Botany and Landscape Ecology, Ernst-Moritz-Arndt University of Greifswald, D-17487, Greifswald, Germany
[b] Department of Biological Sciences, College of Science, University of Santo Tomas, Manila, Philippines
[c] Fungal Biodiversity and Systematics Group, Research Center for the Natural and Applied Sciences, University of Santo Tomas, Manila, Philippines

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ABSTRACT

No profiling of diversity of myxomycetes has ever been conducted in one of the biodiversity hotspot areas in the Philippine archipelago, and this necessitates a swift survey of myxomycetes in Puerto Galera, Oriental Mindoro. An assessment of diversity of myxomycetes collected from seven collecting points of three different forest types in the study area showed a total of 926 records of myxomycetes. Of which, 42 morphospecies belonging to 16 genera are reported in this study. Species richness of myxomycetes was higher in collecting points that were found in inland lowland mountain forests, but the most taxonomically diverse species was found in coastal forests. Myxomycete species, namely, *Arcyria cinerea*, *Diderma hemisphaericum*, *Physarum echinosporum*, *Lamproderma scintillans*, and *Stemonitis fusca*, were found in all the collecting points. Manmade disturbances and forest structure may affect the occurrence of myxomycetes.

Introduction

Puerto Galera (Philippines), with diverse coral reef diving spots and rich presence of endemic species in its nearby mountain areas, was designated by the United Nations Educational, Scientific and Cultural Organization (UNESCO) as a Man and Biosphere Reserve. The general topography of Puerto Galera is characterized by rugged terrains with occasionally dense jungle and an irregular coastline with crystal clear water and white sand beaches. Due to the presence of various types of ecosystems in Puerto Galera, i.e. savannas, grasslands, dipterocarp forests, and coastal ecosystem, the floral and faunal species of this area have been well documented and reported by a large number studies. However, diversity studies on terrestrial protists, which are known to be an abundant community in any soil vegetation, found in this pristine hotspot have not yet been fully documented. This particularly holds true for the understudied myxomycetes, more commonly known as plasmodial slime molds.

Myxomycetes are an enthralling group of amoeboid eukaryotic organisms, which were previously correlated with fungi for many years but are now grouped with the protists (Adl et al 2005; Baldauf 2008). In addition, the role of myxomycetes in the environment is neither as decomposers nor as pathogens (Keller and Braun 1999), but they are assumed to be microbial predators that are utilized in the soil ecosystem by feeding on microorganisms, i.e. bacteria, yeasts, and fungal spores, during their amoeboid stage (Ing 1994). Recent phylogenetic studies suggest that myxomycetes form a monophyletic taxon that belongs to the supergroup Amoebozoa (Pawlowski and Burki 2009; Fiorre-Donno et al 2010). They are characterized by a complex life cycle composed of two trophic stages: a uninucleate amoeboid stage or biflagellate swarm cell, and a multinucleate plasmodial stage or sexual diploid stage (Everhart

* Corresponding author. Tel.: +49 3834 86 4132.
** Corresponding author. Tel.: +63 731 4031.
E-mail addresses: nhadagamac@gmail.com (N.H.A. Dagamac), tedelacruz@mnl.ust.edu.ph (T.E.E. dela Cruz).
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and Keller 2008); intermittently they can occur in any of their three quiescent stages—spores, microcysts, and sclerotium. Under unfavorable conditions, the plasmodium develops into intricately structured fruiting bodies with haploid spores. Myxomycetes are extensively distributed in terrestrial environments; furthermore, they also have remarkable economic significance, for example, the use of myxomycetes as a food source for humans as well as for other lower forms of animals (Keller and Everhart 2010), and as a notable source of biofuels (Poulos and Thompson 1971), and utilization of novel bioactive metabolites for biotechnological uses (Dembitsky et al 2005). However, despite their promising applications, their distribution on a global scale and diversity among the Asia-Pacific tropics, especially the Philippines, still remain a mystery. Earlier studies on Philippine myxomycetes were carried out in the 1970s (Reynolds and Alexopoulos 1971; Uyenco 1973; Dogma 1975). At that time, a total of 107 species of myxomycetes were known from the Philippines. A few published papers on Philippine myxomycetes have been used as checklists of species, with very little attention being focused on the distribution and diversity of these cryptogamic organisms. However, investigations about diversity of myxomycetes in the Philippines started to flourish in the past years, but these studies surveyed sites mostly in the Western and Central parts of the Luzon main Island, which include different montane forest habitats (dela Cruz et al 2014; Dagamac et al 2014), ecoparks (dela Cruz et al 2010; Macabago et al 2010), and geographically isolated islands (Kuhn et al 2013; Macabago et al 2012); more recently selected areas in the Visayas island of the archipelago were also investigated (Alfaro et al 2015). These studies annotated a total of 150 myxomycete records for the country (Dagamac et al 2015a). However, this is still a significantly low account in comparison with the more studied Neotropical countries. Thus, in order to conduct further research on myxomycetes and obtain additional information on their diversity, an intensive research was conducted on the myxomycetes found in accessible forest types in Puerto Galera.

This paper will further increase and broaden the amount of available information on the distribution, species composition, and diversity of myxomycetes in the less explored Asian Paleotropics. The new findings of this study will contribute significantly to future scientific literature reviews of myxomycetes of the Asia-Pacific region, especially for comparing their distribution on a worldwide scale, i.e. Paleo- and Neotropical lowland vegetation.

Materials and methods

Study sites

A field survey and substrate collection were carried out in Puerto Galera, Oriental Mindoro, Philippines. To cover most of the forest vegetation areas on the study site, a total of seven accessible collecting forest points were arbitrarily chosen, including regions on higher elevated mountain forests in Mt Malasimbo, a completely inhabited lower-elevated community forest in the foot of Mt Talipanan, and areas in the selected coastal forest ends of Puerto Galera (Figure 1). Description of each collecting points is given in detail in Table 1.

Field collection of myxomycete specimens

Fruiting bodies of myxomycetes that were 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 were glued on herbarium trays and placed inside matchbox-sized herbarium boxes for permanent storage.

Collection of substrates and preparation of moist chambers

The random sampling technique was used to collect substrate samples of twigs, woody vines, and ground and aerial leaf litter from the seven collecting points in Puerto Galera. The collected substrates were placed inside dry paper bags and labeled properly. The substrates were air dried for at least a week prior to the preparation of moist chambers and then set up following the procedure described by Stephenson and Stempen (1994). In this study, a single moist chamber was prepared for each substrate collected. The moist chambers that were used consisted of disposable plastic Petri dishes, which were 10 cm in diameter and 4 cm deep, lined with filter papers. Samples were moistened with distilled water. After a period of 24 hours, pH of each substrate was checked using a pH meter (Sartorius PB-11) and excess water was removed up to the point such that water was adequate for the chamber to be moist. Following the incubation condition of Dagamac et al (2014), moist chambers were maintained at room temperature (22–25°C) in diffuse daylight. The moist chambers were checked three times every week for the first 2 months to detect the presence of plasmodia and fruiting bodies, and once a week for the next 2 months. If the moist chambers dried out and no plasmodia and fruiting bodies were observed, water was again added to preserve the moisture of the culture and the moist chambers were further incubated until the 16th week.

Characterization and identification of myxomycetes

The fruiting bodies of the plasmodial myxomycetes were air dried and segregated in different herbarium boxes. A Motic Moticam 1000 digital camera (Michigan, USA) was utilized to take photographs of every specimen. Fruiting body characteristics and spore morphology were described, and used as the basis for identification. In order to identify the fruiting body characteristics, the specimens were observed under a binocular stereo dissecting microscope (Amscope SE305R-P) and the following characters were noted: type, size, shape, appearance, and color. Internal structures such as capillitium and columella, and the presence and absence of lime ($CaCO_3$) were also noted. To study the spore morphology, spores from the fruiting bodies were mounted on separate slides, using lactophenol for dark spores and potassium hydroxide (KOH) for light spores, and the slides were viewed under a light compound microscope. The shape, texture, and color of spores were noted for each specimen. After the description and characterization of the fruiting bodies and spore morphology, the specimens were ready for identification. Identification of the specimens was performed by comparing their morphological characters with published data (Stephenson and Stempen 1994; Keller and Braun 1999). Web-based electronic databases, e.g. Eumycetozoan Project (http://slimemold.uark.edu), were also utilized for the verification of some morphological features. Nomenclature followed the online nomenclatural information database for eumycetozoans (http://nomen.eumycetozoa.com) and authorities were cited according to Kirk and Ansell (1992). For specimens that could not be identified fully with certainty due to some malformation but had adequate characteristics to be identified 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.

Data evaluation

A moist chamber that displayed either plasmodial or fruiting body growth was regarded positive for the existence of