

EXPLORING THE DIVERSITY OF
TROPICAL MYXOMYCETES:
A classical ecological assessment and
modern molecular approach

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vorgelegt von

Nikki Heherson A. Dagamac

geboren am 16.05.1987 in

Quezon City, Philippines

Dekan: Prof. Dr. Werner Weitschies

1. Gutachter: Prof. Dr. Martin Schnittler

2. Gutachter: Prof. Dr. Roland Kirschner

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PREFACE

This cumulative dissertation is a product of more than 3 years of field and laboratory work searching for myxomycetes especially in the unexplored areas of the tropics under the supervision of Prof. Dr. Martin Schnittler. All of the information presented henceforth was obtained from my original independent work with collaborations coming from different myxomycete experts around the world. After the abstract in **Chapter 1** and general introduction in **Chapter 2**, a total of 10 papers or manuscripts (7 of which I was the first author and 3 as a coauthor) are presented in **Chapter 3**.

Chapter 3.1 focuses on three surveys that I conducted solely in the Philippines and a review paper about the status of myxomycetes in the country. All of these are in collaboration with the Fungal Biodiversity and Systematic (FBS) Group of Dr. Thomas Edison E. dela Cruz at University of Santo Tomas, Manila, Philippines. **Chapter 3.2** concentrates on the unexplored knowledge in the Pan tropics. Initial surveys from Ethiopia (student excursion of Prof. Schnittler in 2012) and Thailand (student excursion that I participated with Dr. Martin Unterseher in 2013) enabled us to augment information about myxomycete distribution in the old world tropics. The dataset that I have from the Philippines and Thailand (with other dataset coming from Dr. Carlos Rojas [Universidad de Costa Rica, Costa Rica] and Dr. Steve Stephenson [University of Arkansas, USA]) were then assembled together with the dataset in Vietnam coming from Dr. Yuri Novozhilov of Komarov Botanical Institute, St. Petersburg, Russia to represent the Paleotropics. The biogeographical assessment of myxomycetes in the tropics was done by comparing our Paleotropic dataset with the data from the Neotropics (coming from Prof. Schnittler, Dr. Stephenson and Dr. Rojas). **Chapter 3.3** is about the molecular methods, aiming to disentangle the morphological complexities and biogeographic pattern of a well-known distributed tropical myxomycete, which I conducted in the General and Systematic Botany Group of Prof. Schnittler. **Chapter 3.4** is the three included papers, all of which is related to the ecology and diversity of myxomycetes, that I participated in as a coauthor. Two papers came from the graduate and undergraduate projects of the group of Dr. dela Cruz and the other one came from the side project of Prof. Schnittler under the DFG funded project for Dr. Pascal Eusemann. A conclusion in **Chapter 4** synthesizes the hypotheses and important findings that this study significantly contributes to the current knowledge we have in the global distribution of tropical myxomycetes.

List of publications included in this cumulative dissertation

Accepted in peer reviewed journals

- **Dagamac NHA**, Hoffman M, Novozhilov YK, Schnittler M. Myxomycetes diversity from the highlands of Ethiopia. 2016. *Nova Hedwigia* (manuscript accepted)
- Schnittler M, **Dagamac NHA**, Sauke M, Wilmking M, Buras A, Ahlgrimm S, Eusemann P. 2016. Ecological factors limiting occurrence of corticolous myxomycetes - a case study from Alaska. *Fungal Ecology* 21: 16-23.
- **Dagamac NHA**, dela Cruz TEE, Rea-Maminta MAD, Aril-dela Cruz JV, Schnittler M. 2015. Rapid assessment of myxomycete diversity in the Bicol Peninsula, Philippines. *Nova Hedwigia* online first http://dx.doi.org/10.1127/nova_hedwigia/2015/0252.
- **Dagamac NHA**, Rea-Maminta MAD, dela Cruz TEE. 2015. Plasmodial slime molds of a tropical karst forest Quezon National Park, the Philippines. *Pacific Science* 69(3):407– 418.
- **Dagamac NHA**, Rea-Maminta MAD, Batungbacal NS, Jung SH, Bulang CRT, Cayago AGR, dela Cruz TEE. 2015. Diversity of plasmodial slime molds (myxomycetes) on coastal, mountain, and community forest of Puerto Galera, Oriental Mindoro, Philippines. *Journal of Asia Pacific Biodiversity* 8:322–329.
- **Dagamac NHA**, & dela Cruz TEE. 2015. Myxomycete research in the Philippines: Updates & Opportunities. *Mycosphere* 6(6):784–795.
- Rea-Maminta MAD, **Dagamac NHA**, Huyop FS, Wahab RAB, dela Cruz TEE. 2015. Comparative diversity and heavy metal biosorption of myxomycetes from forest patches on ultramafic and volcanic soils. *Chemistry & Ecology* 31(8):741 – 753.
- Alfaro JRA, Alcaide DLIM, Agbulos JB, **Dagamac NHA**, dela Cruz TEE. 2015. The occurrence of myxomycetes from a lowland montane forest and agricultural plantations of Negros Occidental, Western Visayas, Philippines. *Fine Focus* 1: 7 – 20.

Manuscripts submitted

- **Dagamac NHA**, Rojas CA, Moreno GH, Novozhilov YK, Schlueter R, Schnittler M. Speciation in progress? A phylogeographic study among *Hemitrichia serpula* (myxomycetes) (submitted in Fungal Diversity)
- **Dagamac NHA**, Novozhilov YK, Stephenson SL, dela Cruz TEE, Rojas CA, Unterseher M, Schnittler M. Biogeographical assessment of myxomycete assemblages across the Tropics. (submitted in Journal of Biogeography)

DEDICATION

This dissertation is dedicated to my family who has always been my greatest source of inspiration. To my father **Gerardo** and my mother **Rosibe** for being the best parents I could have who raised me to become who I am today. Your wisdom, support and unconditional love have always helped me on every life decisions I made and will make eventually in the future. Because of you, I learned to appreciate that the simplest things in this world are what matters the most.

“Change the way you look at things and the things you look at change”
Wayne Dyers

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Abstract

CHAPTER 1

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1.1. Deutsch

Myxomyceten sind pilzähnliche Protisten der Großgruppe Amoebozoa, die in allen terrestrischen Ökosystemen häufig vorkommen. Hauptsächlich wegen ihrer makroskopisch sichtbaren Fruchtkörper ist die Ökologie und Diversität von Myxomyceten besser bekannt als die vergleichbarer Protistengruppen, aber es gibt noch große Kenntnislücken in den globalen Diversitätsmustern, denn tropische Gebiete, insbesondere die altweltlichen Tropen, sind noch unzureichend untersucht. In dieser Dissertation wurde eine Kombination aus klassischen ökologischen und molekularen Methoden verwendet, um das Wissen um Diversität und Biogeographie der Myxomyceten in der Paleotropis zu erweitern.

Feldarbeiten in verschiedenen Gebieten der Philippinen sollen das Wissen zur Verbreitung von Myxomyceten in Südostasien erweitern. Mit einer Kombination von Aufsammlungen direkt im Feld und ca. 2500 Substratkulturen wurden vier bisher unerforschte Gebiete untersucht: die Bicol Halbinsel (746 Belege, 57 Taxa), Puerto Galera (926 Belege, 42 Taxa), Quezon National Park (205 Belege, 35 Taxa), und die Provinz Negros (193 Belege, 28 Taxa). Damit sind nun 150 Taxa für die Philippinen nachgewiesen, ein Beleg, *Stemonaria fuscoidea*, erwies sich als neu für die Paleotropen Asiens. Gebiete mit artenreicheren Pflanzengesellschaften zeigten ebenfalls eine höhere Diversität für Myxomyceten. Wie auch in den Neotropen sind anthropogene Störungen und Struktur der Wälder entscheidende Faktoren für das Vorkommen von Myxomyceten.

Eine Untersuchung in einer anderen paleotropischen Region, dem Hochland Äthopiens, erbrachte 151 Belege; alle 39 gefundenen Arten sind neu für dieses Land. Drei Nachweise einer eventuell *Diderma miniatum* zuzuordnenden Art mit einer dauerhaften, leuchtend roten Peridie und ein Nachweis einer *Didymium flexuosum* ähnelnden Fruktifikation, deren Sporen aber ein breit gebändertes netzförmiges Ornament haben, wurden ausführlich beschrieben und sequenziert, da es sich um neu zu beschreibende Arten handeln könnte. Eine Reihe selten gefundener Arten wie *Didymium saturnus*, *Metatrichia floripara*, *Perichaena areolata* und *Physarina echinospora* zeigen, dass die ostafrikanischen Gebirge ähnlich ihrer einzigartigen Flora auch für Myxomyceten sehr divers und verschieden von anderen Gebieten sind.

Ein Ansatz dieser Dissertation war die Erstellung eines qualitativ hochwertigen und umfangreichen Datensatzes zu Vorkommen von Myxomyceten aus der Paleotropis, um diesen mit bereits ein Jahrzehnt früher erstellten Daten aus der Neotropis zu vergleichen. Insgesamt acht regionale Artenlisten (mit je zwei aus dem Tiefland, zwei aus dem Hochland für je Neo- und Paleotropis) bilden die Grundlage für eine vergleichende Untersuchung. Jede Artenliste stammt aus seiner Region mit relativ homogener natürlicher Vegetation und setzt sich aus Feldaufsammlungen und Nachweisen aus Substratkulturen zusammen. Eine statistische Auswertung der Artenakkumulationskurven zeigte, dass 70-95% der theoretisch zu erwartenden Artenzahl gefunden wurde. Auch für Artenlisten mit mehr als 1000 Belegen scheint sich dieser Prozentsatz kaum zu verbessern; da ein hoher Anteil der Arten mit nur ein- oder wenige Male nachgewiesen wurde. Sowohl Ordinationsmethoden als auch Clusteranalysen zeigen, dass sich die Unterschiede in den regionalen Artenlisten deutlich besser durch geographische Separation als durch Höhendifferenzen (Tiefland versus Hochland) erklären lassen.

An *Hemitrichia serpula*, einem weitverbreiteten tropischen Myxomyceten, wurde eine biogeographische Untersuchung mit molekularen Markern durchgeführt. Es handelt sich um eine morphologisch unverwechselbare Art mit Fruktifikationen, die ein goldgelbes Netz bilden. Geringfügige Unterschiede in der Ornamentierung der Sporen weisen jedoch auf die Möglichkeit kryptischer Artbildung hin. Mit partiellen Sequenzen zweier unabhängiger Markergene, der kleinen Untereinheit des rRNA Genes (SSU, ein nukleares Gen mit mehreren Kopien in extrachromosomaler DNA, die keine mendelsche Vererbung zeigt, 135 Sequenzen) und dem Proteinverlängerungsfaktor 1 alpha (EF1A, ein nukleares und chromosomal codiertes Gen mit mendelscher Vererbung, 30 Sequenzen) wurden 135 Aufsammlungen weltweit untersucht. Phylogenien beider Marker zeigen übereinstimmend vier Zweige, die jeweils durch eine unverwechselbare Kombination aus SSU- und EF1A-Genotypen charakterisiert sind und daher sehr wahrscheinlich reproduktiv isolierte biologische Arten innerhalb der Morphospezies *H. serpula* darstellen.

Ein Manteltest mit partiellen SSU-Sequenzen weist auf geographische Differenzierung innerhalb der Morphospezies hin: paarweise berechnete geographische und genetische Distanzen korrelieren mit $R=0.467$; dieser Wert liegt klar außerhalb des 95% Konfidenzintervalls aus 999 Permutationen (-0.013 to 0.021). Die biogeographische Analyse zeigt regionenspezifische innerartliche Variation, welches auf limitierten Genfluss innerhalb der Weltpopulation schließen lässt. Dieses Muster wird am besten durch kryptische, jedoch noch nicht abgeschlossene Artbildung erklärt. Eine eventbasierte Rekonstruktion des hypothetischen evolutionären Ursprungsareals mit dem Algorithmus S-DIVA (implementiert in der Software RASP) zeigt, dass die gefundenen Ribotypen auf ein globales Ausbreitungsereignis aus der Neotropis zurückgehen. Zusätzlich aus der Korrelation von Klimavariablen und Vorkommen berechnete potentielle Areale sind für die drei am stärksten getrennten Zweige deutlich verschieden; in diesem Sinne stützen die Befunde für *H. serpula* die "moderate endemism" Hypothese für Protisten.

Zusammenfassend ergeben diese Untersuchungen, dass die Myxomycetengesellschaften der Paleotropis (1) artenreicher als die der Neotropen sind, (2) trotz ähnlicher makrökologischer Bedingungen nur dort vorkommende Arten aufweisen, (3) durch die geographische Separation Artbildung auf dem Niveau morphologischer, aber auch biologischer Arten möglich erscheint, und (4) das Modell ubiquitärer Verbreitung in dem Sinne bestätigen, dass der Genfluss durch Fernverbreitung von Sporen ausreicht, das gesamte potentielle Areal einer Art zu besetzen. Aber, dieser Genfluss reicht (5) nicht aus, um die Variation zwischen regionalen Genpools auszugleichen, welches zu Artbildung nach dem Modell des regionalen Endemismus führen kann. Für ein umfassendes Bild der Diversität tropische Myxomyceten sind die vorhandenen Daten noch nicht ausreichend, aber die Daten dieser Dissertation legen ein erstes Fundament für weitere Untersuchungen.

1.2. English

Myxomycetes are fungus-like protists of the supergroup Amoebozoa found to be abundant in all terrestrial ecosystems. Mainly based on its macroscopically visible fruit bodies, our knowledge on ecology and diversity of myxomycetes is better than for most other protistean groups, but there is still a lacking knowledge about global diversity patterns since tropical regions, especially the old world tropics, are still understudied. In this thesis a combination of classical ecological analyses and modern molecular methods were used to expand the current knowledge on myxomycete diversity and biogeography in the Paleotropics.

A number of surveys in the Philippine archipelago are conducted to provide and to add information about the distribution of myxomycetes in the Southeast Asian region. A combination of field collecting and ca. 2500 moist chamber cultures from four unexplored areas in the Philippines, namely, the Bicol Peninsula (746 records, 57 taxa), Puerto Galera (926 records, 42 taxa), Quezon National Park (205 records, 35 taxa), and Negros Province (193 records, 28 taxa), now brings the number of species recorded for Philippines to 150; with one record, *Stemonaria fuscoidea*, noted as new for the Asian Paleotropics. Collecting localities that have more diverse plant communities showed as well higher species diversity of myxomycetes. In congruence with studies from the Neotropical forests, it seems also that anthropogenic disturbances and the type of forest structure affect the occurrence of myxomycetes for the Philippines.

Another survey carried out in another paleotropical region, the highlands of Ethiopia, revealed a total of 151 records, with all 39 species found as new for the country. Three records of *Diderma* cf. *miniaturum* with a strong bright red peridium and one record of *Didymium* cf. *flexuosum* with a conspicuous broad reticulation in the spore ornamentation were described and barcoded, since both may represent morphospecies new to science. A number of rarely recorded species, like *Didymium saturnus*, *Metatrichia floripara*, *Perichaena areolata*, and *Physarina echinospora* showed that resembling to its unique flora, the east African mountain ranges harbor a diverse and distinctive myxomycete assemblage.

One incentive of this study was to compile a solid large dataset for the Paleotropical region that is comparable to data obtained from comprehensive studies performed in the Neotropical areas a decade ago. A total of eight surveys (with four comprehensive regional surveys, two from lowland and two from highland, for each region, the Neo- and the Paleotropics) were used, to compare the myxomycete assemblages of both regions. Each survey comes from a region with fairly homogenous vegetation, and includes specimens from both field and moist chamber cultures component. A statistical analysis of species accumulation curves revealed that only between 70 and 95% of all species to be expected have been found. Even for >1000 specimens per survey these figures seem hardly to increase with increasing collection effort, since a high proportion of species is always represented by a single or a few records only. Both ordination and cluster analysis suggests that geographical separation explains differences in species composition of the myxomycete assemblages much better than elevational differences.

The molecular component of this thesis is a phylogeographic study of the widely distributed tropical myxomycete *Hemitrichia serpula*. It is a morphologically distinct species with golden-yellow fructifications forming a reticulum. However, subtle variation in spore ornamentation points to cryptic speciation within this myxomycete. Using two independent molecular markers, 135 partial sequences of the small subunit (SSU) rRNA (a nuclear but extrachromosomal gene) and 30 partial sequences of the elongation factor 1 alpha gene (EF1A) (a nuclear gene), a study of 135 *Hemitrichia serpula* specimens collected worldwide revealed the existence of four clades that are likely to represent reproductively isolated biospecies, since each clade shows a unique combination of SSU and EF1A genotypes.

A Mantel test with the partial SSU sequences indicated geographical differentiation, giving a correlation coefficient of 0.467 between the pairwise computed geographic and genetic distances, compared with the 95% confidence interval from 999 permutations (-0.013 to 0.021). Biogeographical analysis of the 40 SSU ribotypes showed clear intraspecific variation and geographic differentiation demonstrating a limited gene flow among the world population. We argue that the distribution of cryptic species in the different clade can be explained by ongoing, but still incomplete speciation. An event-based ancestral area reconstruction using the software S-DIVA employed in RASP showed that the probable origin of the ribotypes was a global dispersal event in the Neotropics. Additional species distribution models that were implemented for the three most prominent clades show different putative ranges. As such *H. serpula* supports the moderate endemism hypothesis for protists.

In summary, myxomycete assemblages in the Paleotropics (1) displayed a higher diversity than for Neotropical forests, (2) harbor unique taxa that differentiates those assemblages in spite of the expected similar macroecological all over the Tropics, (3) are affected by geographical barriers that likely causes speciation both at a morphospecies and biospecies level, and (4) follow the ubiquitous model in the sense that gene flow mediated by long-distance dispersal of spores is high enough that a species can fill out its entire putative range, but (5) the gene flow is not high enough to prevent variation in regional gene pools, which may lead to speciation and is better explained by the moderate endemism model. Our data are still too limited to draw a comprehensive picture of the diversity of tropical myxomycetes, but the baseline information compiled with the aid of both classical ecology and molecular approaches from this study are first major steps towards this goal.

General Introduction

CHAPTER 2

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2.1. BACKGROUND

2.1.1. The slime molds

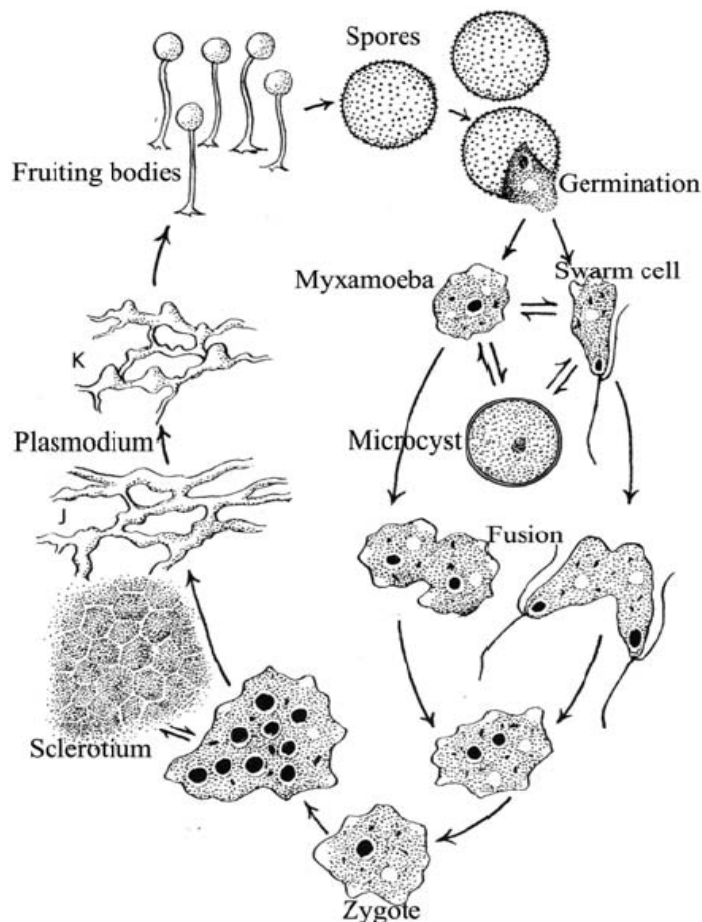


Fig 1. The typical life cycle of a myxomycete. (image adapted from Rollins & Stephenson 2011)

The slime molds or myxomycetes have been recognized for many centuries now when the German mycologist, Thomas Pankow initially described *Lycogala epidendrum* (L.) Fr. in 1654. (Martin & Alexopoulos 1969). Still, the first spore germination studies are accounted to Heinrich Anton de Bary who started to investigate their life cycles starting from spore formation up to the development of fruiting bodies (Everhart & Keller 2008). He then established that the myxomycetes do not belong to the group Gasteromycetes, and coined the term Mycetozoa. Because of the recognized animal-like feature like the capability to move across any substrata through the use of microfilament rearrangements and cytoplasmic streaming (Clark & Haskins 2015; Nagai et al. 1978), they were previously

classified in the Kingdom Animalia (Class Mycetozoa). More so, myxomycetes have been also previously

categorized in the Kingdom Plantae (Class Myxomycota) but since myxomycetes are usually found in habitats where fungi are usually seen and they display fungus-like reproductive stage (Keller and Braun 1999), they were even treated as taxa within the Kingdom Fungi (Class Myxomycetes). However, recent development 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 genetic analysis supported the classification of myxomycetes in the Kingdom Protista along with other amoeboid eukaryotic microorganisms (Fiore – Donno et al. 2010; Spiegel et al. 2004). These eukaryotic, phagotrophic fungus-like protists (Schnittler et al. 2012), with about more than 1 000 species described worldwide (Lado 2005-2015) are remarkably known to inhabit decaying organic matter in terrestrial forest ecosystems (Schnittler et al. 2012; Kosheleva et al. 2008) i.e. decaying logs, twigs and leaf litters, where they ingest soil microorganisms by moving over the surface of the substratum and simultaneously engulfing yeasts, algae, fungal spores, bacteria and other microorganisms as their food source. Thus, they are considered as “microbial predators” in nature (Keller et al. 2008), which are thought to play an important role in maintaining balance among several microbial populations in the soil (Stephenson et al. 2011; dela Cruz et al. 2010). Their ecological role identified from being

more of a scavenger rather than a decomposer poses a major problem to fully understand their ecological behavior since they are not directly affected by any environmental factors that would affect their food source. At present, factors like pH (Snell and Keller 2003; Everhart et al. 2008; Kilgore et al. 2009), soil chemistry (Novozhilov and Schnittler, 2008), moisture and temperature (Stephenson and Stempen, 1994; McHugh 2005) are the only variables that can be explored and be evaluated.

Their typical life cycle involves multifaceted yet fascinating stages. The myxomycete life cycle consists of two vegetative stages: (1) a uninucleate amoeba with or without flagella and (2) a multinucleate, unicellular structure known as a plasmodium (Clark & Haskins 2012) that typically flourishes in cool and shady moist environments (Ko Ko et.al 2011). Upon the release of spores in the fruiting bodies, haploid, unicellular myxamoebae, then germinate from these spores. At this point of their life cycle, the myxamoebae are encapsulated in a cell membrane and if there is adequate amount of water in the surrounding, flagella develops from the myxamoebae (at this point it is usually referred as the amoeboflagellated cells or the swarm cells). The myxamoebae undergo mitosis and cytoplasmic division creating large amount of clonal population. During this stage of their life cycle, the swarm cells or myxamoebae at times undergo a dormant stage and form microcysts. However, not much evidence proves that microcysts are formed directly from swarm cells, as it is clearly in the case of myxamoebae (Everhart & Keller, 2008). When the myxamoebae or two swarm cells of genetically compatible mating types fuse, the gametes form then a diploid zygote. The zygote starts to feed, initiates cell division without cytokinesis and form an assimilative, multinucleated, amorphous mass of cytoplasm identified as the plasmodium. Essentially, the macroscopic plasmodium is just a single cell (Rollins & Stephenson 2011) that moves out of its habitat and into a drier, more exposed location where it develop into either solitary or aggregate forms of fruiting bodies (Clark & Haskins 2013; Everhart & Keller 2008). During unfavorable conditions, the plasmodium can either creep to a new location or transforms into its resting state known as sclerotium, for a long period of time until conditions become favorable again. Once the suitable conditions return, the sclerotium will once again feed, crawl out and gives rise to the reproductive stage, a somewhat fungus-like fruiting body (Clark & Haskins 2013; Rojas & Stephenson 2007). These fruiting bodies shows elaborate and intricate morphological structures, each are containing many amounts of spores that are primarily thought to be dispersed by air currents (Rojas & Stephenson 2012; Stephenson & Tesmer 2008). Fruiting bodies form one of four general types: (1) sporangium, (2) plasmodiocarp, (3) pseudoaethalium, or (4) aethalium. Spores from the fruiting bodies are formed by some portions of the plasmodial protoplasm that houses individual nuclei. Meiosis in the spores may (resulting to a haploid spores) or may not (apomixis) happen during the spore formation. When spores mature, they will germinate under the appropriate conditions and the life cycle starts again (Everhart & Keller 2008; Stephenson & Stempen 1994,). This remarkable life strategy from an animal-like to a fungus-like form makes the myxomycetes as promising biological model systems for cellular development in lower life forms (Kuhn et al. 2013; Keller & Everhart 2010).

There are six orders previously recognized as myxomycetes. Ceratiomyxales is characterized by having an erected sporangia, each with many stalked spores borne externally and is represented by the genus *Ceratiomyxa*. Traditional classifications of myxomycetes exclude Ceratiomyxales as an order since they are distinctly different from members of the other orders, and many modern workers have removed these organisms from the myxomycetes and reassigned them to the protostelids (Stephenson et al, 2008). *Echinosteliales* is a little order characterized by a protoplasmodium, single, tiny sporangiate fruiting body (usually less than one mm), light coloured spores in mass, and are normally found on the bark of living trees and vines as their microhabitat. The *Liceales* is a heterogeneous assemblage of species that have either a phanerosplasmodium or protoplasmodium, fruiting bodies that lack a columella, true capillitium, and calcium

carbonate. The *Trichiales* are characterized by having fruiting bodies with light coloured spores in mass, the presence of a true capillitium, and absence of a columella and calcium carbonate. The *Stemonitales* are characterized by an aphanoplasmodium and sporangia that may appear separated from each other or pseudoaethaloid due to nearly closed or clustered grouping. The sporangia have dark coloured spores in mass and are characterized by occurrence of a true capillitium and absence of calcium carbonate (Keller and Braun, 1999). The *Physarales* is by far the biggest order and has large phaneroplasmodia and fruiting bodies with dark coloured spores in mass. Fruiting bodies have a true capillitium and granular or crystalline calcium carbonate (Keller and Braun, 1999). The phylogeny of myxomycetes consisted previously of two major groupings based on their spore color (Lister & Lister 1925). The order Amaurosporales consisted of taxa with violet–brown, purplish gray or colorless spores and included species from the present Orders *Physarales*, *Stemonitales* and *Echinosteliales*. The other order, Lamprosporales, was characterized by their various colored spores and included species from the present Orders *Liceales* and *Trichiales*. However, in 1973, Ross classified the orders primarily base on their unique presumed ancient, mode of fruiting body formation, and thus proposed that the order *Stemonitales* should be estranged out and be placed as a sister group to a super-assemblage of the four remaining orders. However, molecular data analysis of their phylogenetic relationships using EF-1 α and small subunit RNA gene sequences supported the existence of five orders (Fiorre-Donno et al. 2005). It also proved that the *Echinosteliales* was a basal clade and a sister clade to the other orders. The four other orders branch off in pairs, *Stemonitales* with *Physarales*, and *Liceales* with *Trichiales*. As such, the five orders were divided into three distinct groups, consistent with the color of the spores as described by Lister & Lister (1925) signifying the relationship of spore coloration and DNA sequence variations.

2.1.2 Economic importance of slime molds

There is still quite a little knowledge about the economical significant of myxomycetes. For the last decades, much of researches concentrated on slime molds as sources of novel organic metabolites that are of medical importance. In a review of Dembitsky in 2005, a total of almost 100 natural compounds were identified coming from the myxomycetes including their chemical structures and biological activities. The metabolites with well-defined structures were included on their list namely lipids, fatty acids amides and derivatives, alkaloids, amino acids, naphthoquinone pigments, aromatic compounds, carbohydrate compounds and terpenoid compounds. From a plasmodial culture of *Didymium bahiense*, a new monoalkyl-glycerol with antimicrobial activity against *Bacillus subtilis* was identified and named as bahiensiol (Misono et al. 2003). Another novel bioactive lipid, the cyclic phosphatidic acid was isolated from *Physarum polycephalum*, which had been identified with many biological functions i.e. antimitogenic regulation of the cell cycle, regulation of actin stress fiber formation and rearrangement, inhibition of cancer cell invasion and metastasis, regulation of differentiation and viability of neuronal cells, and mobilization of intracellular calcium (Kobayashi et al. 2002). Two new polypropionate lactone glycosides from myxomycetes *Lycogala epidendrum* was also isolated and showed growth inhibitory activities against Gram positive bacteria (Rezanka & Dvorakova, 2003).

The red colored sporophores from *Arcyria denudata* had been identified to possess the major pigment arcyrarubins and arcyriflavins whose chemical structures can be synthesize into biologically active substances that is verified to have an antimicrobial activity against *Bacillus cereus*, anti-tumor activity against P388 leukemia cell lines, and has inhibition properties against protein kinase A, topoisomerases I and II and tyrosine and serine kinases (Pereira et al. 1996). Pyroloimmينوquinone alkaloids i.e. makaluvamines that can be extracted from myxomycetes *Didymium sp.* (Ishibashi et al. 2001) was found to exhibit in

in vitro cytotoxicity against the human colon tumor cell-line HCT 116, can show differential toxicity against the topoisomerase II - sensitive CHO cell-line xrs, can inhibit topoisomerase II in vitro and can exhibit in vivo antitumor activity against the human ovarian carcinoma (Ovar 3) implanted in athymic mice (Radisky et al. 1993; Barrows et al. 1993; Matsumoto et al. 1999). Another medically important compound that was isolated from the myxomycetes is the aromatic compounds like the glycosidic dibenzofuran metabolite from the slime mold *Fuligo cinerea*, fulicineroside (Rezanka et al. 2005). The said compound was found to be highly active against Gram-positive bacteria and crown gall tumors.

2.1.3. Myxomycetes researches in the tropics

Much of what is currently known about myxomycete assemblages has been derived from various intensive local surveys carried out in the temperate regions of the world that are based on the morphology of the fruiting bodies. These surveys had explored major vegetations like alpine and subalpine mountains (Novozhilov et al. 2013; Ronikier & Ronikier 2009), tundra (Stephenson et al. 2000; Novozhilov et al. 1999), winter – cold deserts (Novozhilov & Schnittler 2008; Schnittler 2001), Asian temperate forests (Schnittler et al. 2013; Takahashi 2013; Takahashi & Hada 2012) and temperate grassland (Rollins & Stephenson 2013). For the last years, diversity and ecological studies had also been accounted for the tropics, but mostly coming only from the Neotropical countries. From species listings of myxomycetes at a local scale (Rojas et al. 2013, 2012a, 2010) to comprehensive ecological studies of forest reserves (Rojas et al. 2012b) and highlands (Lado et al. 2003; Schnittler et al. 2002), these various studies adds to the current understanding we have about the worldwide distribution of myxomycetes. Available data in the tropics leads to comparably different ecological pattern among the temperate myxomycetes. Like for an instance, aerial microhabitat in the tropics harbor more myxomycete than the forest floor while to the temperate forest, it is commonly observed that myxomycete diversity is greatest in microhabitats associated with the ground litter (Stephenson et al. 2008). The unique microhabitats in the tropics i.e. inflorescence of tropical plants (Schnittler & Stephenson 2002), epiphytic liverworts (Schnittler 2001) apparently support rich species of myxomycetes, with some species like *Physarum didermoides* showing strong preference for these microhabitats.

The current knowledge about the global pattern of distribution among myxomycetes defies the traditional concept for other macroorganism that “as latitude increases, species richness decreases”. This is due to the evidences that showed higher diversity of myxomycete among the temperate areas in comparison to the tropics but perhaps this can be also attributed with the relatively small body of information regarding myxomycetes in major tropical zones. Tropical study areas play a substantial part in comprehending biodiversity distribution because (1) major percentage of the biodiversity, at least for macroscopic organisms, of the planet is expected to be high (Davis et al. 1997) and (2) of its quick rate of exposure to habitat dilapidation and biodiversity loss (Tittensor et al. 2011). In fact almost half of the known species of myxomycetes have been already recorded overall from the Neotropics (431 taxa coming from 51 genera, Lado & Wrigley de Basanta 2008) but, unfortunately, the Paleotropical counterpart in the globe still does not have a concrete number.

From the review of Rojas & Doss (2013) about the history of myxomycete research in the Neotropics, the earliest known reports for myxomycetes in the Neotropics were accounted from Chile (Bertero 1828) and Peru (Rudolphi 1829) but it was not until the end of the 19th century that the first publication about Neotropical myxomycetes appeared. For centuries, myxomycete listings in the region grow steadfastly, but it was only in the late 70s

when a comprehensive monograph of Farr (1976), for the Neotropical myxomycetes was made available for the public. In a review of myxomycetes distribution from 558 publications in the Neotropics by Lado & Wrigley de Basanta (2008), Mexico (323) has by far the largest number of myxomycete species records, including many new species like *Calonema foliicola* Estrada, J.M. Ramirez & Lado, *Cribraria fragilis* Lado & Estrada, *Diderma acanthosporum* Estrada & Lado, *Didymium tehuacanense* Estrada, D. Wrigley & Lado, *Perichaena stipitata* Lado, Estrada & D. Wrigley to name a few. However, it is important to stress out, that Mexico has a wide range of vegetation types investigated by many myxomycetologist since this is a country where, boreal, “strictly” Neotropical and caribbean vegetation tends to overlap. Another strongly surveyed area in the Neotropic for myxomycetes would be the Central America. First reports regarding the myxomycetes of Central America begun in 1893 when the American mycologist Thomas Macbride started to report novel species for that ecoregion (Rojas & Doss 2013). Much of the established ecological researches conducted for the Central America, and perhaps in the Neotropical region as well, occurred in countries like Costa Rica (with 208 species known; Schnittler & Stephenson 2002; Rojas et al. 2010b, Rojas & Stephenson 2007, 2012b), Ecuador (Mchugh 2005; Schnittler 2001) and Puerto Rico (Novozhilov et al. 2000; Wrigley de Basanta et al. 2008). These papers resulted with too many systematics surveys on different forest types mainly concentrated among the highlands. A recent paper about environmental niche modeling in commonly occurring Costa Rican myxomycetes recommended that more systematic survey should be conducted also among lowland forest in the country (Rojas et al. 2015). Needless to say that up to this moment, the number of other studies that explores the Neotropical myxomycetes is continuously increasing.

However, a counterpart for the intensive research efforts from the Paleotropics is still in its beginnings. For instance, the investigations about the distribution of myxomycetes in the tropical region of Africa can still be considered as relatively scarce. In fact, from a comprehensive checklist by Ndiritu et al. (2009), a total of only 294 species represented by 49 genera were reported overall in 31 countries in African continent. The most intensive study in a tropical African country is by far done in Madagascar by Wrigley de Basanta et al. (2013). That survey reported 124 myxomycete species belonging to 22 genera, 106 of which are new records for Madagascar and one new species, *Perichaena madagascariensis* D. Wrigley, Lado, Estrada & S.L. Stephenson, was reported new to science. Several investigations had also been conducted in the Southeast Asian Paleotropics countries like Thailand (132 taxa, Ko Ko et al. 2010; Tran et al. 2008), Singapore (92, Rosing et al. 2011), Myanmar (67, Ko Ko et al. 2013), Vietnam (57, Tran et al. 2014), Laos (44 Ko Ko et al. 2012) and Philippines (150, Dagamac et al. 2015a, Macabago et al. 2012). By far, Thailand and the Philippines had the more in depth studies conducted for ecological research of myxomycetes for the region in comparison to the other countries that published merely species lists. Such factors like seasonality (Takahashi & Hada 2012; Dagamac et al. 2012; Ko Ko et al. 2011), disturbance (Dagamac et al. 2015b; Rea-Maminta et al. 2015), and litter heterogeneity (Alfaro et al. 2015, Tran et al. 2006) were investigated to explain the occurrence and distribution of myxomycetes in the region. In the past 10 years alone, more interesting new species were accounted to this less explored region e.g. *Comatrachia spinispora* Y. Novozhilov & D.W. Mitchell, *Craterium retisporum* G. Moreno, D.W. Mitch. & S.L. Stephenson, *Cribraria tecta* Hooff, *Perichaena echinolophospora* Y. Novozhilov & S.L. Stephenson. But despite this growing attention in the Paleotropics, the knowledge about myxomycete in this region is still very far from complete. As a result, there is still a wide gap to fill to fully understand the taxonomy and ecology of the assemblages of myxomycetes that occur in the tropical region of the world and in particular to have a more complete understanding about their distribution in a global scale.

2.1.4. Biogeographic hypothesis about myxomycetes

Similar with other protists, there are two controversial hypotheses that is use to explain the biogeographic distribution of myxomycetes. Either they follow the Baas-Becking model of ubiquity (Finlay 2002; Finlay and Clarke 1999) or the model of moderate endemism (Foissner 1999). The former model argue that the ability of protists to have almost unlimited dispersal due to their small cell sizes cause them to be found everywhere (Finlay et al. 2004) and geographical barriers does not limit such global dispersal (Finlay 2002) but rather it is driven by random events i.e. wind, human migration, ocean currents. In that case, habitat suitability influences the occurrence of a protist species in a location and not of restricted dispersion. On the other hand, the latter model infers that some protist may be cosmopolitan but others have their own geographically restricted distribution (Foissner 2006) and some may even be endemic on that particular locality (Cotterill et al. 2008). These model attenuates the tenet of “everything is everywhere” by proposing that (a) the abundances and thus the migration rates are low in ~90% of the species, (b) extinction rates are moderate, (c) the proportion of the global species pool found locally is moderate, and (d) ~30% of the species are endemics (Foissner 2008). Understanding which appropriate model fits the myxomycetes is deemed necessary especially on the light of estimating world’s total biodiversity. If all myxomycetes are indeed cosmopolitan in distribution, then it is assumable that their global diversity is low. However, if indeed some myxomycetes can be restricted in terms of their geographic distribution and their pattern of occurrence is not by merely because of their expected habitat requirement, then their global diversity should be considerably high (Mitchell & Meisterfeld 2005).

The relatively small spore size of the myxomycetes is of course expected to be capable of such long dispersals; however, it appears that there are some species that seems to be restricted to the temperate and tropical zones (Stephenson & Stempen 1994). For instance the two species of the myxomycete *Ceratiomyxa* i.e. *C. morchella* and *C. sphaerosperma* are thought to be restricted only in the tropics (Rojas et al. 2008). Also, the patchy range of *Barbeyella minutissima* seems to be exclusive to temperate montane *Picea* and/or *Abies* forests (Schnittler et al. 2000). This kind of restriction is affecting the total species composition in a myxomycete assemblage like for the case of the paper of Rojas et al. 2012b that evaluated highland myxomycetes in the Northern America to Central America, it showed that as latitude decreases, in that case from Mexico to Costa Rica, the similarity of species composition in the temperate zone, represented by the United States, also decreases.

In another biogeographic paper of Schnittler & Mitchell (2000) that considered abundance values as a determinant, out of the 446 myxomycetes species they examined, more than half of the taxa were assigned to be rare (found in less than collection in a certain locality). This rarity and restriction to some myxomycetes species are affected by microhabitat availability and macroenvironmental parameters as suggested by Stephenson et al. 2008. Nevertheless, most biogeographic studies conducted in the Americas not only supported this line of thought where abundances of myxomycetes are highly reliant to the forest structures (Rojas et al. 2011) but also with historical-geographical events (Estrada-Torres et al. 2013). All of these biogeographic studies, of which employs only morphospecies data, lead to the generalization that myxomycetes conform to moderate endemism. At the biospecies level, allopatric speciation among cryptic species population of the dark spored myxomycetes *Badhamia melanospora* resists cosmopolitanism hypothesis too (Aguilar et al. 2013). Since most of these biogeographic studies done so far use dataset from the America, what would be interesting to look at now is if moderate endemism also applies at a morphospecies and biospecies level on myxomycete assemblages along a longitudinal geography represented by the Pantropics.

2.2. GOALS OF THE STUDY

Given the current knowledge regarding the diversity of tropical myxomycetes as described in the previous section, this present study is outlined based on the following independent questions:

Question 1: Due to fairly few amount of knowledge about the Paleotropics, particularly in the local scale setting i.e. Philippine lowland mountainous vegetation, this phase of the research project wants to answer the question: How diverse are the myxomycete communities in an unexplored Paleotropic archipelago?

To answer this problem, our study specifically aims to:

- collect field specimens and set up moist chamber cultures coming from substrates collected randomly in accessible lowland forest areas in the Philippines
- create an annotated species list for some selected surveyed area
- characterized, describe and determine myxomycetes that is collected in the field and moist chamber cultures
- prepared SEM and LM photograph of slide micromorphology and fruiting body phenology of some taxonomically ambiguous specimens
- perform classical diversity assessments, with the aid of various software, in different lowland forest areas in the Philippines

Question 2: The second phase of the research study is about having a compilation of large datasets from different parts of the tropics (both the Paleo and Neotropics) and eventually comparing this huge amount of data (using diversity and evenness indices, multivariate statistics). Because of limited understanding about the distribution of myxomycetes in the tropical region, this phase of the research project attempts to answer the question: Is there a difference in myxomycete communities between the Neotropic and Paleotropic?

To answer this problem, our study specifically aims to:

- update myxomycete data in two highland forests in the Paleotropics namely (a) afro-montane areas in Ethiopia and (b) tropical montane areas in Thailand
- assemble a compiled extensive database conducted in the Southeast Asian Paleotropics that would be used for a comparative study of collated myxomycete surveys done in Central American Neotropics
- compare the species composition and species diversity using classical ecological methods,
- use multivariate analysis to compare myxomycete communities

Question 3: Most of genus belonging to the myxomycetes is of cosmopolitan in distribution. Like the well-known pretzel slime mold, *Hemitrichia serpula* (Scop.) Rost. (Trichiaceae) that is a widely spread species in the tropics, it still hints morphological variation despite of it being a clear cut morphospecies across regions. Since recent molecular studies among the myxomycetes had already revealed some hidden diversities it is interesting to posed the question: Can geographical separation influence gene flows in a known tropical myxomycete population?

To answer this problem, our study specifically aims to:

- investigate intraspecific variation among the genotypes of *Hemitrichia serpula* population by means of molecular methods
- correlate geographic distances with genetic distance using Mantel Test
- test the moderate endemism hypothesis using species distribution modeling
- infer an event based ancestral area reconstruction that would explain the distribution of *Hemitrichia serpula* genotypes using Statistical Dispersal Vicariance Analysis.

2.3. SIGNIFICANCE OF THE STUDY

There is still a major gap of understanding with regards to the global pattern of distribution for myxomycetes that need to be filled. This is the reason why the major focus of my research work is about using both the conventional ecological assessment tools (species richness, species diversity, community analysis) and modern molecular methods to explore the ecology and biogeography of the unexplored tropical myxomycetes.

To accomplish this, it is of course necessary to have reference point of information covering at least a good amount of surveys for major forest types across Pantropics. Much data are available for the well-studied Neotropics, but unfortunately this is not the case in the Paleotropics. Thus, this is one incentive of my research work: to establish baseline information in a place known as a megahotspot for biodiversity. Since microbial diversity in the Philippines is not a popular choice of interest, not much is known for the country's myxoflora. My research work are able to establish local survey in three ideal but unstudied areas in the Philippines namely (1) lowland forest areas in one whole region of the country, the Bicol Peninsula, (2) a karst landscape known for high endemic floral and faunal communities, the Quezon National Park and (3) a UNESCO Biosphere site, Puerto Galera, that will add to the current knowledge about myxomycete diversity for the Philippines. The comprehensive discussions regarding the surveys for the Philippines are shown in the published papers presented in Chapter 3.1.

Moreover, my research work enables to also have additional surveys in two highland forests in the Paleotropics namely (1) the unexplored highland Afromontane forest in Ethiopia and (2) the tropical montane area in Thailand. These surveys now poses another incentive for the current knowledge we have about tropical myxomycetes because together with the dataset I have from the Philippine lowlands, I was able to generate a good amount of analogous dataset that can be compared with the readily available dataset in the Neotropics. Comparing the myxomycete communities in the tropics in an ideally "almost" similar environmental setting is a good starting point to test if myxomycetes follow moderate endemism model of protistean biogeography. Also the unique habitats in the highlands of the Paleotropical areas hint towards evidences of possible restricted species. With my research study, we can able to test these suppositions. More detailed explanations are given in the succeeding papers presented in Chapter 3.2.

However, beyond testing moderate endemism via morphological evaluation, my research work had also investigated a clear cut population of *Hemitrichia serpula* to test the influence of geographic separation at a biospecies level. This myxomycete is a widely distributed species globally and since no studies had yet to use a bright spored myxomycete to evaluate gene flow in a geographical context, this makes *Hemitrichia serpula* an ideal model. Molecular biogeography combined with species distribution modeling in myxomycetes is still a budding field. The results of my research work confirm cryptic speciation and how wide geographic separation can lead to intraspecific variation among genotypes. More elaborate discussions are presented in a manuscript prepared in Chapter 3.3.

Lastly my research work adds information about myxomycete ecology. From the collaborative studies that I participated, knowledge about myxomycetes potential for bioremediation, comparative diversity between heterogenous plant communities and monotypic agricultural plantation and habitat suitability for corticolous myxomycetes are thoroughly shown in papers written in Chapter 3.4.

2.4. LITERATURE CITED

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CHAPTER 3.1

Myxomycetes diversity of the Philippine archipelago

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Plasmodial Slime Molds of a Tropical Karst Forest, Quezon National Park, the Philippines¹

Nikki Heberson A. Dagamac,^{2,5} Maria Angelica D. Rea-Maminta,^{3,4} and Thomas Edison E. dela Cruz^{3,4}

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 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 was 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 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.

PLASMODIAL SLIME MOLDS or myxomycetes are phagotrophic eukaryotes that have bewildered many taxonomists and ecologists all over the world. Previously, myxomycetes were classified under the Kingdom Animalia (Class Mycetozoa) because 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 rearrange-

ments and cytoplasmic streaming across any substrate (Nagai et al. 1978). Because myxomycetes are usually seen in habitats where fungi are typically found and because 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- α (EF-1 α) 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 other than 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

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² Institute of Botany and Landscape Ecology, Ernst-Moritz-Arndt Universität Greifswald, D-17487 Greifswald, Germany.

³ Fungal Biodiversity and Systematics Group, Research Center for the Natural and Applied Sciences, University of Santo Tomas, España 1015, Manila, Philippines.

⁴ The Graduate School, University of Santo Tomas, España 1015, Manila, Philippines.

⁵ Corresponding author (e-mail: nhadagamac@gmail.com).

et al. 2014) of the main island of Luzon. With these intensive diversity assessments, 127 records of myxomycetes were documented in the country (dela Cruz et al. 2013), a noteworthy 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 with the biodiversity studies on myxomycetes that have been carried out in other ecoregions worldwide. 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 (13° 59' 35.4" N, 121° 49' 25.0" E) 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 ha is a lowland rain forest 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* (Department of Environment and Natural Resources 2014). The province has two pronounced seasons, dry from November to April and wet during the rest of the year, with an annual average temperature range between 23.3°C and 30.2°C and 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 were not yet in contact with the ground (aerial litter [AL]), leaves found on the forest floor (ground litter [GL]), and pieces of twigs (TW) along the forest trail, were haphazardly collected along the west and east Atimonan trail of the study area. At each part of the trail, three accessible sampling points that are approximately 200 m apart were assigned, making a total of six collecting points for the whole study (Figure 1). GPS coordinates of each sampling point 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 3 to 4 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

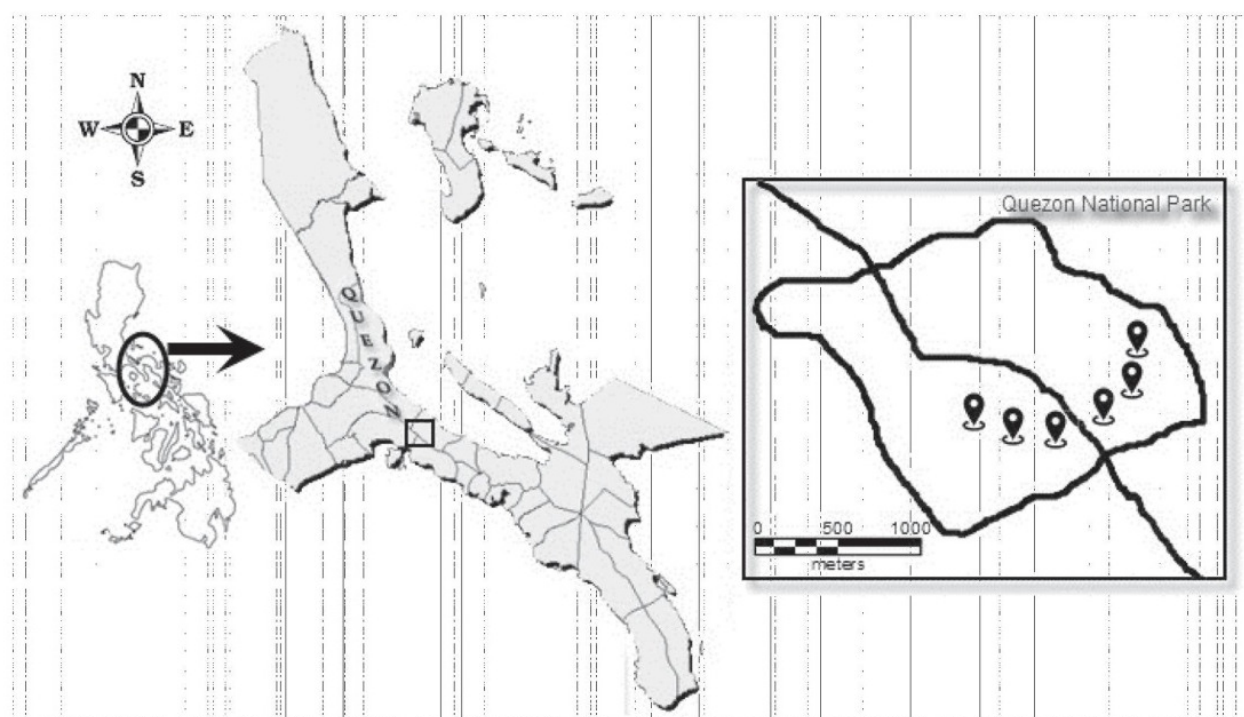


FIGURE 1. Map of the general study area showing the west and east trails of Quezon National Park with three sampling points each.

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°C–25°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 such as 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 noncalcareous-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 400× to 1,000× magnification. Identification of the species was done to the species level using Web-based identification keys (e.g., SynKey [D. Mitchell, 2008, Synoptic key to the myxomycetes, United

Kingdom] and the Eumycetozoa 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 Chao2 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, 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 percentage yield from MC in the study area. Percentage yield was then calculated as the number of MCs positive for myxomycetes divided by the total number of MCs 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 CC index is based solely on the presence

or absence of a species in the two communities being compared, whereas the PS index considers both the presence and absence of a species and its relative abundance (Stephenson et al. 1993).

RESULTS

Percentage Yield of the Moist Chambers and Species Accumulation Curve

In this study, a total of 205 records was 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 myxomycete species (Chao2) in the area is around 45.9 (Figure 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 (Figure 2b). The rarefaction curves for the three substrates

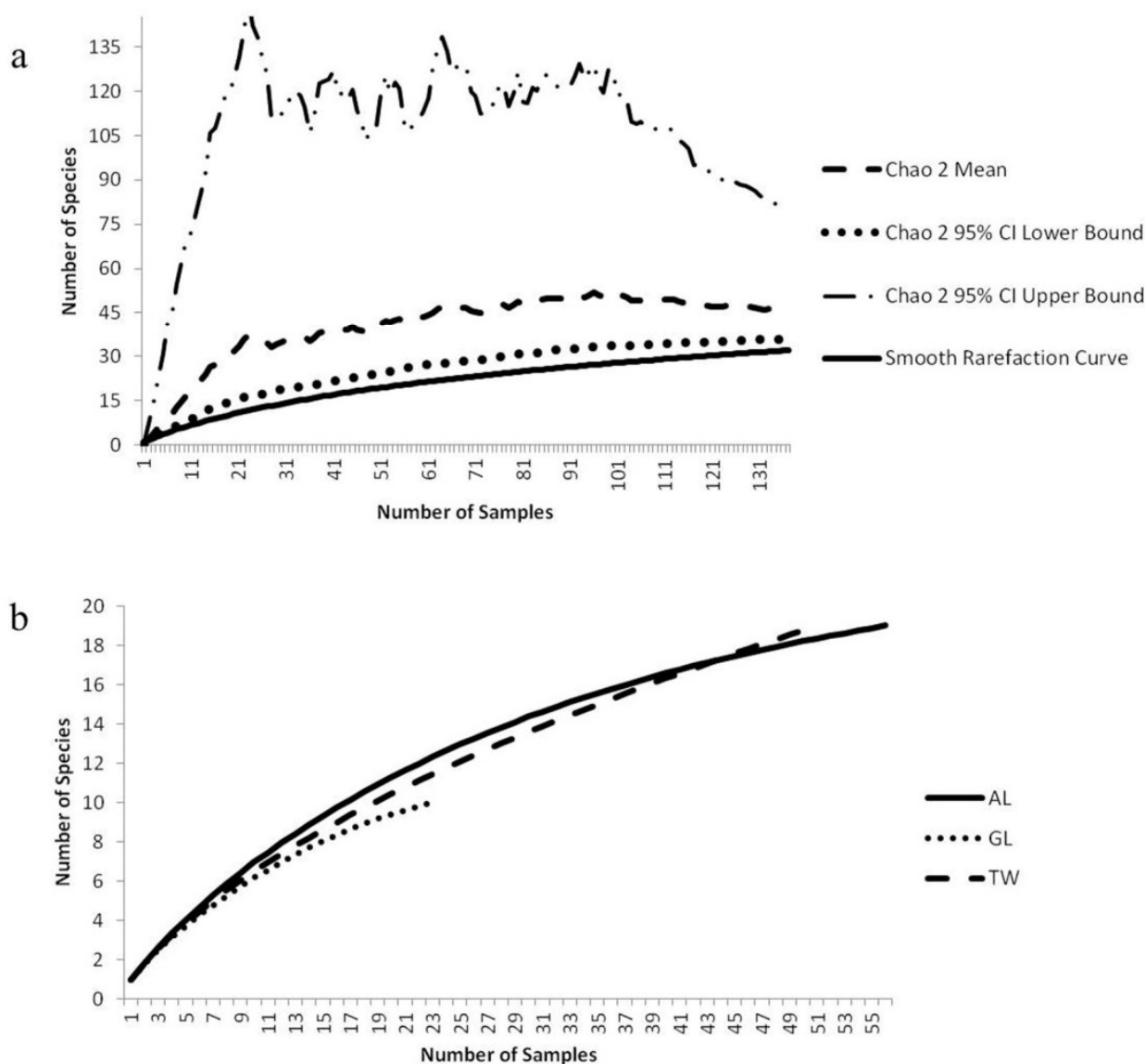


FIGURE 2. *a*, Species accumulation curve of myxomycetes sampled from the Atimonan trail of Quezon National Park and generated using EstimateS and Chao2 Estimator. *b*, Coleman rarefaction curve for the three different microhabitats used in the moist-chamber setup.

(aerial litter, ground litter, and twigs) are still progressing, suggesting that more species of myxomycetes are still to be found (Figure 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 was identified from the rapid field survey and moist-chamber cultures. From these 35 species, four species, *Arcyria denudata*, *Ceratiomyxa fruticulosa*, *Lycogala exiguum*, and *Physarum pezizoides*, were found only in 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 be identified only to the genus level because 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: 15 species had a relative abundance of less than 0.5%. Nine myxomycete species were occasional, and seven myxomycete species were common (Table 1).

Taxonomic and Species Diversity

Considering only the myxomycete 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, and ground leaf litter had 10 species belonging to eight 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 = .843, α = .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 (Figure 3). When comparing the myxomycete communities found on the different substrates, ground litter and twigs have one common species, *Cribraria violacea*. Four myxomycete species were found on all three substrates, *Arcyria cinerea*, *Diderma effusum*, *Lamproderma scintillans*, and *Perichaena depressa* (Figure 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. In this study we report 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

TABLE 1

Occurrence of Myxomycetes in the Entire Study Area, Their Abundance Index (AI), and Abundance Based on Number of Records from the Three Collected Substrates, Including Sampling Effort and Species Diversity Indices Generated from SPADE

Species	Mean pH Value of MC	AI ^a	Frequency (Pooled Data)	Records			
				Field	AL	GL	TW
<i>Arcyria afro-alpina</i> Rammeloo	5.80	R	1		1		
<i>Arcyria cinerea</i> (Bull.) Pers.	5.61	A	43	1	17	9	16
<i>Arcyria denudata</i> (L.) Wettst.		R	1	1			
<i>Ceratiomyxa fruticulosa</i> (Müll.) T. Macbr.		R	1	1			
<i>Collaria arcyrionema</i> (Rostaf.) Nann-Bremek. ex. Lado	5.73	C	4		3		1
<i>Comatricha laxa</i> Rostaf.	3.50	R	1				1
<i>Comatricha nigra</i> (Pers. ex J. F. Gmel.) Schroet.	5.60	R	1		1		
<i>Craterium minutum</i> (Leers) Fr.	5.85	O	2			2	
<i>Cribraria</i> sp.	5.30	R	1				1
<i>Cribraria violacea</i> Rex	5.82	C	5			2	3
<i>Didymium iridis</i> (Ditmar) Fr.	5.57	O	3		2	1	
<i>Didymium nigripes</i> (Link) Fr.	3.50	O	2	1	1		
<i>Didymium</i> cf. <i>ochroideum</i> G. Lister	6.00	R	1				1
<i>Didymium squamulosum</i> (Alb. & Schwein.) Fr.	6.20	O	2		2		
<i>Diderma effusum</i> (Schwein.) Morgan	5.87	C	6		3	2	1
<i>Diderma hemisphaericum</i> (Bull.) Hornem.	5.38	C	4		3	1	
<i>Echinostelium</i> sp.	6.20	R	1		1		
<i>Hemitrichia serpulata</i> (Scop.) Rostaf.	5.70	R	1				1
<i>Lamproderma scintillans</i> (Berk. & Broome) Morgan	5.99	A	9		4	2	3
<i>Lycogala exiguum</i> Morgan		O	2	2			
<i>Perichaena chrysosperma</i> (Currey) Lister	5.88	C	4		3		1
<i>Perichaena depressa</i> Libert	5.67	A	9		2	2	5
<i>Perichaena dictyonema</i> Rammeloo	4.85	O	2		2		
<i>Perichaena pedata</i> (Lister & G. Lister) G. Lister	6.07	C	6		4		2
<i>Physarella oblonga</i> (Berk. & M. A. Curtis) Morgan	4.90	R	1				1
<i>Physarum</i> sp.	5.70	R	1				1
<i>Physarum album</i> (Nees) Fr.	6.30	R	1				1
<i>Physarum cinereum</i> (Batsch) Pers.	5.70	R	1				1
<i>Physarum compressum</i> Alb. & Schwein.	5.65	O	2		1	1	
<i>Physarum decipiens</i> M. A. Curtis	5.60	O	2				2
<i>Physarum melleum</i> (Berk. & Broome) Massee	5.88	C	5		4	1	
<i>Physarum</i> cf. <i>notabile</i> T. Macbr.	6.20	R	1		1		
<i>Physarum pezizoideum</i> (Jungh.) Pavill & Lagerh.		R	1	1			
<i>Stemonitis fusca</i> Roth	4.88	A	8				8
<i>Stemonitis pallida</i> 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
Chao2 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
Fisher'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

^a A, abundant; C, common; O, occasional; R, rare.

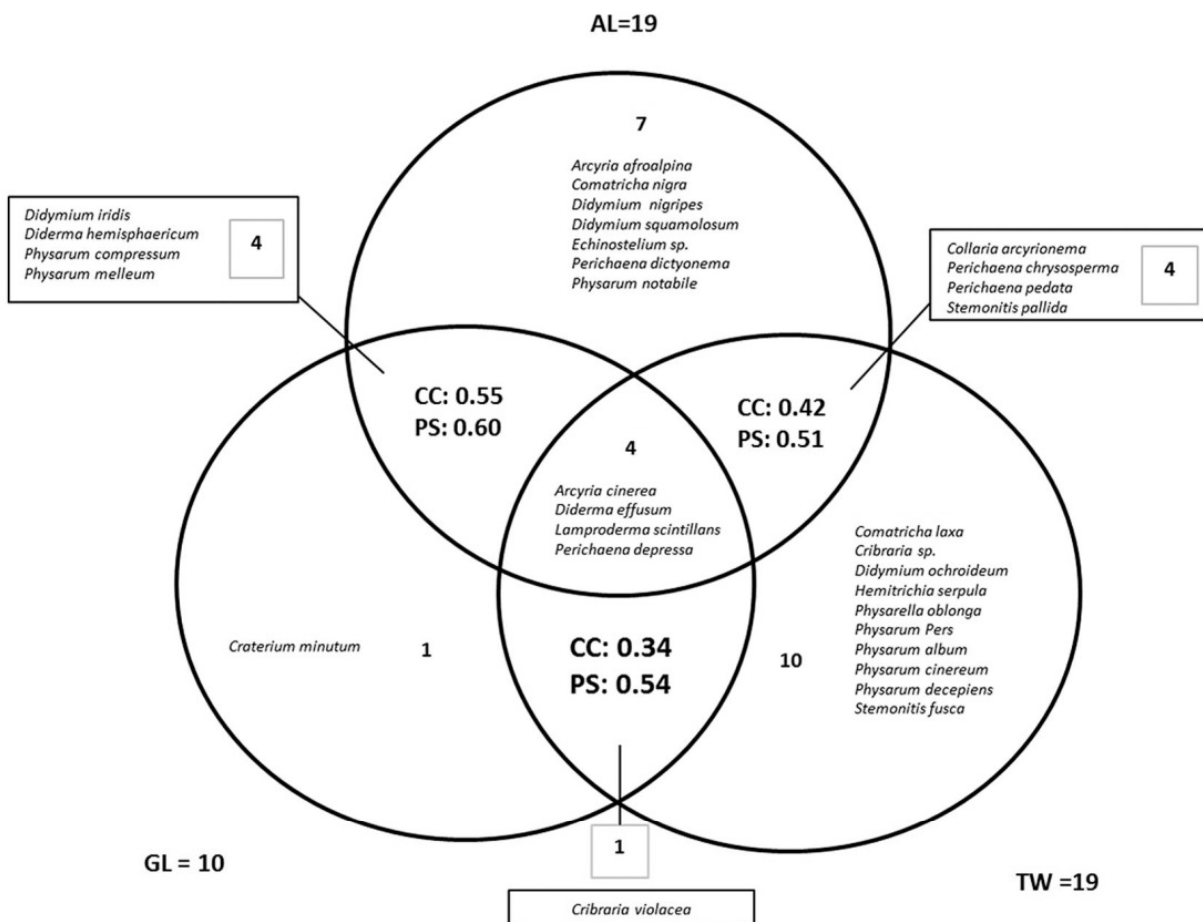


FIGURE 3. Venn diagram showing distribution of myxomycete assemblages collected from three different substrates (AL, GL, TW) and the β diversity values (CC and PS) between the communities.

has ever recorded the myxomycetes in this site.

Moist-Chamber Productivity

The high percentage yield (82%) reported in this article is comparable with 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 percentage 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

The 35 species identified in the study area are comparably greater than the number found in previous surveys conducted in other mountain forests in the Philippines. For example, a total of only 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 that 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 Amazon (Rojas and Stephenson 2012), in which aerial litter, ground litter, and twigs were also the only substrate types collected, because they harbor the most common myxomycete 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 important to note that our sampling and the number of species recovered were comparable with 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 this study were decaying organic matter randomly collected along an 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 2012). 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 twigs, 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 example, temperature and moisture are considered to be the primary factors limiting the occurrence of myxomycetes in nature (Alexopolous 1963). In addition, 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 myxomycete assemblages: the myxomycete species *Diderma hemisphaericum*, *D. effusum*, and *Physarum compressum* were 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 the plant and animal communities makes the Philippines a hot spot of global biodiversity. But this supposition is not applicable to microbial biota

such as the myxomycetes. Although all of the myxomycete species were previously reported in the country, the myxomycete assemblages reported in this article are the first for this unique tropical karst forest landscape. Of the 35 myxomycete species reported in this article, 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 with 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], and Myanmar [Ko Ko et al. 2013]), the number of myxomycetes reported 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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RAPID ASSESSMENT OF MYXOMYCETE DIVERSITY IN THE BICOL PENINSULA, PHILIPPINES

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NIKKI HEHERSON A. DAGAMAC^{*1}, THOMAS EDISON E. DELA CRUZ^{2,3},
MARIA ANGELICA D. REA-MAMINTA^{2,3}, JEANE V. ARIL-DELA CRUZ³
& MARTIN SCHNITTTLER¹

¹ Institute of Botany and Landscape Ecology, Ernst Moritz Arndt University Greifswald,
D-17489 Greifswald, Germany

² Fungal Biodiversity and Systematics Group, Research Centre for the Natural and Applied Sciences
and

³ The Graduate School, University of Santo Tomas, España 1015 Manila, Philippines

ABSTRACT

A myxomycete survey combining field collections and moist chamber cultures from seven collecting areas in the Bicol Peninsula (Philippines) yielded 57 species belonging to 18 genera. Of these, eight are reported as new records for the Philippines; *Stemonaria fuscoidea* is noted as a first record for the Asian Paleotropics. The comparison of the seven collecting localities showed higher numbers of records from areas with lowland forest characterized of having either old growth forest or successional forest patches where there is more heterogeneity of plant communities. This study is the first comprehensive report of myxomycete distribution in the Bicol Peninsula.

Keywords: Paleotropics, moist chamber culture technique, plasmodial slime molds, species list

Corresponding author: Nikki Heherson A. Dagamac, Institute of Botany and Landscape Ecology,
Ernst Moritz Arndt University Greifswald, D-17489 Greifswald, Germany. Email:
nhadagamac@gmail.com

INTRODUCTION

The myxomycetes (also known as myxogastrids or plasmodial slime molds) are phagotrophic amoeboid protists forming fruit bodies (sporocarps) that release airborne spores (Schnittler *et al.* 2012). Many species inhabit forest ecosystems and fruit on woody debris and plant litter (Stephenson & Stempen 1994). The strong partitioning of resources and surprisingly narrow niche breadths at least for the formation of fruiting bodies (Rojas *et al.* 2009, Schnittler *et al.* 2006) suggest that most species show limited patterns of distributions at both regional and local level (Rojas *et al.* 2011, Lado *et al.* 2013). Although, in comparison to other protistean groups, rather extensive data on myxomycete diversity are available (Stephenson *et al.* 2008) and the number of studies on myxomycete ecology is growing, many ecosystems have not yet been adequately surveyed (Ndiritu *et al.* 2009, Spiegel *et al.* 2004). In comparison to the Neotropics (Lado & Wrigley de Basanta 2008) the Paleotropical region of the world is severely understudied, which holds particularly true for Southeast Asia (Ko Ko *et al.* 2013, Dagamac *et al.* 2011). A first systematic survey on myxomycete diversity was conducted in Thailand (Ko Ko *et al.* 2011), whereas studies in the Philippines (de la Cruz *et al.* 2014, Macabago *et al.* 2012), Laos (Ko Ko *et al.* 2012) and Myanmar (Ko Ko *et al.* 2013) are still preliminary by nature. As a whole, none of the Southeast Asian countries shows survey intensity comparable to that of well-studied Neotropical countries like Costa Rica (Rojas *et al.* 2010). Therefore, the main goal of this study was to establish a study area in the Paleotropics to compile an annotated species list, including data on abundance and substrate preferences of species.

Unfortunately, most surveys on myxomycete diversity in the Philippines (de la Cruz *et al.* 2014, Kuhn *et al.* 2013, Dagamac *et al.* 2011), were only limited to plain species lists and targeted

upland forests. From this reason we have chosen the Bicol Peninsula as an area for intensive studies employing the moist chamber culture technique (Gilbert & Martin 1933), since it comprises large tracts of both secondary and old growth lowland forest. As such, this paper also presents the first systematic survey generating comprehensive baseline data on the composition of myxomycete assemblages and microhabitat distribution in the Philippines. In future, such data will help to test the moderate endemism hypothesis that are put forward for other eukaryotic protists (e.g., Ciliates, Foissner 2006, Aguilar 2013) by a comparison of Neo- and Paleotropical myxomycete assemblages.

MATERIALS AND METHODS

Study area and sampling localities

The Bicol Peninsula covers 12 070 km² in the south-eastern part of the Philippine archipelago (Fig 1). It gave the whole region its name and is composed of four provinces (Camarines Norte, Camarines Sur, Albay and Sorsogon). Its northern border connects to Luzon main island (province of Tayabas, Quezon). Deep coastal bays form an extended coastline around slightly rolling plains with a large lowland agricultural area and remaining pockets of forest in the hilly parts. Tropical seasons are not very pronounced but there is a relatively dry period lasting from November to April; the rest of the year is constantly wet. The area receives an average rainfall of 3 013 millimeters; annual mean temperature ranges between 27.4 °C and 29.6 °C (Department of Agriculture Region V 2014). With focus on the remaining forested areas, a northwest-southeast running transect of seven collecting localities was established during a survey conducted within May 20 to July 24, 2013:

- (1) *Daet* (14°06'19"N 122°57'06"E, ca. 230 m a.s.l.), lowland tropical moist forest, a highly disturbed remaining pocket near the town of Burgos;
- (2) *Basud* (14°03'39"N 122°57'50"E, ca. 175 m a.s.l.), lowland tropical wet forest including old growth patches with tall dipterocarps and bamboos along the strip of Bicol National Park;
- (3) *Isarog* (13°39'05"N 123°25'38"E, ca. 410 m a.s.l.), tropical mountain rainforest slightly disturbed, along a steep trail in the town of Tigaon, covered with large trees like *Shorea* spp., *Sandoricum* spp., *Vitex parviflora* and *Koordersiodendron pinnatum*;
- (4) *Asog* (13°26'44"N 123°26'07"E, ca. 380 m a.s.l.), tropical mountain wet forest trail with secondary forest patches dominated by tall forbs (Musaceae, Poaceae) and palms;
- (5) *Malilipot* (13°18'14"N 123°44'25"E, ca. 450 m a.s.l.), tropical wet forest with a rocky steep forest trail along the seven tiered waterfalls;
- (6) *Castilla* (12°57'12"N 123°52'56"E, ca. 175 m a.s.l.), lowland tropical moist forest within a wide agricultural plain maintained by local inhabitants and scattered small open canopy forest patches;
- (7) *Bulusan* (12°45'18"N 124°05'44"E, ca. 350 m a.s.l.), tropical wet forest, moderately disturbed by tourists, around a volcanic lake surrounded with tall trees, orchids and endemic giant ferns.

Field survey and moist chamber cultures

All determinable fruiting bodies of myxomycetes found along logs and litter patches over 1 km of forest trails were recorded for each of the seven collecting sites using an opportunistic sampling method (Cannon & Sutton 2004). Myxomycete specimens were initially placed on compartmentalized plastic boxes, and placed at the same day on clean herbarium trays and stored in matchboxes for permanent storage.

Material for moist chamber cultures was sampled from aerial litter, ground litter and small twigs 5–10 cm in diam. on the ground along a ca. 300 m transect established for each sampling site. By pooling enough pieces of substrate to fill a Petri Dish of 9 cm diam., one sample was gathered over a distance of every 10 m. This effort resulted in a total of 30 samples for each substrate group, giving 90 samples per study site and 630 samples in total. A single moist chamber (MC) was prepared for

each sample to avoid pseudoreplicates. Material was spread on clean Petri dishes over three layers of white toilet paper and allowed to soak with distilled water over night. After 24h excess water was poured off and pH values were determined at three different points of the wet substrate surface using an Orion model 610 pH meter with a touch down probe. All cultures were incubated under ambient light at room temperature (ca 20–24°C) for up to 90 days and were checked regularly (days 4, 9, 13, 25, 45, 60, 90) for the growth of plasmodia and/or fruiting bodies using a dissecting microscope. To maintain moisture, distilled water was added over the first six weeks of the incubation period. Mature sporocarps were directly transferred to herbarium boxes, with one record defined as a colony of fructifications from one taxon developing in one culture.

Myxomycete determination

Specimens were determined morphologically using web-based identification keys [Eumycetozoa Project (<http://slimemold.uark.edu/>)] and monographs (Neubert *et al.*, 1995–2000, Poulain *et al.*, 2011). For Trichales Liu *et al.* (2007) was consulted. Sporocarps were mounted as permanent slides in lactophenol or Hoyer's medium to preserve structures containing lime and studied with bright field and differential interference microscopy using a Leica DFC450 microscope. For the *Arcyria* complex, microscopic structures were viewed using a Scanning electron microscope (Zeiss EVO LS10). Colours are given according to the NBS/ISCC standard (Anonymous, 2012). Nomenclature follows Lado (2005–2014); authorities are cited according to Kirk and Ansell (1992). Voucher specimens are deposited in the herbarium collection of the second author in the Pure and Applied Microbiology Laboratory, Research Center for the Natural and Applied Sciences, University of Santo Tomas in Manila, Philippines; duplicates remained in the private collection of the last author deposited at the Botanical State Collection Munich (M).

Data evaluation

To estimate the extent to which the survey was exhaustive, individual-based species accumulation curves were constructed for each of the collecting localities according to the rarefaction formula using the program EstimateS (Version 9.1, Colwell 2013, 100 randomizations), which computes as well some estimators of species richness. In accordance with Unterseher *et al.* (2008), the Chao2 estimator (Chao *et al.* 2005) was chosen and calculated using the “classical settings” of EstimateS. All comparisons of abundance data for fruit bodies among collecting localities were calculated using Kruskal Wallis test employed in PAST (Hammer *et al.*, 2001). Subsequently, figures for α -diversity of the seven collecting localities and its corresponding 95% confidence interval were calculated using the classical formula for the Fisher's index generated in SPADE (Chao & Shen 2010). The same program was used to compute the Morisita index for dissimilarity values among the myxomycete assemblages from the seven collecting localities. This index ranges from 0 (identical assemblages) to 1 (total dissimilarity between the communities).

RESULTS

A total of 746 myxomycete records, including 33 from field collections and 713 from moist chamber cultures, were obtained during the course of the whole investigation. The individual-based species accumulation curves for each of the collecting localities constructed with records from the field and from moist chamber cultures (Fig. 2) show that the sampling strategy employed was still inadequate, especially in Bulusan and Daet, to recover most of the myxomycete species from each of the collecting localities. Approximately 74% (468 of 630) moist chambers were positive for myxomycetes. Among the 713 myxomycete records from moist chamber cultures (MC), 116 plasmodia did not develop into fructifications, whereas 597 fruitings could be determined to species. With 33 determinable records from field collections, the total number of records increases to 630.

Considering both records from the field and MC, among the seven collecting localities Basud, Malilipot and Isarog were most productive, accounting for 19.4%, 18.1% and 16.0% of the total number of records, respectively. Least productive were Daet and Bulusan with 10.9% and 7.4% of all records, respectively (Tab. 1). For both cases, the difference to the rich localities was significant

($H(\chi^2)=21.8$, $p<0.001$). Consequently, the three localities mentioned first had as well yielded the highest value for the Fisher's diversity index. However, these differences regard only abundance, not species diversity (Fig. 3). Some common species like *Arcyria cinerea* (both white and yellow form), *Diderma hemisphaericum*, *Didymium squamulosum*, *Perichaena depressa* and *Stemonitis fusca* were observed in all localities. Basud and Isarog had as well the highest number of unique species (six per site). Based on the computed Morisita dissimilarity index (Tab. 2), the highest dissimilarities were observed between the sites Malilipot / Daet, and Basud / Daet with 0.55 and 0.51, respectively.

Annotated species list

The following annotated species list includes 57 taxa belonging to 18 different genera. Well-developed specimens that cannot be assigned to a known species without doubt are denoted as "cf." (confer), scanty or weathered specimens not allowing safe determination are indicated by a question mark "?". After each name, an estimation of abundance (Stephenson *et al.* 1993) is given in brackets, followed by the total number of records. This is based upon the proportion of a species to the total number of determinable fruiting body records for each group: R – rare (< 0.5 %, <4 records), O – occasional (> 0.5–1.5 %, 4–9 records), C – common (> 1.5–3 %, 10–19 records), A – abundant (> 3 %, more than 20 records). With the denominators llae (aerial litter), llgr (ground litter), tw (twigs) and w (wood, all field collections) the number of records made on the main substrate types are indicated. After a slash in a similar way the number of records in the seven sampling localities is indicated with the abbreviations DA (Daet), BA (Basud), IS (Isarog), AS (Asog), MA (Malilipot), CA (Castilla) and BU (Bulusan). In parentheses the substrate pH (mean±SEM, minimum–maximum value) for all species with collections obtained from moist chamber cultures is given. An asterisk (*) indicates a species reported as new for the Philippines. Two asterisks (**) mark the species reported as new for the Asian Paleotropics.

Arcyria cinerea (Bull.) Pers. (Figs. 4, 7) [A, 115] fc: 1 mc: 114 / llae: 49 llgr: 22 tw: 43 w: 1 / BA 10 IS 14 CA 15 MA 10 AS 20 BU 14 DA 32 (6.4±0.08, 3.5–7.6)

Arcyria cinerea* var. *digitata Schwein. [R, 1] fc: 1 / w: 1 / MA 1

***Arcyria cinerea*, dwarf form** (Bull.) Pers. (Fig. 6, 8) [A, 21] mc: 21 / llae: 10 llgr: 5 tw: 6 / BA 12 IS 3 CA 2 MA 2 DA 2 (6.5±0.14, 5.2–7.5) This form is usually collected in MC from tropical aerial litter and often mistaken as *Arcyria pomiformis*. Sporocarps possess a much slender stalk than the normal form (stalk 5–8 times exceeding sporocarp length), are 0.3–1 mm tall with an ovoid to subglobose sporocyst, colours are paler as in the normal-sized form, ranging from light grey (264) to yellowish white (92), sometimes nearly white (263).

***Arcyria cinerea*, yellow form** (Bull.) Pers. (Figs. 5, 7) [A, 53] mc: 53 / llae: 22 llgr: 21 tw: 10 / BA 12 IS 3 CA 11 MA 12 AS 9 BU 3 DA 3 (6.5±0.09, 4.4–7.9) This form shows the same size and shape (1–4 mm total height, elongated cylindrical sporotheca, stalk 0.3–1 times sporotheca length) as the cosmopolitan *Arcyria cinerea* but sporocarps are strong yellow (84) to dark yellow (88) and scattered or densely crowded. The calyculus is minute, often smaller than the sporotheca diameter, radially furrowed and minutely punctuated. Capillitium ornamentation includes dense reticulations of mainly spinules or cogs at the superior part of the sporotheca and smoother, thinner spinules in the inferior part. The colour of these fructifications resembles *A. pomiformis*, but the dimorphic capillitium and the cylindrical to subcylindrical sporotheca are characters of *A. cinerea*.

Arcyria denudata (L.) Wettst. [O, 4] fc: 3 mc: 1 / tw: 1 w: 3 / IS 1 MA 2 BU 1

Arcyria globosa Schwein. (Fig. 10) [R, 2] mc: 2 / llae: 1 llgr: 1 / CA 1 MA 1 (5.8±2.05, 3.8–7.9) Stout ovoid sporocarps of about 0.6–0.8 mm height on a short, slightly rugulose stalk reaching 0.3–1 times sporotheca length, the latter is globose in shape, showing a paler, cream colour that makes it clearly distinct from the common *A. cinerea*.

Arcyria incarnata (Pers.) Pers. [R, 1] mc: 1 / tw: 1 / MA 1

Arcyria insignis Kalchbr. & Cooke [R, 1] mc: 1 / llae: 1 / BA 1

Badhamia affinis Rostaf. [R, 1] mc: 1 / tw: 1 / AS 1

Ceratiomyxa fruticulosa var. *fruticulosa* (Müll.) T. Macbr. [O, 7] fc: 6 mc: 1 / llgr: 1 tw: 1 w: 5 / IS 1 CA 1 MA 1 AS 1 BU 1 DA 2

Clastoderma debaryanum A. Blytt [R, 1] mc: 1 / tw: 1 / BA 1

Collaria arcyrionema (Rostaf.) Nann.-Bremek. ex Ing [O, 7] mc: 7 / llgr: 3 tw: 4 / BA 1 AS 2 BU 3 DA 1 (5.7±0.32, 4.2–7.1) First reported by Reynolds (1981) using the name *Lamproderma arcyrionema* (Sommerfeld) Rostaf.

**Comatricha fragilis* Meylan [R, 2] mc: 2 / tw: 2 / MA 2 (7.1±0.32, 6.7–7.4) Crowded dark stalked sporocarps, 2–4 mm high. Stalk thin, black (267), shining, cylindric, reaching one half of total sporocarp height but running as a columella until the apex of the sporotheca. Capillitium dark brown (59), flexuose, branched, attached to the columella but soon breaking off and falling away from it, looped at the periphery with nearly no free ends. Spores brownish grey (64) to dark purplish gray (234), (5.5–)6.8–7.6(–8.3) µm diam., covered with regular warts.

Comatricha nigra (Pers. ex J.F. Gmel.) Schroet. [R, 3] mc: 3 / llae: 1 tw: 2 / MA 1 AS 1 DA 1 (6.8±0.28, 6.4–7.3)

**Comatricha pulchella* (C. Bab. & Berk.) Rostaf. (Fig. 14) [R, 1] mc: 1 / llae: 1 / CA 1 Sporocarps minutely stalked; total height about 0.7–1.5 mm. Stalk black (267), reaching nearly half of the total height. Sporotheca globose, about 0.3 mm diam, deep reddish brown (41). Columella tapering into a thin thread but reaching the end of the sporotheca. Capillitium light reddish brown (42), branching from the columella, looped at the surface without forming a flat surface net, but as well without free ends. Spores relatively small, (6.5–)7.4–8.2(–9.1) µm diam, minutely spinulose.

Comatricha tenerrima (M.A. Curtis) G. Lister [C, 12] fc: 1 mc: 11 / llae: 2 llgr: 1 tw: 9 / BA 3 IS 3 MA 2 AS 3 DA 1 (6.1±0.28, 4.2–7.0)

Craterium leucocephalum (Pers.) Ditmar [R, 2] fc: 1 mc: 1 / llae: 1 llgr: 1 / IS 1 AS 1

Cribraria microcarpa (Schräd.) Pers. [R, 2] mc: 2 / tw: 2 / BA 2 (5.9±0.41, 5.5–6.3)

Cribraria violacea Rex [O, 7] mc: 7 / llgr: 2 tw: 5 / BA 1 IS 1 MA 4 BU (6.6±0.11, 6.2–7.0)

Diachea bulbilosa (Berk. & Broome) Lister ex Penzig [O, 5] mc: 5 / llae: 1 llgr: 4 / BA 1 IS 1 MA 3 (6.6±0.23, 5.8–7.2)

Diachea leucopodia (Bull.) Rostaf. [C, 17] mc: 17 / llae: 11 llgr: 4 tw: 2 / BA 6 CA 3 MA 3 AS 3 BU 1 DA 1 (5.2±0.23, 4.0–6.7)

Diderma effusum (Schwein.) Morgan [A, 20] mc: 20 / llae: 6 llgr: 14 / BA 1 IS 3 CA 4 MA 2 AS 7 DA 3 (6.4±0.18, 3.9–7.4)

Diderma hemisphaericum (Bull.) Hornem. [A, 35] mc: 35 / llae: 22 llgr: 8 tw: 5 / BA 3 IS 5 CA 10 MA 6 AS 7 BU 1 DA 3 (6.02±0.16, 3.5–7.5)

**Didymium floccosum* G.W. Martin, K.S. Thind & Rehill [R, 1] fc: 1 / llgr: 1 / MA 1

Didymium nigripes (Link) Fr. [O, 5] fc: 1 mc: 4 / llae: 2 llgr: 3 / CA 2 AS 1 BU 2 (4.3±1.01, 3.3–5.9)

Didymium squamulosum (Alb. & Schwein.) Fr. (Alb. & Schwein.) Fr. [A, 55] fc: 1 mc: 54 / llae: 31 llgr: 16 tw: 8 / BA 9 IS 8 CA 5 MA 8 AS 6 BU 4 DA 15 / (6.7±0.12, 3.5–7.7)

**Didymium verrucosporum* Welden [O, 5] fc: 2 mc: 3 / llae: 3 llgr: 2 / BA 1 IS 1 CA 1 MA 2 (6.6±1.93, 6.0–7.5) Sporocarps long stalked, often appearing in clusters, 1–2 mm tall. Sporotheca globose, nodding, white (263), 0.2–0.5 mm in diam. Stalk limeless, dark in the base but becoming

light brown (57) as it tapers upward towards the apex. Peridium covered with unevenly scattered stellate lime crystals. Columella distinctly globose, white (263), comprising about half the height of the sporotheca. Capillitium greyish yellowish brown (80), branched with few bearing dark swellings. Spores dark purplish grey (234), coarsely warted, (7.0–)8.0–8.7(–9.2) μm in diam.

Hemitrichia calyculata (Speg.) M.L. Farr [R, 2] fc: 2 / w: 2 / IS 2 First reported by Reynolds (1981) using the name *Hemitrichia stipitata* (Massee) T. Macbr.

Hemitrichia serpula (Scop.) Rostaf. [O, 5] fc: 2 mc: 3 / llae: 1 llgr: 1 tw: 2 w: 1 / IS 1 MA 2 AS 2 (5.3±1.72, 4.3–6.9)

Lamproderma scintillans (Berk. & Broome) Morgan [A, 23] mc: 23 / llae: 7 llgr: 11 tw: 5 / BA 12 IS 4 MA 1 AS 1 BU 1 DA 4 (6.6±0.17, 4.0–7.5)

Lycogala exiguum Morgan [R, 1] fc: 1 / w: 1 / DA 1

Perichaena chrysosperma (Currey) Lister [C, 13] mc: 13 / llae: 4 llgr: 1 tw: 8 / BA 2 IS 6 CA 2 MA 1 BU 1 DA 1 (6.3±0.13, 5.5–6.9)

Perichaena depressa Libert [A, 66] mc: 66 / llae: 21 llgr: 16 tw: 29 / BA 14 IS 12 CA 13 MA 21 AS 4 BU 1 DA 1 (6.4±0.08, 3.5–7.4)

Perichaena dictyonema Rammeloo [C, 14] mc: 14 / llae: 4 llgr: 2 tw: 8 / BA 4 IS 2 MA 6 DA 2 (6.7±0.21, 4.8–7.6). Sporocarps sessile on a constricted base, scattered or in small groups, globose to ellipsoidal or somewhat elongated in shape, 0.5–1.0 mm in diam. Peridium only at parts of the sporocarp double, with a thick, opaque layer of amorphous material firmly attached to the translucent, membranous inner layer, therefore the sporocarp appears blackish and yellow. Dehiscence indefinitely operculate. Capillitium long, sometimes branched. Spore mass vivid yellow (82). Spores globose, verruculose, (9.5–)10.2–11.9(–13.4) μm in diam.

Perichaena minor* var. *pardina (Minakata) Hagelst [R, 1] mc: 1 / tw: 1 / BA 1

Perichaena pedata (Lister & G. Lister) G. Lister [A, 20] mc: 20 / llae: 15 llgr: 2 tw: 3 / BA 2 IS 7 MA 11 (6.7±0.07, 6.1–7.5). Sporocarps solitary, 0.2–0.8(–1.0) mm tall, with a comparatively long (0.1–0.7 mm) and rather stout stalk composed of granulated, black material. Sporotheca globose, ochraceous, ca. 0.2–0.5 mm in diam. Peridium cartilaginous, thick, opaque and uniform in appearance. Dehiscence usually by polygonal areolae, sometimes leaving a smooth and persistent base where the capillitial threads remain to be attached. Capillitium abundant, slightly elastic, branched and forming anastomosing, densely interwoven threads arising from the peridium and base, with some short, free ends, ornamentation verrucose, sometimes constricted or marked with many spines. Spores pale yellow (89), (7.1–)7.6–9.0(–10.4) μm diam., verruculose by small warts.

****Perichaena vermicularis*** (Schwein.) Rostaf. (Fig. 13) [C, 10] mc: 10 / llae: 3 llgr: 2 tw: 5 / BA 7 IS 1 MA 2 (6.8±0.12, 6.3–7.7)

Physarum album (Nees) Fr. [R, 1] mc: 1 / llae: 1 / DA 1 Reported by Reynolds (1981) as *Physarum nutans* Pers.

Physarum bivalve Pers. [R, 2] mc: 2 / llae: 1 llgr: 1 / BU 2 (5.1±0.40, 4.7–7.3)

Physarum cinereum (Batsch) Pers. [R, 1] mc: 1 / llae: 1 / DA 1

Physarum compressum Alb. & Schwein. [O, 7] fc: 4 mc: 3 / llae: 1 llgr: 3 tw: 2 w: 1 / MA 3 AS 3 DA 1 (7.1±2.9, 6.8–7.4)

Physarum decipiens M.A. Curtis [C, 18] mc: 18 / llae: 1 tw: 17 / BA 5 IS 6 CA 4 MA 1 AS 1 BU 1 (6.3±0.10, 5.5–6.9)

Physarum didermoides (Pers.) Rostaf. [R, 2] mc: 2 / llae: 1 tw: 1 / IS 1 MA 1 (6.7±0.05, 6.6–6.8)

Physarum echinosporum Lister (Fig. 12) [R, 2] mc: 2 / llgr: 2 / AS 1 BU 1 (4.4±0.45, 3.9–4.9)

This rare myxomycete is first reported from the Philippines by Reynolds (1981). It is widely distributed in tropical zones, particularly in Asia (Thailand, Taiwan, and India), Africa (Kenya, Cameroon) but the species seems to be rare in the Central American Neotropics (Rojas *et al.* 2010) and the Caribbean region (Jamaica, Dominica).

Physarum globuliferum (Bull.) Pers. [R, 1] mc: 1 / tw: 1 / BA 1

Physarum melleum (Berk. & Broome) Massee [O, 5] mc: 5 / llae: 1 llgr: 3 tw: 1 / BA 2 CA 1 BU 2 (6.0±0.49, 4.4–7.1)

Physarum* cf. *oblatum T. Macbr. [O, 6] mc: 6 / llae: 1 tw: 5 / CA 6 (6.6±0.20, 6.2–7.5) Sporocarps stalked, gregarious. Sporotheca globose to subglobose or oblate, (0.3–)0.4–0.6(–0.8) mm in diam. Peridium roughened with lime scales, these moderate orange yellow (71) to strong yellow (84) sometimes nearly white with a dark greyish yellow (91) base of nearly contiguous lime scales persisting like a cup. Stalk making up for three fourth of the sporocarp height, slender, deep reddish brown (41). Columella absent. Capillitium not radiating from the sporotheca centre, rather irregularly branched, with large, angular shaped lime nodes. Spores in mass nearly black, deep brown (56) in transmitted light, warted with groups of conspicuously darker warts, (9.2–)10.2–12.7(–13.6) µm in diam. The stout habit excludes *P. flavicomum* Berk, but we see no clear differences between *P. galbeum* Wingate, *P. limonium* Nann. Bremek. and *P. oblatum* and thus cannot assign one of these names to our specimens without doubt.

Physarum pezizoideum (Jungb.) Pavill. & Lagerh. (Fig. 16) [R, 1] mc: 1 / tw: 1 / BA 1

Physarum pulcherrimum Berk. & Ravenel in Berk. [R, 1] fc: 1 / w: 1 / IS 1

****Physarum pusillum*** (Berk. & M.A. Curtis) G. Lister [R, 1] mc: 1 / tw: 1 / MA 1

Physarum stellatum (Massee) G.W. Martin [R, 1] fc: 1 / tw: 1 / IS

Physarum superbum Hagelst. (Fig. 11) [R, 1] mc: 1 / llae: 1 / IS 1

Physarum tenerum Rex [R, 1] mc: 1 / tw: 1 / CA 1

*****Stemonaria fuscoidea*** Nann.-Bremek. & Y. Yamam. (Fig. 15) [O, 7] mc: 7 / tw: 7 / IS 1 CA 1 MA 1 AS 3 BU 1 (5.7±0.28, 4.7–7.1) Sporocarps densely clustered over a common hypothallus, stalked, dark, 4–5 mm tall. Sporotheca long, cylindrical, rounded at the base and apex, about 0.5 mm in diam., 2–4 mm long. Stalk black (267), hollow, horny and appearing homogenous. Columella dissipates into 2–3 branches at the apex. Capillitium moderate olive brown (95), forming an internal net with 2–3(–4) meshes around the radius, with many expansions mostly at the sides, threads anastomosing at the periphery but not even forming an incomplete surface net. Spores dark olive brown (96), (8.0–)8.5–9.3(–10.4) µm in diam., with an incomplete reticulum consisting of rows of darker spines about 0.5 µm in length.

****Stemonitis flavogenita*** Jahn [R, 1] fc: 1 / w: 1 / IS 1

Stemonitis fusca Roth [C, 14] fc: 3 mc: 11 / llae: 3 llgr: 1 tw: 8 w: 2 / BA 2 IS 2 CA 2 MA 1 AS 1 BU 2 DA 4 (6.2±0.54, 3.6–7.4)

Stemonitis pallida Wingate [R, 1] mc: 1 / llae: 1 / BU 1

DISCUSSION

The first studies of Philippine myxomycetes were carried out in the late Seventies (Reynolds 1981; Dogma 1975; Uyenco 1973), resulting in a total of 107 known from the Philippines. These first papers included checklists of species, usually without data on distribution and abundance. Recently, a number of surveys were carried out in the Western and Central part of Luzon main Island, including different types of tropical montane forests (dela Cruz *et al.* 2014, Dagamac *et al.* 2014); tropical moist

forest (Macabago *et al.* 2010, dela Cruz *et al.* 2010) and geographically isolated islands with lowland tropical forest (Kuhn *et al.* 2013, Macabago *et al.* 2012). Altogether, these studies increased the species list for the Philippines to 143; much less than reported for Costa Rica (208, Rojas *et al.* 2010). The present study added another eight species, updating the number of taxa known for the country to 151. In comparison to other areas in the Paleotropic Southeast Asia, this figure now outnumbers data for Thailand (132 taxa, Ko Ko *et al.* 2010), Singapore (92, Rosing *et al.* 2011), and Myanmar (67, Ko Ko *et al.* 2013).

This is the first myxomycete survey for the Bicol Peninsula. With vast stretches of diverse tropical lowland forests as the natural vegetation, species richness in the Bicol Peninsula is most likely higher than reflected by this study; especially when taking into account that myxomycetes from the field are still undersampled in comparison to records from MC. Both anthropogenic activities and natural disturbances like typhoons and volcanic eruptions create today a diverse landscape of cultivated areas, secondary forests and remaining pockets of old-growth forest. With 746 records amounting to 57 taxa, this survey yielded more species than all carried out previously in other stretches of lower montane forests in the Philippines, like Mt. Gonting in Lubang Island (45 taxa, Macabago *et al.* 2012) or Mt. Arayat in Pampanga (30, Dagamac *et al.* 2014). However, with only 33 records, the field component of this survey is certainly underrepresented, and so are estimates on species richness, since numerous species of myxomycetes do never or rarely fruit in moist chambers.

The sites Basud, Isarog and Malilipot showing the highest species diversity are characterized by either old growth or older, slightly or moderately disturbed successional forests. These lowland forests seem to have more diverse vegetation, and the forest cover is interrupted by emergent trees and treefall gaps. Similarly, Takahashi (2013) noted higher numbers of myxomycete species in Japanese mixed evergreen/deciduous compared in comparison to pure bamboo or pine forests. Tran *et al.* (2006) report a higher number of taxa from plots with mixed litter than from plots with uniform litter, composed by leaves of a single tree species. This mounts evidence for the hypothesis that indeed forest structure in the tropics affects myxomycete occurrence (Rojas & Stephenson 2012). Species richness in myxomycete may follow the intermediate disturbance hypothesis, reaching its maximum in forests with intermediate disturbance levels. Daet, with highly degraded forest patches due to urbanization in the area, was much poorer than Basud and Malilipot; and the latter sites had a higher number of unique species. In a recent study of Rojas & Stephenson (2013) from the southwestern Peruvian Amazon showed that forest loss and high disturbance decreases the abundance of myxomycete fructifications. It would be interesting to test the other end of a disturbance gradient: in homogenous, undisturbed forests, myxomycete assemblages should be more depauperated than in forests disturbed by frequent treefall gaps, or at forest fringes.

So far, most data on myxomycete distribution come from temperate regions (Stephenson *et al.* 2008). For the tropics, the Neotropical region is much better studied than the African or Southeast Asian Paleotropics. Further in-depth studies with a focus on fairly complete surveys (including both a field and a moist chamber component) from selected regions are needed to reveal differences in species composition and abundance between Neo- and Paleotropical myxomycetes. Apart from the morphospecies level, such studies must be augmented by molecular barcoding to unveil differences at the biospecies level, which are much more likely to occur. For such a program, this basic floristic survey for a hitherto neglected area like the Bicol Peninsula is one of the initial steps.

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Table & Figures

Table 1: Statistical data for seven collecting sites showing the description of the forest in terms of age: old growth (OG) or successional forest (SF), canopy closure: closed (C) or open (O), and estimated degree of disturbance: virtually undisturbed (UND), disturbed (DIS) or highly disturbed due to human settlements (HDI); proportion of moist chamber cultures positive for myxomycetes (90 cultures prepared per site); records of myxomycetes yielded from both field and moist chambers; and the taxonomic diversity index (TDI) computed as the number of species divided by the number of genera

<i>Collecting Sites</i>	Forest			Moist Chamber	Myxomycete Records				Taxonomic Diversity		
	<i>Age</i>	<i>Canopy</i>	<i>Dist</i>	<i>Percent</i>	<i>Fruiting bodies</i>	<i>Plasmodia</i>	<i>Both</i>	<i>Percent of the total</i>	<i>No. of species</i>	<i>No. of genera</i>	<i>TDI</i>
Basud	OG	C	UND	94.4	121	24	145	19.4	30	12	2.50
Asog	SF	O	HDI	71.1	79	17	96	12.9	22	15	1.47
Castilla	SF	O	HDI	88.9	89	26	115	15.4	22	10	2.20
Bulusan	SF	C	DIS	51.1	45	10	55	7.4	21	12	1.75
Daet	SF	O	HDI	56.7	80	1	81	10.9	20	11	1.82
Isarog	SF	C	DIS	76.7	98	21	119	16.0	32	14	2.29
Malilipot	SF	O	DIS	81.1	118	17	135	18.1	34	13	2.62

Table 2: Morisita index for dissimilarity of myxomycete communities between collecting sites (upper right) and numbers of species shared by two collecting sites (lower left)

	Asog	Basud	Bulusan	Castilla	Daet	Isarog	Malilipot
Asog	–	0.34	0.01	0.06	0.12	0.18	0.31
Basud	12	–	0.42	0.20	0.51	0.11	0.15
Bulusan	13	14	–	0.19	0.02	0.23	0.46
Castilla	12	14	13	–	0.34	0.10	0.12
Daet	14	14	11	11	–	0.34	0.55
Isarog	14	19	13	13	13	–	0.06
Malilipot	16	20	14	16	15	24	–

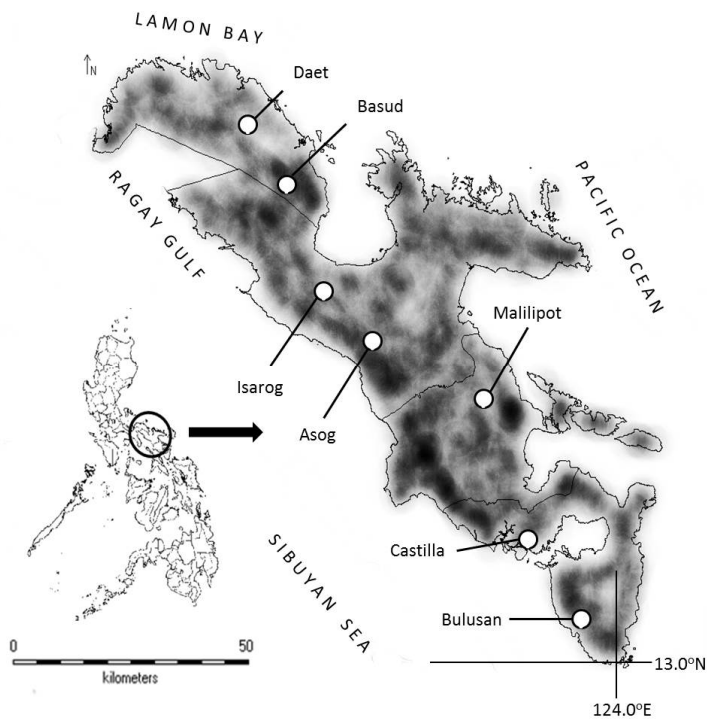


Fig 1: Position of the study area within the Philippine archipelago (inset) and geographic map of the Bicol peninsula showing the seven collecting sites. Higher elevations are shaded in grey.

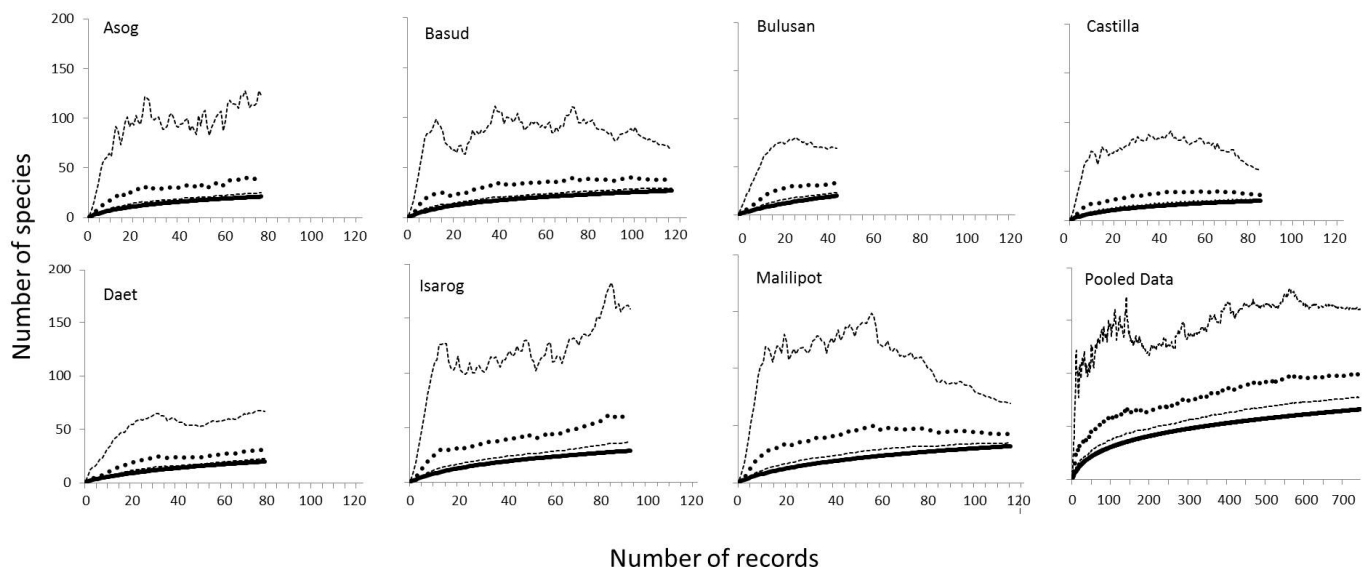


Fig 2: Species accumulation curves generated for each of the seven collecting localities and the pooled data set (lower right). Shown are individual-based species accumulation curves generated by EstimateS (thick solid lines) and figures for the Chao 2 estimator (thick dotted lines) and its 5% and 95% confidence interval (thin dotted lines).

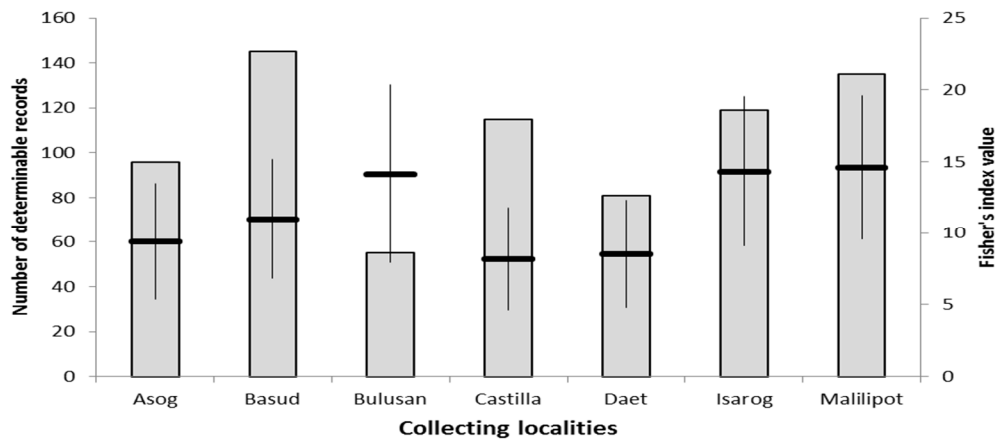
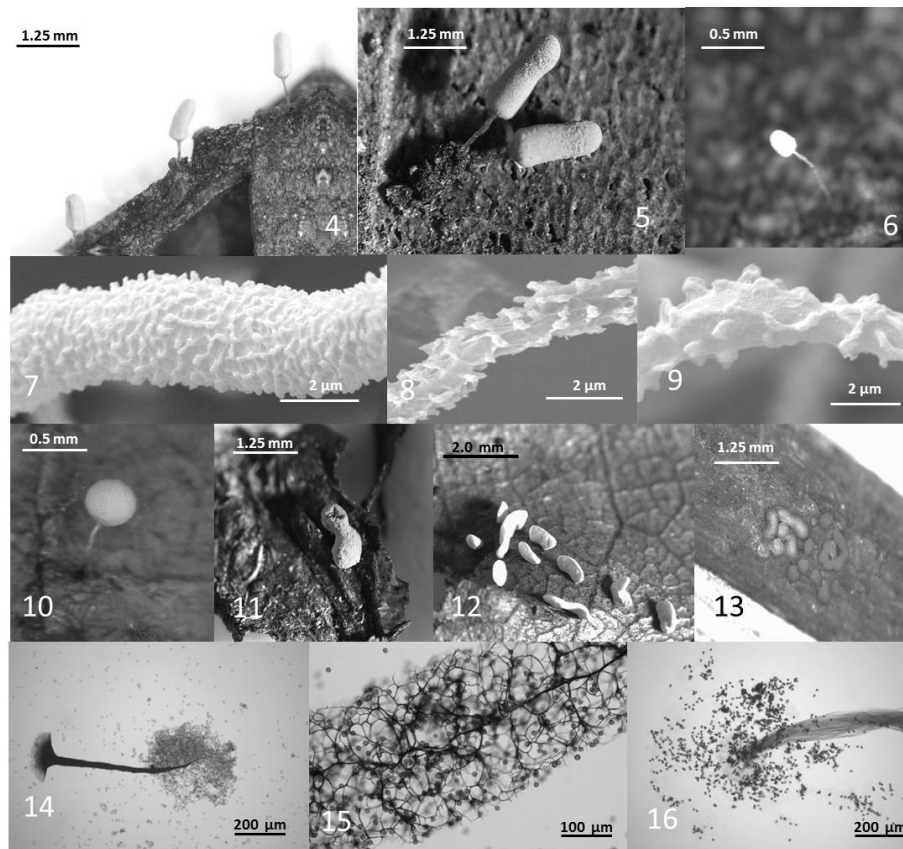


Fig 3: Alpha diversity in the seven collecting localities. Grey bars denote the number of determinable records; horizontal black lines show the Fisher's index of diversity with its 95% confidence interval (thin vertical lines) as computed by SPADE.



Figs 4–16: Myxomycete species. **4–6.** Specimens of the *Arcyria cinerea* complex (dissecting microscope). 4. Typical form. 5. Yellow form. 6. Dwarf form. **7–9.** Capillitial threads of the *Arcyria* complex (SEM). 7. Typical form. 8. Yellow form. 9. Dwarf form. **10–13.** Fruiting bodies of some rare species. 10. *Arcyria globosa*. 11. *Physarum superbum*. 12. *Physarum echinosporum*. 13. *Perichaena vermicularis*. **14–16.** Mounted slides (Hoyers medium, compound microscope). 14. *Comatricha pulchella*. 15. *Stemonaria fuscoides*. 16. *Physarum pezizoideum*.



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.

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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@mnlu.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 (CaCO₃) 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. Eumycetozoa Project (<http://slimemold.uark.edu>), were also utilized for the verification of some morphological features. Nomenclature followed the online nomenclatural information database for eumycetozoa (<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

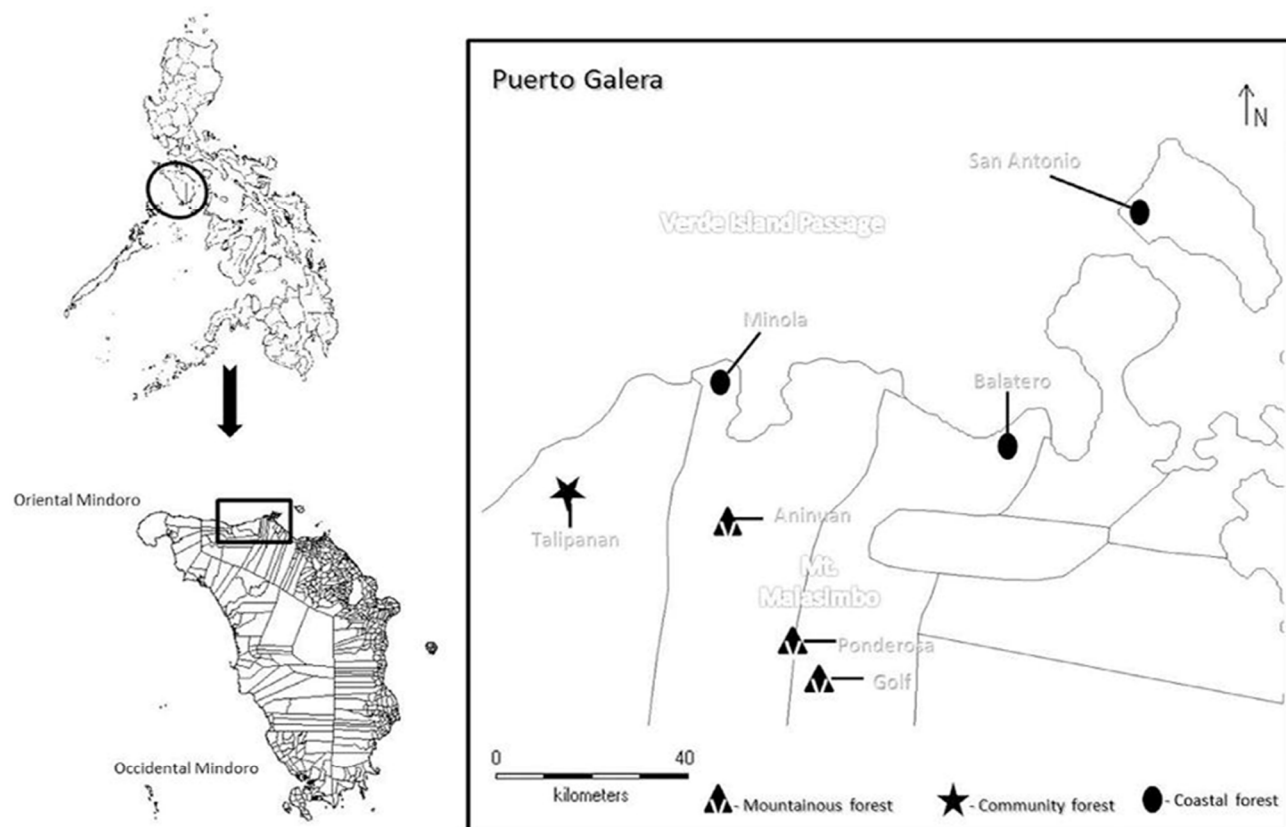


Figure 1. Study area Puerto Galera, Oriental Mindoro, showing the seven collecting points.

Table 1. Habitat description, elevation, and geographic coordinates of the seven collecting points in Puerto Galera, Oriental Mindoro, the Philippines.

Site number	Site name	Latitude	Longitude	Elevation (masl)	Description
1	Aninuan	N 13° 29' 01.3"	E 120° 54' 19.6"	177	With steep, slippery, & stony paths with forest floor litter along the stream
2	Golf	N 13° 28' 36.5"	E 120° 54' 46.4"	406	With numerous leeches surrounding the grass areas, tall tree forbs & small shrubs
3	Ponderosa	N 13° 28' 38.4"	E 120° 54' 42.2v	467	Surrounded by old & high dipterocarp trees, & vegetation
4	Talipanan	N 13° 29' 52.9"	E 120° 53' 9.8"	24	Dominated by indigenous tribes & big dipterocarp trees
5	San Antonio	N 13° 31' 26.6"	E 120° 57' 4.5"	16	Dominated by dense, thorny shrubs & old growth trees
6	Balatero	N 13° 30' 39.3"	E 120° 55' 37.9"	22	Dominated by shrubs & trees with various manmade wastes
7	Minola	N 13° 30' 30.4"	E 120° 54' 55.3"	10	A steep terrain completely surrounded by plenty coconut trees

E = East; N = North.

myxomycetes, and noted as one positive collection. The number of positive collections was counted. Then, the total number of positive collections was divided by the total number of moist chambers prepared, which was expressed as moist chamber productivity (Macabago et al 2012). Subsequently, 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 was constructed based on the records obtained from the collection in the field and moist chambers, according to the rarefaction formula using the default settings of the program EstimateS (version 9.1; 100 randomizations; <http://purl.oclc.org/estimates>). The Chao 2 estimator was then chosen as the best estimator, in accordance with the findings of Unterseher et al (2008). The percentage of completeness for the study area and each collecting locality 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. The species composition of the collection sites was then determined simply by recording the different species collected from the study sites. The occurrence of various myxomycete species in each moist chamber was then determined. Remember that the moist chamber that displayed a fruiting body of a particular species was considered a positive collection. The recurrent presence of a specific species of myxomycetes in a positive moist chamber was termed as its occurrence. A collection was now regarded as an individual unit. The value obtained by dividing the total number of collections for each specific species of myxomycetes by the total number of myxomycetes collected was defined as the relative abundance for each species (Stephenson et al 1993). The relative abundance of each species of myxomycetes was interpreted as the abundance index. The computed relative abundance for each species was then translated to an abundance index that was described

by Stephenson et al (1993), namely, rare for species < 0.5% of the total number of collections, occasional for species > 0.5% but < 1.5% of the total number of collections, common for species > 1.5% but < 3% of the total number of collections, and abundant for species > 3% of the total number of collections. All abundance data for fruit bodies obtained from collecting localities were compared using Kruskal–Wallis test employed in Paleontological Statistics (PAST) software (Hammer et al 2001). Then, the taxonomic diversity was computed by calculating the ratio of the number of species to the number of genera. The value of this ratio was inversely proportional to the taxonomic diversity. Therefore, the lower the ratio, the more diverse the particular biota. A biota in which the species were divided among numerous genera is “intuitively” more diverse in a taxonomic basis than a biota wherein the species belong to only a few genera (Stephenson et al 1993). In this study, species diversity of myxomycetes was then calculated using the software SPADE (Species Prediction And Diversity Estimation; <http://chao.stat.nthu.edu.tw>) by generating the maximum likelihood estimator and the classic formula for the more intuitive indices, Simpson and Fisher indices. Additionally, the taxonomic diversity between collecting sites was also computed. The number of species and genera for each moist chamber was counted primarily. The same program was also used for a β analysis using the Morisita index for dissimilarity values among the myxomycete assemblages from the seven collecting localities. The values of this index range from 0 to 1, and a lower value indicates a higher degree of similarity between the communities being evaluated.

Results

Using the combined opportunistic sampling in the field and moist chamber culture preparation, a total of 926 records of myxomycetes were obtained for this survey. Of these, 71 records were from the field survey and 855 from the moist chamber component. Moreover, of these 926 records, a total of 42 morphospecies belonging to 16 genera of myxomycetes are accounted and reported in this study. In terms of species composition, the occurrence of the 42 morphospecies recorded in the area showed that nine species were abundant, five were common, 14 were occasional, and 14 were reported to be occurring as rare (Table 2).

The individual-based species accumulation curve constructed using the software EstimateS showed that the mean Chao 2 estimator reached a constant value of 47.2 (Figure 2). Using the formula of Ndiritu et al (2009) to calculate the exhaustiveness of the sampling effort for the whole study, our result gave us a computed sampling effort of 89%, as evident from the nearly asymptotic curves for the whole study area. Regarding our sampling efforts in the seven collecting localities, the sampling effort was least in San Antonio (35.2%) followed by in Aninuan (51.5%), suggesting the potential of recovering more myxomycete species from these collecting sites (Table 2).

An assessment of the α diversity of myxomycetes in the seven collecting localities showed that, on the one hand, the inland montane forests from Golf (26 species) and Ponderosa (22 species) were most species rich. On the other hand, when considering the evenness of myxomycetes species, the only community forest Talipanan had the highest values of Simpson and Fisher indices, followed by Golf and Minolo (Figure 3). However, there were no statistically significant differences in the species diversity among the seven collecting localities ($p = 0.443$, $\alpha = 0.05$). Furthermore, our result showed that, among the seven collecting localities, the highest taxonomic diversity index (TDI) is observed in the least species-rich area of San Antonio (TDI = 1.30).

A comparison of the similarities of myxomycetes species among the seven localities showed that the species *Arcyria cinerea*, *Didymium hemisphaericum*, *Physarum echinosporum*, *Lamproderma scintillans*, and *Stemonitis fusca* were observed in all collecting localities. Based on the computed Morisita dissimilarity index, highest dissimilarities were observed between the sites San Antonio/Ponderosa and Talipanan/Ponderosa (0.350 and 0.271, respectively). By contrast, the lowest dissimilarity (0.001) of myxomycete assemblages was observed between the coastal Balatero and Talipanan (Table 3).

Discussion

The pristine habitats of Puerto Galera in Oriental Mindoro merit its designation as a UNESCO Man and Biosphere Reserve. Many flora and fauna from its terrestrial and marine habitats were documented. This study is the first to assess myxomycete diversity in Puerto Galera. The topography and landscape of forest patches from lowland mountain areas and coastal vegetation in the study area make it an ideal site to further investigate the species composition of myxomycetes on the basis of forest structures and/or forest disturbance, since a booming ecotourism industry exists in Puerto Galera.

In this survey, seven major collecting points were systematically chosen to represent a particular forest type: (1) the *lowland montane* areas (Aninuan, Golf, and Ponderosa) described as composed of the semideciduous rainforest dominated by a vast number of trees that lose their leaves during the dry season and the evergreen or semievergreen rainforest found in areas with a semi-high altitude (~400 masl); (2) a slightly disturbed forest portion at the foot of *community* settlements (Talipanan) characterized by altered forms of vegetation because of the increased activities of local people living in this area; and (3) *coastal* forest habitats (San Antonio, Balatero, and Minolo) characterized by plants and trees found in the offshore of an island (Figure 1). In a coastal forest, vegetation is influenced and altered by the wind behavior along the shore. Gaps are observed in between scattered trees and plants. It has increased light, soil, water, and airflow exchange, but decreased litter and canopy cover compared to a mountain forest.

Species composition of myxomycetes in Puerto Galera

With a relatively high sampling effort (89%) for the whole study (Figure 2), several species of myxomycetes, namely, *Cribraria violacea*, *A. cinerea*, *Perichaena depressa*, *D. hemisphaericum*, *Didymium nigripes*, *Didymium squamulosum*, *Physarum album*, *Physarum bivalve*, *Physarum compressum*, *P. echinosporum*, *Physarum leucophaeum*, *Collaria arcyrionema*, *Diachea leucopodia*, *L. scintillans*, *Stemonitis axifera*, *S. fusca*, and *Stemonitis herbatica*, were common to all forest types, regardless of the collecting points. In this study, a total of 42 morphospecies of myxomycetes belonging to 16 genera were collected from the mountain, community, and coastal forests of Puerto Galera. This outnumbers the species reported in recent studies conducted in other areas of the Philippines where myxomycete survey was conducted, i.e. from forest areas in Negros (Alfaro et al 2015), karst forest of Quezon National Park (Dagamac et al 2015b), and the northern slope of Mt Makulot (Cheng et al 2013).

Myxomycetes diversity in different forest types

The seemingly different forest conditions may play a role in assessing the dynamics of myxomycete diversity. Most species of myxomycetes from the mountains usually fruited from decaying parts of trees. They are collected from under or even inside the logs,

Table 2. Occurrence of myxomycetes in the entire study area, their AI (A = abundant, C = common, O = occasional, R = rare), and abundance based on the number of records from the seven collecting points, including the sampling effort and taxonomic diversity index.

Taxon	AI	Frequency	Field collection	Moist chamber						
				Aninuan	Golf	Ponderosa	Talipanan	San Antonio	Balatero	Minolo
Order Echinosteliales										
<i>Echinostelium minutum</i> de Bary	O	5	—	0	2	2	0	1	0	0
Order Liceales										
<i>Cribraria violacea</i> Rex	O	10	—	0	4	3	2	1	0	0
<i>Lycogala epidendrum</i> (J. C. Buxb. ex L.) Fr.	R	1	1	0	0	0	0	0	0	0
<i>Lycogala exiguum</i> Morgan	R	2	2	0	0	0	0	0	0	0
Order Trichiales										
<i>Arcyria cinerea</i> (Bull.) Pers.	A	275	13	45	66	34	33	14	48	22
<i>Arcyria denudata</i> (L.) Wettst.	O	13	7	1	4	1	0	0	0	0
<i>Arcyriacarnata</i> (Pers ex J. F Gmel) Pers.	O	9	7	0	0	0	2	0	0	0
<i>Arcyria pomiformis</i> (Leers) Rostaf.	R	2	1	1	0	0	0	0	0	0
<i>Hemitrichia calyculata</i> (Speg.) M.L. Farr	R	1	1	0	0	0	0	0	0	0
<i>Hemitrichia serpulata</i> (Scop.) Rostaf.	R	4	2	0	0	0	0	0	2	0
<i>Perichaena chrysosperma</i> (Currey) Lister	O	5	—	0	1	0	1	0	3	0
<i>Perichaena depressa</i> Libert	C	19	1	0	5	1	0	5	5	2
<i>Perichaena vermicularis</i> (Schwein.) Rostaf.	R	2	—	0	2	0	0	0	0	0
Order Physarales										
<i>Diderma effusum</i> (Schwein.) Morgan	R	1	1	0	0	0	0	0	0	0
<i>Diderma hemisphaericum</i> (Bull) Hornem	A	39	—	7	10	5	4	2	5	6
<i>Didymium iridis</i> (Ditmar) Fr.	C	17	2	2	4	3	0	0	1	5
<i>Didymium nigripes</i> (Link) Fr.	O	13	3	1	2	2	1	0	2	2
<i>Didymium ochroideum</i> G. Lister	O	5	—	0	0	1	3	1	0	0
<i>Didymium squamololum</i> (Alb. & Schwein.) Fr.	A	69	3	14	12	23	3	0	10	4
<i>Physarum album</i> (Bull) Chevall	A	68	—	9	7	36	2	0	7	7
<i>Physarum bivalve</i> Pers.	C	19	—	8	1	3	2	0	4	1
<i>Physarum bogoriense</i> Racib.	O	10	—	1	2	1	0	1	3	2
<i>Physarum cinereum</i> (Batsch) Pers.	O	11	—	1	3	0	0	1	2	4
<i>Physarum compressum</i> Alb. & Schwein.	A	75	—	13	20	9	6	0	13	14
<i>Physarum echinosporum</i> Lister	A	43	—	11	4	9	2	4	9	4
<i>Physarum leucophaeum</i> Fr.	A	29	—	10	5	4	1	0	4	5
<i>Physarum melleum</i> (Berk. & Broome) Massee	O	7	5	0	0	0	0	0	0	2
<i>Physarum pusillum</i> (Berk. & M.A. Curtis) G. Lister	R	1	1	0	0	0	0	0	0	0
<i>Physarum pulcherrimum</i> Berk & Ravenel	R	4	4	0	0	0	0	0	0	0
<i>Physarum roseum</i> Berk. & Broome	R	1	1	0	0	0	0	0	0	0
<i>Physarum superbum</i> Hagelst.	R	1	1	0	0	0	0	0	0	0
<i>Physarum viride</i> (Bull.) Pers.	O	8	3	0	1	0	2	0	0	2
Order Protosteliomycetes										
<i>Ceratomyxa fruticulosa</i> (Müll.) Mac	R	4	4	0	0	0	0	0	0	0
Order Stemonitales										
<i>Collaria arcyrionema</i> (Rostaf.) Nann-Bremek ex Lado	C	16	—	4	5	4	1	1	0	1
<i>Comatrichanigra</i> (Pers. ex J.F. Gmel.) Schroet.	C	16	—	3	3	3	3	0	2	2
<i>Craterium aureum</i> (Schum.) Rostaf.	R	1	1	0	0	0	0	0	0	0
<i>Craterium leucocephalum</i> (Pers.) Ditmar	R	3	3	0	0	0	0	0	0	0
<i>Diachea leucopodia</i> (Bull.) Rostaf.	O	12	1	0	1	4	2	0	1	3
<i>Lamprodermascintillans</i> (Berk. & Broome) Morgan	A	30	—	6	4	4	5	6	1	4
<i>Stemonitis axifera</i> (Bull). T. Macbr.	O	11	1	0	6	1	1	1	1	0
<i>Stemonitis fusca</i> Roth	A	54	—	10	19	11	3	4	4	3
<i>Stemonitis herbatica</i> Peck.	O	10	2	1	4	0	2	0	1	0
Total number of records		926	71	148	197	164	81	42	128	95
Number of species		42		19	26	22	21	13	21	20
Number of genera		16		8	12	12	11	10	10	10
Chao 2-estimated mean number of species		47.2		36.9	27.1	25.3	22.1	36.9	23.0	20.1
% sampling effort		89.0		51.5	95.9	87.0	95.0	35.2	91.3	99.5
Taxonomic diversity index		2.63		2.00	2.17	1.83	1.91	1.30	2.10	2.00

AI = abundance index (A = abundant, C = common, O = occasional, R = rare).

especially under conditions when the logs are extremely air dried (Schnittler et al 2013). Myxomycetes found in various forests differ from one mountain to another, as well as up and down the meridians of the earth. However, the myxomycetes species found in one mountain is typically more comparable with those found in a mountain closer to it than in one that is situated farther, because geographical position affects environmental conditions (Novozhilov and Schnittler 1997). In the Philippines, several studies on mountain myxomycetes were conducted. For instance, 33 species of myxomycetes were recorded from Mt Arayat in Pampanga, five of those being new records for the country (Dagamac et al 2011), and 21 species were recorded from Mt Makulot in Batangas (Cheng et al 2013).

Myxomycetes also thrive in coastal areas, and live most favorably in habitats such as old, rotting piles of coconut husks and nuts (Ing and Hnatiuk 1981). Coconut husks retain plenty of water and dry out only gradually, providing a condition that is conducive for the growth of myxomycetes over an extended period of time. This is principally evident during the start of the dry season when the majority of other habitats, where myxomycetes grow, have already dried out (Ing and Hnatiuk 1981). Succulent plants in areas near the coast were also productive substrates for myxomycetes in the field (Lado et al 2007). Adamonyte and Mitchell (2000) recorded *Licea clarkii* as a species of myxomycetes commonly found in coastal areas. Furthermore, Bosselaers (2004) cited several other species that grow in this type of habitats, e.g. *A. cinerea*, *Arcyria incarnata*, *Arcyria*

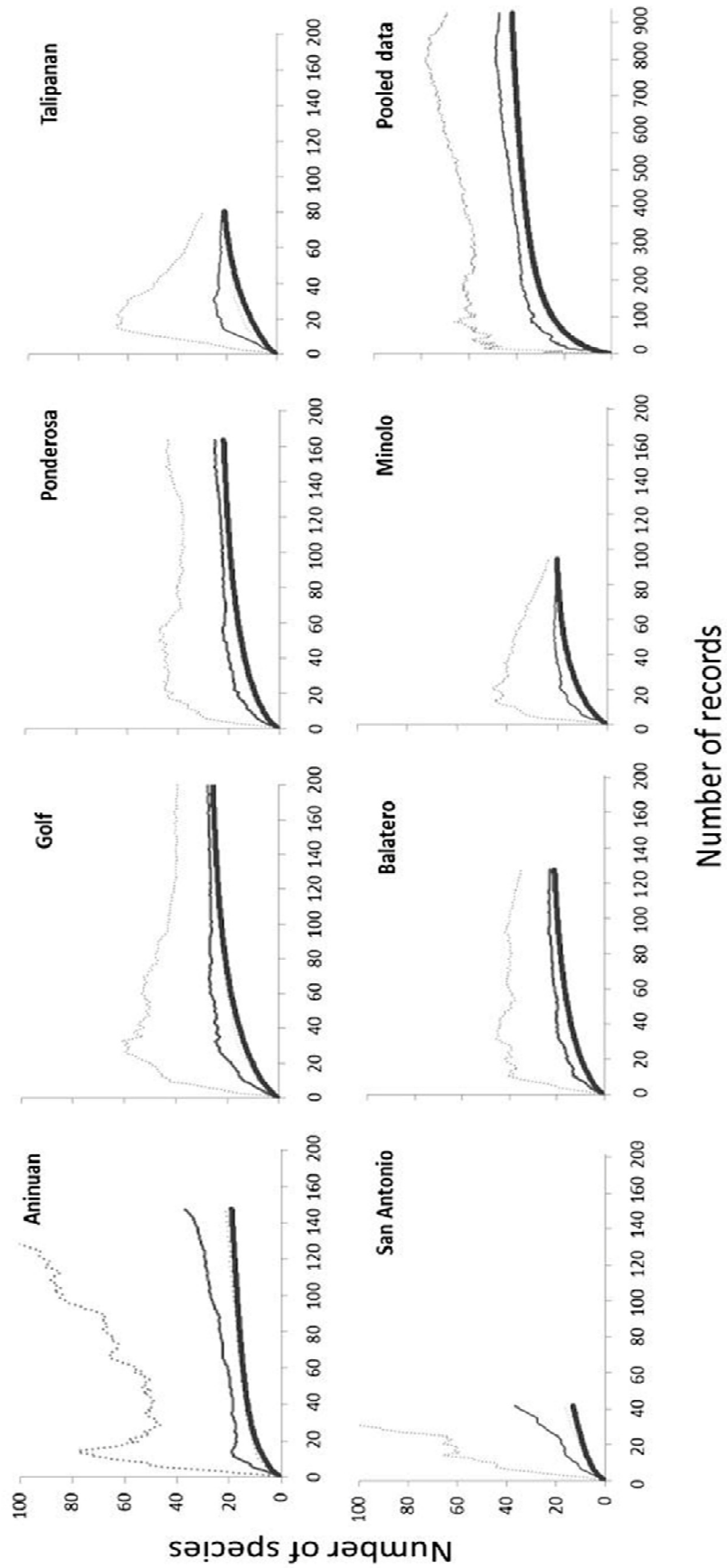


Figure 2. Species accumulation curves generated for each of the seven collecting localities including pooled data. The thick solid line represents individual-based species accumulation curve generated by EstimateS. Thin solid line: Chao 2 estimator and its 5% and 95% confidence interval (thin dotted lines).

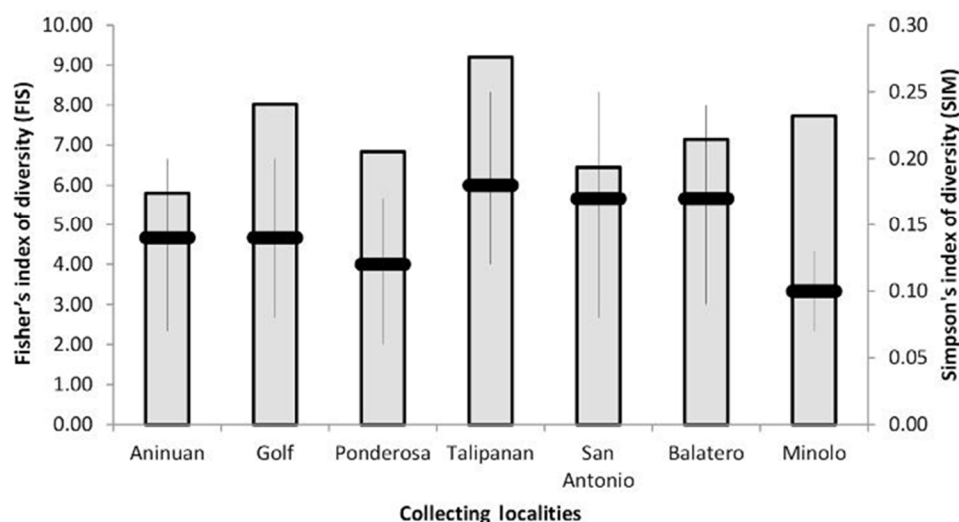


Figure 3. The α diversity in the seven collecting localities. Gray bars denote FIS; horizontal black lines show the SIM with its upper and lower 95% confidence interval (thin vertical lines) as computed by SPADE. FIS = Fisher's index of diversity; SIM = Simpson's index of diversity; SPADE = Species Prediction and Diversity Estimation.

obvelata, *Comatricha nigra*, *Cribraria cancellata*, *Enerthenema papillatum*, *Enteridium lycoperdon*, *Enteridium splendens*, *Fuligo septica*, *Lycogala epidendrum*, *Stemonaria fuscoidea*, *Stemonitis flavogenita*, *S. fusca*, *Stemonitis splendens*, and *Stemonitopsis subcaespitosa*.

In fact, by merely considering the richness of species in each collecting locality in our study, the forest patches found on lowland montane (Golf and Ponderosa) and community (Talipanan) forests had a relatively higher number of myxomycete species in comparison to the collecting points in coastal forests (Table 2). However, the evenness of species found among the three forest types is not statistically different among each other. If taxonomic diversity is to be considered, the lesser species but greater genera ratio in the coastal forest of San Antonio is intuitively the most taxonomically diverse among the seven collecting points. These findings now support the hypothesis that forest structure in the tropics affects myxomycete diversity (Rojas and Stephenson 2012).

Anthropogenic disturbance may influence myxomycete communities

A comparison of the communities of myxomycetes found in collecting localities (Table 3) showed that the highest value of dissimilarity were obtained from pairwise combination of disturbed collecting locality namely, Ponderosa-San Antonio and Ponderosa-Talipanan. As what was observed, Ponderosa has forest patches that are significantly disturbed because of different anthropogenic activities, i.e. trekking and animal domestication. The two other localities (coastal forest in San Antonio and community forest of Talipanan) are also exposed to high disturbance

due to human activities, the former being exposed to water sport activities for the tourists and the latter being a location where the indigenous tribes settle.

The higher dissimilarity can be attributed to the facts that (1) a lower number of myxomycete species were collected in San Antonio and (2) Ponderosa harbors more unique species with greater abundance than Talipanan. Although it is still highly speculative from our findings, it seems that human interference may be a reason why there was a lesser yield of myxomycetes in coastal forests in San Antonio and why Ponderosa had a higher number of unique species compared to the other collecting points. Disturbances most often affect biological diversity. The recent study of Rojas and Stephenson (2013) on myxomycete assemblages in the Neotropics, particularly in southwestern Peruvian Amazon, suggested that forest disturbances and habitat loss lead to differences in occurrences of the fruiting bodies of myxomycetes. A similar observation was also noted for the myxomycete communities found in disturbed areas of the Bicol Peninsula in the Philippines (Dagamac et al 2015a). Species richness in myxomycetes may follow the intermediate disturbance hypothesis (Connell 1978), wherein the diversity of species is greatest in ecosystems with intermediate degree of disturbances, while in ecosystems with a high or low degree of disturbances, it becomes less. In such cases, it is interesting to further investigate the anthropogenic disturbance gradient in different forest types in Puerto Galera, which being a tourist hotspot of the Philippines is most often exposed to many urban development projects, to further understand forest dynamics using myxomycetes assemblages as the model organisms.

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Table 3. Morisita index for dissimilarity of myxomycete communities between collecting sites (upper right) and numbers of species shared by two collecting sites (lower left).

	Aninuan	Golf	Ponderosa	Talipanan	San Antonio	Balatero	Minolo
Aninuan	—	0.014	0.127	0.041	0.115	0.004	0.031
Golf	18	—	0.206	0.012	0.081	0.009	0.055
Ponderosa	16	21	—	0.271	0.350	0.193	0.159
Talipanan	14	19	17	—	0.057	0.001	0.116
San Antonio	8	12	12	8	—	0.094	0.196
Balatero	16	20	17	16	9	—	0.067
Minolo	16	18	17	15	9	17	—

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Myxomycete research in the Philippines: Updates and opportunities

Dagamac NHA¹ and dela Cruz TEE²

¹*Institute of Botany and Landscape Ecology, University of Greifswald, Soldmannstr. 15, D-17487, Greifswald, Germany*

²*Department of Biological Sciences, College of Science and Fungal Biodiversity and Systematics Group, Research Center for the Natural and Applied Sciences, University of Santo Tomas, España 1008 Manila, Philippines*

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Abstract

Myxomycetes, commonly known as slime molds, are phagotrophic, eukaryotic organisms that exhibit both fungal and protozoan characteristics. They are widely distributed both in temperate and tropical ecoregions, where they usually occur on dead plant substrates, such as bark, twigs, dried leaves, woody vines, and even decayed inflorescences or fruits. Their unique, diverse morphologies and fascinating life strategies make them ideal model organisms to study life processes. However, despite the high potential diversity in tropical systems, little is known about them particularly in archipelagic countries, such as the Philippines. In fact, previous studies on myxomycetes in the Philippines in the late 70s and early 80s by Reynolds encompassed the most comprehensive listing for the country. A total of 107 species were recorded at that time for the Philippines and roughly 50% of these species represented new records for the country. But the paper was mainly an extensive, annotated species listing. In recent years, myxomycete research in the country has progressed beyond species lists to diversity and ecological studies. Several papers by the UST RCNAS Fungal Biodiversity and Systematics group have documented the occurrence and distribution of slime molds in several habitat types, e.g. in forest parks, coastal and inland limestone forests, lowland mountain forests, and from varied substrata – grass litter, aerial and ground leaf litter, twigs, and bark. These studies updated the list of species of myxomycetes in the Philippines to 150. These also provided baseline information on the ecological patterns and geographic distribution of slime molds in the tropics. This paper presents an update on slime mold research in the Philippines for the 35 years following Reynolds' publication in 1981 and discusses challenges and opportunities for further studies.

Key words – archipelago – checklist – Paleotropics – slime molds – taxonomy

This paper is written in honor of two mycologists who started and contributed significantly to the myxomycete research in the Philippines, Don Reynolds and Irineo J. Dogma Jr.

Introduction

Myxomycetes, also known as plasmodial slime molds or true slime molds, are a small, relatively homogenous group of phagotrophic fungus-like protists (Schnittler et al. 2012, Rojas & Stephenson 2013), with about 1 000 species known and described worldwide (Lado 2005-2015). These species are often abundant in many forested regions, particularly in areas with decaying logs,

twigs and leaf litter (Stephenson & Stempen 1994, Rojas & Stephenson 2013). These substrata provide them with a sufficient supply of potential food, such as bacteria, yeasts, algae and other microscopic organisms (Lado et al. 2013). Myxomycetes are also recognized as producers of bioactive secondary metabolites. For example, Dembitsky et al. (2005) listed about 100 natural compounds, e.g. fatty acids, lipids, naphthoquinone pigments, aromatic compounds, alkaloids and terpenoids, isolated and obtained from several species of myxomycetes. Several of these compounds are known to exhibit antitumor activities, e.g. cyclic phosphatidic acid from *Physarum polycephalum* (Kobayashi et al. 2002) and pyrroloiminoquinone from *Didymium bahiense* (Ishibashi et al. 2001). Myxomycetes are also key components in soil microbiota (Feest & Madelin 1988, Stephenson et al. 2011, Hoppe & Schnittler 2015) and may play a role in maintaining balance in soil ecosystems (Feest 1987; Foissner 1999). Many insects also depend on slime molds for food (Keller & Snell 2002). Despite their numerous applications and important role in nature, the study of the biodiversity of myxomycetes has not been given much attention particularly in the Southeast Asian Paleotropics such as the Philippines, where higher species diversity can be expected (Dagamac et al. 2014).

The earliest report of myxomycetes in the Philippines started from Uyenco (1973) who originally claimed to have published the first report on Philippine myxomycetes. From the 314 specimens collected by Uyenco from Luzon (Quezon City and Laguna) and Mindanao (Basilan and Zamboanga) during the year 1961 to 1973, 18 species were reported belonging to 10 genera. However, Dogma (1975) noted that Martin & Alexopoulos (1969) already credited the Philippines with 22 species in their book entitled "*The Myxomycetes*". Dogma (1975) then listed 46 species of myxomycetes from 20 genera for the Philippines. The most wide-ranging annotation on Philippine myxomycetes however, was conducted by Don Reynolds in 1981. Reynolds (1981) initially reported the myxomycete collections from Mindanao (Davao, Cotabato and Zamboanga) by E. B. Copeland, and from Luzon by A. D. E. Elmer (Benguet) and E.D. Merrill (Bataan, Manila, Cavite and Laguna). These collections can now be found in the British Museum in London. He also presented an annotated list of 107 species of Philippine myxomycetes based on published and unpublished records. In this list, 53 species were listed as novel records for the country. Myxomycetes that are part of the species list were from the genera *Arcyria*, *Badhamia*, *Ceratiomyxa*, *Clastoderma*, *Comatricha*, *Craterium*, *Cribaria*, *Diachea*, *Dictydium*, *Diderma*, *Didymium*, *Echinostelium*, *Fuligo*, *Hemitrichia*, *Lamproderma*, *Licea*, *Lycogala*, *Metatrichia*, *Perichaena*, *Physarella*, *Physarum*, *Stemonitis*, *Trichia*, and *Tubifera*. Most of these myxomycetes were collected from substrates obtained from several sites in the country: (1) Luzon: Batanes, Ilocos, Mt. Province, Benguet, Bataan, Laguna, Quezon and Palawan, (2) Visayas: Camarines Sur, Sorsogon, Albay, Iloilo, Antique, Leyte, Negros and Cebu, and (3) Mindanao: Agusan, Cotabato, Surigao del Sur, Davao, Zamboanga and Sulu. The 107 myxomycete species reported in this study also included one unidentified species of *Didymium* collected from Cebu that resembles *D. squamulosum*, except that the spores are larger than the typical *D. squamulosum*. Moreover, two myxomycete species listed were currently synonyms of two other myxomycete species found on that same list. *Stemonitis nigrescens* and *S. smithii* are basionyms of *S. axifera* and *S. fusca*, respectively. The list also corresponded to 25% of known species at that time and to 60% of the estimated total myxomycete flora in the Philippines (Reynolds, 1981). Since then, myxomycete survey remained stagnant.

For several decades, there were no published literatures about any myxomycete records since the last comprehensive annotation of Reynolds (1981). Thus in 2009, dela Cruz et al. initially looked at the myxomycetes collection at the Museum of Natural History Mycological Herbarium at the University of the Philippines in Los Baños, Laguna. Myxomycete specimens here were mainly deposited by Dogma, Reynolds and Quimio and belonged to the 21 genera: *Arcyria*, *Ceratiomyxa*, *Cienkowskia* (now identified as *Willkommlangea*), *Clastoderma*, *Comatricha*, *Craterium*, *Cribaria*, *Diachea*, *Dictydium*, *Diderma*, *Didymium*, *Fuligo*, *Hemitrichia*, *Lamproderma*, *Lycogala*, *Perichaena*, *Physarella*, *Physarum*, *Stemonitis*, *Trichia* and *Tubifera*. Interestingly, the collection also included specimens from the USA, Ecuador and Costa Rica dating from pre-World War II, i.e.

1888 to 1939. These deposited specimens were found to be generally in good condition. On the same year, a specimen found in Anda Island, Pangasinan was identified as a new species, *Craterium retisporum* (Moreno et al. 2009). This new myxomycete species were morphologically erected as new to science because of its distinguishing prominent reticulations found on the spore ornamentation. Dagamac et al. (2010) added five new records of myxomycetes for the Philippines, i.e. *Clastoderma microcarpum*, *Dianema harveyi*, *Diderma subasteroides*, *Physarum leucophaeum*, and *Stemonitis pallida*. These species were collected from bark samples of *Samanea samans* (Jacq) Merr. obtained from 21 different sites in Luzon Island. Furthermore, Dagamac et al. (2011) reported another 5 new records, namely, *Arcyria afroalpina*, *Collaria arcyronema*, *Craterium concinnum*, *Enerthenema papillatum*, and *Licea biforis* from Mount Arayat National Park, Pampanga. Two additional records, *Lepidoderma tigrinum* and *Perichaena pedata*, were also reported by dela Cruz et al. (2011) as new records for the Philippines. Macabago et al. (2012) reported seven newly recorded species of myxomycetes in the Philippines which were identified as *Arcyria globosa*, *Collaria rubens*, *Comatricha robusta*, *Craterium atrolucens*, *Lamproderma cacographicum*, *Oligonema schweinitzii*, and *Perichaena microspora* from Lubang Island, Occidental Mindoro. Additionally, three new records were reported by Kuhn et al. (2013a) from Pangasinan and additional 17 new records by dela Cruz et al. (2014) from Bataan, Cavite and Subic. Recently, Dagamac et al. (2015a) found 8 new records in Bicol Peninsula which now brings the total of myxomycetes recorded in the Philippines to 150.

With such additions to the myxomycete flora accounted for a biodiversity hotspot like the Philippines, this paper (1) provides an updated checklist of the slime molds reported in the Philippines and (2) discusses the challenges and opportunities of engaging one's interest in myxomycete research in the country.

General Study Area

The Philippines is an archipelago composed of 7,107 islands that lies in the Southeast Asian region. Majority of these islands are assumed to be volcanic in origin as it is geographically part of the Pacific Ring of Fire (Hall 2002). General topography in the area is hilly and mountainous typically having constricted coastal plains with abundant rivers, streams and lakes (Catibog-Sinha & Heaney 2006). Every island is endowed with sand beaches, clear coast lines and tropical rain forested mountains wherein the highest point in the country is the peak of Mt. Apo in Mindanao which is ca. 2,954 masl. The country's climate can be described to have a relatively high temperature and humidity with plentiful amount of annual rainfall (Lantican 2001). Similar to other tropical countries, the seasonality in the Philippines is divided into wet and dry seasons. Due to erratic rainfall distribution in different areas in the Philippines, four types of climate were designated on different parts of the archipelago (Philippine Institute for Development Studies, 2005). Type I are characterized of having two pronounced season that is dry during November to April, and wet during all the other months of the year. Areas included in this climate type are part of the Northwestern Luzon (Ilocos region, Western Mt. Provinces, and some part of the Southern Tagalog region). Type II climate has no clear dry period but with a very pronounced rainy season from the months of December to February. There is no single dry month and the minimum annual rainfall occurs during the months of March to May. The Southeastern Luzon areas (Bicol region and Quezon Province) are some examples of localities exposed to such climate type. Type III has no clear seasonality, but is relatively dry from November to April. Areas in the Philippines that experiences such climate type includes the northeastern part of the Ilocos region, scattered islands of the Central Visayas (Aklan, Capiz, Iloilo, Siquijor) and Western part of Mindanao (Agusan, del Sur, Bukidnon, and east Maguindanao). Lastly, Type IV climate is defined to have more or less an evenly distributed rainfall throughout the year and major Visayas islands like Cebu, Bohol, Western Samar and Southern Mindanao (Zamboanga, Davao, Sultan Kudarat) typically experience this climate.

Due to such promising topography and tropical climate distribution in the Philippines, the country is popularly known to be a biodiversity megahotspot (Catibog-Sinha & Heany 2006). With

its tropical climate, the country is gifted with numerous forest ecosystems: lowland rainforest, montane - mossy forest, pine forest and coastal, beach or mangrove forest. It is also completely bordered by tropical seas, thus isolating the archipelago from other Asian landmass by hundreds of kilometers of open water. Geological evidence has shown that the Philippines, with the exception of the Palawan and Mindoro regions, have always been isolated (Heaney 1998). Such geographic isolation and ideal climatic conditions resulted in floral and faunal endemism, as high as 80% (Catibog-Sinha & Heaney 2006). However, with its annual deforestation rate estimated to be 1.4% or about 89,000 hectares removed per year, the country is now listed as one of the most threatened ecosystems on the planet. In 2004, only 24% of the total land area (only about 7.2 million hectares of about 30 million hectares) remained covered with forests (Catibog-Sinha & Heaney 2006). Thus, this necessitates an urgent assessment of the country's biodiversity since many areas remained un- or under-explored and many species remain undiscovered including the myxomycetes.

List of myxomycetes for the Philippines

Table 1 In the myxomycete listing that follows, we present a table for all myxomycetes recorded for the Philippines. Information is provided on the source(s) of each record where the species was first mentioned, along with some general comments. Nomenclature basically follows Lado (2005-2015).

Myxomycete species		Sources
1	<i>Alwisia bombarda</i> Berk. & Broome	First reported as <i>Tubifera bombarda</i> (Berk. & Broome) G.W. Martin by Reynolds (1981)
2	<i>Arcyria afroalpina</i> Rammeloo	First reported from Dagamac et al. (2011)
3	<i>Arcyria cinerea</i> (Bull.) Pers.	First reported from Reynolds (1981)
4	<i>Arcyria denudata</i> (L.) Wettst.	First reported from Reynolds (1981)
5	<i>Arcyria globosa</i> Schwein.	First reported from Macabago et al. (2012)
6	<i>Arcyria incarnata</i> (Pers. ex J.F. Gmel.) Pers.	First reported from Reynolds (1981)
7	<i>Arcyria insignis</i> Kalchbr. & Cooke	First reported from Reynolds (1981)
8	<i>Arcyria magna</i> Rex	First reported from Reynolds (1981)
9	<i>Arcyria marginoundalata</i> Nann.-Bremek. & Y. Yamam.	First reported from dela Cruz et al. (2014)
10	<i>Arcyria obvelata</i> (Oeder) Onsberg	First reported as <i>Arcyria nutans</i> (Bull.) Grev. by Reynolds (1981)
11	<i>Arcyria virescens</i> G. Lister	First reported from Reynolds (1981)
12	<i>Badhamia affinis</i> Rostaf.	First reported from dela Cruz et al. (2014)
13	<i>Badhamia utricularis</i> (Bull.) Berk.	First reported from Reynolds (1981)
14	<i>Calomyxa metallica</i> (Berk.) Nieuwl.	First reported from Reynolds (1981)
15	<i>Ceratiomyxa fruticulosa</i> (O.F. Müll.) T. Macbr.	First reported from Reynolds (1981)
16	<i>Clastoderma debaryanum</i> A. Blytt	First reported from Reynolds (1981)
17	<i>Clastoderma microcarpum</i> (Meyl.) Kowalski	First reported from Dagamac et al. (2010)
18	<i>Collaria arcyronema</i> (Rostaf.) Nann.-Bremek. ex Lado	First reported from Dagamac et al. (2011) however Reynolds (1981) first reported this species as <i>Lamproderma arcyronema</i>
19	<i>Collaria rubens</i> (Lister) Nann.-Bremek.	First reported from Macabago et al. (2012)
20	<i>Comatricha fragilis</i> Meyl.	First reported from Dagamac et al. (2015a)
21	<i>Comatricha longipila</i> Nann.-Bremek.	First reported from Reynolds (1981)
22	<i>Comatricha nigra</i> (Pers. ex J.F. Gmel.) J. Schröt.	First reported from Reynolds (1981)
23	<i>Comatricha pulchella</i> (C. Bab.) Rostaf.	First reported from Dagamac et al. (2015a)
24	<i>Comatricha robusta</i> (T.N. Lakh. & K.G. Mukerji) Nann.-Bremek. & Y. Yamam.	First reported from Macabago et al. (2012)
25	<i>Comatricha tenerrima</i> (M.A. Curtis) G. Lister	First reported from dela Cruz et al. (2014)
26	<i>Craterium atrolucens</i> Flatau	First reported from Macabago et al. (2012)
27	<i>Craterium concinnum</i> Rex	First reported from Dagamac et al. (2011)
28	<i>Craterium leucocephalum</i> (Pers. ex J.F. Gmel.) Ditmar	Reynolds (1981)
29	<i>Craterium microcarpum</i> H.Z. Li, Yu Li & Shuang L. Chen	First reported from Kuhn et al. (2013a)
30	<i>Craterium minutum</i> (Leers) Fr.	First reported from Reynolds (1981)
31	<i>Craterium paraguayense</i> (Speg.) G. Lister	First reported from Reynolds (1981)

	Myxomycete species	Sources
32	<i>Craterium retisporum</i> G. Moreno, D.W. Mitch. & S.L. Stephenson	First reported from Moreno et al. (2009). This new species was described from collections in the Philippines and Western Australia.
33	<i>Cribraria atrofusca</i> G.W. Martin & Lovejoy	First reported from Reynolds (1981)
34	<i>Cribraria cancellata</i> (Batsch) Nann.-Bremek.	First reported as <i>Dictydium cancellatum</i> (Batsch) T. Macbr. by Reynolds (1981)
35	<i>Cribraria microcarpa</i> (Schrad.) Pers.	First reported from Reynolds (1981) as <i>Cribraria pachydictyon</i> Nann.-Bremek.
36	<i>Cribraria piriformis</i> Schrad.	First reported from Reynolds (1981)
37	<i>Cribraria violacea</i> Rex	First reported from Reynolds (1981)
38	<i>Diachea bulbilosa</i> (Berk. & Broome) Lister	First reported from Reynolds (1981)
39	<i>Diachea leucopodia</i> (Bull.) Rostaf.	First reported from Reynolds (1981)
40	<i>Diachea radiata</i> G. Lister & Petch	First reported from Reynolds (1981)
41	<i>Diachea splendens</i> Peck	First reported from Reynolds (1981)
42	<i>Dianema harveyi</i> Rex	First reported from Dagamac et al. (2010)
43	<i>Dictydiaethalium plumbeum</i> (Schumach.) Rostaf.	First reported from Reynolds (1981)
44	<i>Diderma chondrioderma</i> (de Bary & Rostaf.) G. Lister	First reported from dela Cruz et al. (2014)
45	<i>Diderma effusum</i> (Schwein.) Morgan	First reported from Reynolds (1981)
46	<i>Diderma fallax</i> (Rostaf.) E.Sheld.	First reported as <i>Diderma lyallii</i> (Masse) T. Macbr. by Reynolds (1981)
47	<i>Diderma hemisphaericum</i> (Bull.) Hornem.	First reported from Reynolds (1981)
48	<i>Diderma rugosum</i> (Rex) T. Macbr.	First reported from Reynolds (1981)
49	<i>Diderma saundersii</i> (Berk. & Broome ex Masse) E. Sheld.	First reported from dela Cruz et al. (2014)
50	<i>Diderma subasteroides</i> M.L. Farr	First reported from Dagamac et al. (2010)
51	<i>Didymium anellus</i> Morgan	First reported from Reynolds (1981)
52	<i>Didymium anellus</i> Morgan	First reported from dela Cruz et al. (2014)
53	<i>Didymium clavus</i> (Alb. & Schwein.) Rabenh.	First reported from Reynolds (1981)
54	<i>Didymium floccosum</i> G.W. Martin, K.S. Thind & Rehill	First reported from Dagamac et al. (2015a)
55	<i>Didymium iridis</i> (Ditmar) Fr.	First reported from Reynolds (1981)
56	<i>Didymium leoninum</i> Berk. & Broome	First reported from Reynolds (1981)
57	<i>Didymium megalosporum</i> Berk. & M.A. Curtis	First reported from Reynolds (1981)
58	<i>Didymium melanospermum</i> (Pers.) T. Macbr.	First reported from Reynolds (1981)
59	<i>Didymium minus</i> (Lister) Morgan	First reported from Reynolds (1981)
60	<i>Didymium nigripes</i> (Link) Fr.	First reported from Reynolds (1981)
61	<i>Didymium ochroideum</i> G. Lister	First reported from dela Cruz et al. (2014)
62	<i>Didymium serpula</i> Fr.	First reported from dela Cruz et al. (2014)
63	<i>Didymium squamulosum</i> (Alb. & Schwein.) Fr. & Palmquist	First reported from Reynolds (1981)
64	<i>Didymium verrucosporum</i> A.L. Welden	First reported from Dagamac et al. (2015a)
65	<i>Echinostelium minutum</i> de Bary	First reported from Reynolds (1981)
66	<i>Elaeomyxa miyazakiensis</i> (Emoto) Hagelst.	First reported from Kuhn et al. (2013a)
67	<i>Enerthenema papillatum</i> (Pers.) Rostaf.	First reported from Dagamac et al. (2011)
68	<i>Fuligo aurea</i> (Penz.) Y.Yamam.	First reported as <i>Erionema aureum</i> Penz. by Reynolds (1981)
69	<i>Fuligo cinerea</i> (Schwein.) Morgan	First reported from dela Cruz et al. (2014)
70	<i>Fuligo septica</i> (L.) F.H. Wigg.	First reported from Reynolds (1981)
71	<i>Hemitrichia calyculata</i> (Speg.) M.L.Farr	First reported from Dagamac et al. (2015a) however Reynolds (1981) first reported this species as <i>Hemitrichia stipitata</i> (Masse) T. Macbr
72	<i>Hemitrichia intorta</i> (Lister) Lister	First reported from Reynolds (1981)
73	<i>Hemitrichia leiocarpa</i> (Cooke) Lister	First reported as <i>Arcyria leiocarpa</i> (Cooke) Masse by Reynolds (1981)
74	<i>Hemitrichia minor</i> G. Lister	First reported from Dagamac et al. (2015a) however Reynolds (1981) first reported this species as <i>Perichaena minor</i> (G. Lister) Hagelst.
75	<i>Hemitrichia serpula</i> (Scop.) Rostaf. ex Lister	First reported from Reynolds (1981)
76	<i>Lamproderma cacographicum</i> Bozonnet, Mar. Mey. & Poulain	First reported from Macabago et al. (2012)
77	<i>Lamproderma scintillans</i> (Berk. & Broome) Morgan	First reported from Reynolds (1981)
78	<i>Lepidoderma tigrinum</i> (Schrad.) Rostaf.	First reported from dela Cruz et al (2011)
79	<i>Licea biforis</i> Morgan	First reported from Dagamac et al. (2011)

	Myxomycete species	Sources
80	<i>Licea erecta</i> K.S. Thind & Dhillon	First reported from Reynolds (1981)
81	<i>Licea floriformis</i> T.N. Lakh. & R.K. Chopra	First reported as <i>Licea floriformis</i> var. <i>aureospora</i> M.T.M. Willemse & Nann.-Bremek by dela Cruz et al. (2014)
82	<i>Licea operculata</i> (Wingate) G.W. Martin	First reported from dela Cruz et al. (2014)
83	<i>Lycogala epidendrum</i> (L.) Fr.	First reported from Reynolds (1981)
84	<i>Lycogala exiguum</i> Morgan	First reported from Reynolds (1981)
85	<i>Meriderma cribrarioides</i> (Fr.) Mar.Mey. & Poulain	First reported as <i>Lamproderma cribrarioides</i> (Fr.) R.E. Fr. By Reynolds (1981)
86	<i>Metatrichia vesparia</i> (Batsch) Nann.-Bremek. ex G.W. Martin & Alexop.	First reported from Reynolds (1981)
87	<i>Oligonema schweinitzii</i> (Berk.) G. W. Martin	First reported from Macabago et al. (2012)
88	<i>Perichaena chrysosperma</i> (Curr.) Lister	First reported from Reynolds (1981)
89	<i>Perichaena corticalis</i> (Batsch) Rostaf.	First reported from Reynolds (1981)
90	<i>Perichaena depressa</i> Lib.	First reported from Reynolds (1981)
91	<i>Perichaena dictyonema</i> Rammeloo	First reported from dela Cruz et al. (2014)
92	<i>Perichaena microspora</i> Penz. & Lister	First reported from Macabago et al. (2012)
93	<i>Perichaena minor</i> G. Lister	First reported from Reynolds (1981)
94	<i>Perichaena pedata</i> (Lister & G. Lister) Lister ex E. Jahn	First reported from dela Cruz et al. (2011)
95	<i>Perichaena reticulospora</i> H.W. Keller & D.R. Reynolds	First reported from Reynolds (1981)
96	<i>Perichaena vermicularis</i> (Schwein.) Rostaf.	First reported from Dagamac et al. (2015a) however dela Cruz et al. (2014) first reported this species as <i>Physarum vermiculare</i> Schwein. (Schwein.) Rostaf.
97	<i>Physarella oblonga</i> (Berk. & M.A. Curtis) Morgan	First reported from Reynolds (1981)
98	<i>Physaru nucleatum</i> Rex	First reported from Reynolds (1981)
99	<i>Physarum album</i> (Bull.) Chevall.	First reported as <i>Physarum nutans</i> Pers. by Reynolds (1981)
100	<i>Physarum bivalve</i> Pers.	First reported from Reynolds (1981)
101	<i>Physarum bogoriense</i> Racib.	First reported from Reynolds (1981)
102	<i>Physarum cinereum</i> (Batsch) Pers.	First reported from Reynolds (1981)
103	<i>Physarum compressum</i> Alb. & Schwein.	First reported from Reynolds (1981)
104	<i>Physarum crateriforme</i> Petch	First reported from dela Cruz et al. (2014)
105	<i>Physarum decipiens</i> M.A. Curtis	First reported from Kuhn et al. (2013a)
106	<i>Physarum didermoides</i> (Pers.) Rostaf.	First reported from Reynolds (1981)
107	<i>Physarum echinosporum</i> Lister	First reported from Reynolds (1981)
108	<i>Physarum flavicomum</i> Berk.	First reported from Reynolds (1981)
109	<i>Physarum globuliferum</i> (Bull.) Pers.	First reported from Reynolds (1981)
110	<i>Physarum gyrosum</i> Rostaf.	First reported from Reynolds (1981)
111	<i>Physarum lakhanpalii</i> Nann.-Bremek. & Y. Yamam.	First reported from dela Cruz et al. (2014)
112	<i>Physarum leucophaeum</i> Fr. & Palmquist	First reported from Dagamac et al. (2010)
113	<i>Physarum melleum</i> (Berk. & Broome) Massee	First reported from Reynolds (1981)
114	<i>Physarum nicaraguense</i> T. Macbr.	First reported from Reynolds (1981)
115	<i>Physarum notabile</i> T. Macbr.	First reported from Reynolds (1981)
116	<i>Physarum oblatum</i> T. Macbr.	First reported from Reynolds (1981)
117	<i>Physarum pezizoideum</i> (Jungh.) Pavill. & Lagarde	First reported from Reynolds (1981)
118	<i>Physarum polycephalum</i> Schwein.	First reported from Reynolds (1981)
119	<i>Physarum psittacinum</i> Ditmar	First reported from Reynolds (1981)
120	<i>Physarum pulcherrimum</i> Berk. & Ravenel	First reported from Reynolds (1981)
121	<i>Physarum pusillum</i> (Berk. & M.A. Curtis) G. Lister	First reported from Dagamac et al. (2015a)
122	<i>Physarum retisporum</i> G.W. Martin, K.S. Thind & Rehill	First reported from Reynolds (1981)
123	<i>Physarum rigidum</i> (G. Lister) G. Lister	First reported from Reynolds (1981)
124	<i>Physarum roseum</i> Berk. & Broome	First reported from Reynolds (1981)
125	<i>Physarum rubiginosum</i> Fr. & Palmquist	First reported from Reynolds (1981)
126	<i>Physarum stellatum</i> (Massee) G.W. Martin	First reported from Reynolds (1981)
127	<i>Physarum superbum</i> Hagelst.	First reported from dela Cruz et al. (2014)
128	<i>Physarum tenerum</i> Rex	First reported from Reynolds (1981)
129	<i>Physarum viride</i> (Bull.) Pers.	First reported from Reynolds (1981)
130	<i>Reticularia lycoperdon</i> Bull.	First reported from Reynolds (1981)
131	<i>Stemonaria fuscoides</i> Nann.-Bremek. & Y. Yamam.	First reported from Dagamac et al. (2015a)
132	<i>Stemonaria longa</i> (Peck) Nann.-Bremek., R.Sharma & Y. Yamam.	First reported as <i>Comatricha longa</i> Peck by Reynolds (1981)

Myxomycete species		Sources
133	<i>Stemonitis axifera</i> (Bull.) T. Macbr.	First reported from Reynolds (1981). <i>Stemonitis smithii</i> T. Macbr. was reported as a separate species by Reynolds (1981)
134	<i>Stemonitis flavogenita</i> E. Jahn	First reported from Dagamac et al. (2015a)
135	<i>Stemonitis fusca</i> Roth	First reported from Reynolds (1981). <i>Stemonitis nigrescens</i> Rex was reported as a separate species by Reynolds (1981)
136	<i>Stemonitis herbatica</i> Peck	First reported from Reynolds (1981)
137	<i>Stemonitis pallida</i> Wingate	First reported from Dagamac et al. (2010)
138	<i>Stemonitis splendens</i> Rostaf.	First reported from Reynolds (1981)
139	<i>Stemonitis uvifera</i> T. Macbr.	First reported from dela Cruz et al. (2014)
140	<i>Stemonitopsis subcaespitosa</i> (Peck) Nann.-Bremek.	First reported as <i>Comatricha subcaespitosa</i> Peck by Reynolds (1981)
141	<i>Stemonitopsis typhina</i> (F.H.Wigg.) Nann.-Bremek.	First reported as <i>Comatricha typhoides</i> (Bull.) Rostaf. by Reynolds (1981)
142	<i>Trichia botrytis</i> (J.F. Gmel.) Pers.	First reported from Reynolds (1981)
143	<i>Trichia contorta</i> (Ditmar) Rostaf.	First reported as <i>Hemitrichia karstenii</i> (Rostaf.) Lister by Reynolds (1981)
144	<i>Trichia decipiens</i> (Pers.) T. Macbr.	First reported from Reynolds (1981)
145	<i>Trichia erecta</i> Rex	First reported from Reynolds (1981)
146	<i>Trichia favoginea</i> (Batsch) Pers.	First reported from Reynolds (1981)
147	<i>Trichia verrucosa</i> Berk.	First reported from Reynolds (1981)
148	<i>Tubifera ferruginosa</i> Batsch) J.F. Gmel.	First reported from Reynolds (1981)
149	<i>Tubifera microsperma</i> (Berk. & M.A. Curtis) G.W. Martin	First reported from Reynolds (1981)
150	<i>Willkommlangea reticulata</i> (Alb. & Schwein.) Kuntze	First reported as <i>Cienkowskia reticulata</i> (Alb. & Schwein.) Rostaf. by Reynolds (1981)

Discussion

The updated number of myxomycetes records reported for the Philippines is 150. Comparably this number is still much less than the neotropical country, Costa Rica with 208 recorded species (Rojas et al. 2010). It is of course necessary to point out that Costa Rica is more subjected to much more sampling effort both temporally and spatially. Nevertheless, with regards to the other countries in the Southeast Asian region that were surveyed so far for myxomycetes, this number is considerably more numerous than the species listing data reported for Thailand (132 taxa, Ko Ko et al. 2010), Singapore (92, Rosing et al. 2011), Myanmar (67, Ko Ko et al. 2013), Vietnam (57, Tran et al. 2014) and Laos (44, Ko Ko et al. 2012). Since earlier literature about Philippine myxomycetes were mere species lists and no information about diversity and ecological studies had been conducted in most of the myxomycete surveys, classical approaches of rapid myxomycete surveys were employed at the UST RCNAS (University of Santo Tomas - Research Center for the Natural and Applied Sciences).

Surveys conducted in different forest types in the Philippines

For most of this survey in the Philippines, a major habitat type investigated for myxomycetes were the lowland mountain forests in the country. Mt. Arayat in Pampanga was used as a model area to observe the occurrence of myxomycetes along an elevational gradient, seasonality and anthropogenic disturbance (Dagamac et al. 2012; Dagamac et al. 2014). The northern slope of Mt. Makulot in Batangas yielded 21 species belonging to 11 genera (Cheng et al. 2013). So far, the highest report of myxomycete diversity for lowland forests were reported in the collective records from Bicol Peninsula with 62 species of myxomycetes reported, of which one species is a first report of *Stemonaria fuscoidea* in the Asian Paleotropics (Dagamac et al. 2015a). National ecoparks in the country were another suitable area for myxomycete surveys because of the intensive environmental protection that at least leads to the development of a secondary type of forest habitat. Baseline information on myxomycete assemblages were conducted in the La Mesa Watershed Ecopark (Macabago et al. 2010), Subic Bay Forest Reserve (dela Cruz et al. 2010), and karst landscape of Quezon National Park (Dagamac et al. 2015b). Furthermore, highland areas were

also studied by Kuhn et al. 2013b wherein a total of 25 species were reported in Benguet and forest parks in Baguio City.

The coastal forests of the Philippines were another type of habitat that were intensely surveyed since the archipelagic landscape of the Philippines makes it ideal for island biogeographic study of myxomycetes. Among the coastal islands that were already surveyed were several small islands in Hundred Islands National Park and Anda island in Pangasinan (dela Cruz et al. 2011; Kuhn et al. 2013a), Lubang Island in Occidental Mindoro (Macabago et al. 2012) and Polilio Island in Quezon (Viray et al. 2014). Besides simple surveys, comparative assessments of myxomycete diversity based on their forest types were conducted in the recent years. A comparative study between agricultural land and protected forest area of Mt. Kanlaon in Negros Oriental showed that a heterogenous plant community harbored higher myxomycete diversity than monotypic agricultural plantations (Alfaro et al. 2015). Dagamac et al. (2015c) observed how myxomycete communities are similar in terms of composition between mountain forests and coastal forest habitats in Puerto Galera, a UNESCO Man and Biosphere Reserve. Rea-Maminta et al. (2015) also compared myxomycete assemblages from forest patches of the Philippines that were characterized of having either ultramafic or volcanic soils. Such finding is deemed interesting because despite the higher heavy metal content, ultramafic forest patches yielded higher species diversity as compared to volcanic soils.

Opportunities on myxomycete research in the Philippines

Though admittedly, investigations in terms of ecological patterns for myxomycetes in the Philippines can still be considered in its infancy, several results from different myxomycete surveys have provided interesting insights about the knowledge of Paleotropical myxomycetes. For instance, it has been speculated that myxomycetes follow the intermediate disturbance hypothesis since myxomycete diversity in areas like Bicol Peninsula (Dagamac et al. 2015a) and Puerto Galera (Dagamac et al. 2015c) seem to be affected by either man made activities or natural disturbances. Future research should seek to establish a series of plots along a disturbance gradient in the Philippines to test this hypothesis. In terms of seasonality, the study of Dagamac et al. (2012) in Mt. Arayat National Park is the only one in the country that attempted to compare the diversity of myxomycetes in the clear pronounced wet and dry seasons. The study concluded that the drier season had more species of myxomycetes in comparison to the wet season. It was of course noted that the measures of diversity used in the study were geared more towards standardized substrate sampling design by using solely the moist chamber components. This is due to the fact that erratic rainfalls in the country represent an obstacle in collecting actual fruiting bodies in the field since they can easily be washed away due to strong typhoons that can hit the country during any time of the year. Finding the most appropriate time to search for fruiting bodies in the field is one of the major challenges for myxomycete survey in the Philippines. In order to detect the influence of seasonality on myxomycete diversity, a multi-year survey that utilizes monthly field and substrate collection for moist chambers across a series of standardized plots is recommended. Another aspect that was investigated in Mt. Arayat National Park was the influence of elevation on myxomycete diversity (Dagamac et al. 2014). In that study, no clear pattern was observed since the highest elevation, about 800masl, still had almost the same general vegetation as the lowland elevation (200masl). Such gradients are not as high in comparison to the studies of elevation gradients in Cocos Island, Costa Rica (Rojas & Stephenson 2008). Investigations on highland areas in the Philippines are still unexplored and such surveys in the future could help fill the missing gaps in the understanding of myxomycete ecology for the Paleotropical Asia.

Other studies that utilized myxomycetes in the country

Most of the myxomycete diversity studies conducted in the UST – RCNAS included both basic and applied research goals. Field guides for myxomycetes are rare since many tourists and naturalists fail to recognize microorganisms, as such, local interactive keys (Dagamac et al. 2011) and photoguides (Macabago & dela Cruz 2012) were developed so that myxomycete research could

at least be accessible for enthusiasts and students. The interactive key based on the DELTA software represented the first for myxomycetes in Southeast Asia. Industrial application of myxomycetes is still unpopular but can be of great potential to many possible research endeavors for the future. The successful in vitro culture of myxomycetes could lead to potential mass production of their unknown natural products. Thus, Macabago & dela Cruz (2014) initialized an attempt to store and preserve cultures of amoebflagellates from myxomycetes. Moreover, their study had showed that *Physarum compressum* was able to excrete extracellular enzymes such as amylase and protease. A recent study by Rea-Maminta et al. (2015) reported that myxomycetes could have higher levels of chromium and manganese relative to their substrate. This provides preliminary evidence about the potential of myxomycetes for bioabsorption or bioremediation.

Additional insights and concluding remarks

Many areas in the world still remained un- or under-studied for myxomycetes (e.g. Madagascar, Wrigley de Basanta et al. 2013; Papua New Guinea and Caledonia, Kylin et al. 2013). This particularly holds true for the Philippines which so far have covered only 26 provinces of the 81 total provinces or a total of about 20 large islands and small islets from the 7,107 islands the country is known for. Many habitat types have not been fully explored, e.g. grasslands, mangrove or beach forest, volcanic forest, mossy or cloud forest, high elevation forests dominated by dwarf trees, to name a few. These all represent unique macrohabitats for myxomycetes. The country is also home to more than 6,000 species of endemic plants. If a single species of myxomycetes may be found restricted to a particular substrata or a host plant, then in theory, this could mean that the country could be home to far more species than previously estimated. Reynolds (1981) in his annotated list estimated that the 107 species recorded for the Philippines at that time represented 60% of the possible species of myxomycetes in the country. Again, if this predication will be accepted as accurate, with the new list of 150 species, adding 43 species in the last 7 years, we are now looking at additional 30 species still waiting to be discovered. Nevertheless, whatever the figures or numbers may be, it is certain that many species of myxomycetes await discovery in the Philippines. Could these unaccounted species be found in many of the unexplored habitats and/or unique substrata in the country? Lastly, the geographic isolation of the Philippines resulted in high endemism among its flora and fauna. Could this also be true for its microbial flora as exemplified by myxomycetes despite their high potential for dispersal? And with some islands previously linked to mainland Asia through the land bridges during the recent and past Ice ages, similarities with some plants and animals in the region have already been documented. Can we also expect similarities in the assemblages of myxomycetes between many parts of Asia and the Philippines? Indeed, the islands of the country offers good sites to explore genetic diversity or study gene flow, speciation, population dynamics or even spore dispersal among myxomycetes. The islands of the Philippines can therefore be a living laboratory to test many hypotheses on key concepts in genetics, ecology and even on interactions between myxomycetes and the endemic host plants or associated insects.

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CHAPTER 3.2

Filling the gaps of myxomycete diversity in the Paleotropics

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MYXOMYCETES FROM THE HIGHLANDS OF ETHIOPIA

NIKKI HEHERSON A. DAGAMAC¹, MICHAEL HOFFMANN², YURI K. NOVOZHILOV³ & MARTIN SCHNITTLER *¹

¹ Institute of Botany and Landscape Ecology, University of Greifswald, Soldmannstr. 15, D-17487, Greifswald, Germany

² Huysstrasse 110, 38822 D-Athenstedt, Germany

³ Komarov Botanical Institute of the Russian Academy of Sciences, Laboratory of Systematics and Geography of Fungi, Prof. Popov Street 2, 197376 St. Petersburg, Russia

ABSTRACT

A rapid assessment of myxomycete diversity in the northern Ethiopian highlands revealed 39 species belonging to 14 genera, all new to the country. Most of the 151 records come from the highlands around Lake Tana and the Simien Mountains. According to species accumulation curves, more species (83) can be expected, but this is the first fairly comprehensive annotation of myxomycetes from the country. We describe and barcode three records of *Diderma* cf. *miniatum* with a strong bright red peridium and one record of *Didymium* cf. *flexuosum* with a conspicuous broad reticulation in the spore ornamentation, both findings may represent species new to science. A number of rarely recorded species, like *Didymium saturnus*, *Metatrichia floripara*, *Perichaena areolata*, and *Physarina echinospora* mount evidence that similar to its unique flora, the east African mountain ranges harbor a diverse and distinctive myxomycete assemblage. Especially interesting as substrate plants were the hollow decaying trunks of giant tree-like lobelia, working as natural moist chambers.

Keywords: moist chamber cultures, old world tropics, plasmodial slime molds, species list

*Corresponding author: Martin Schnittler, Institute of Botany and Landscape Ecology, Ernst Moritz Arndt University Greifswald, Soldmannstr. 15, D-17487 Greifswald, Germany. Email: martin.schnittler@uni-greifswald.de

INTRODUCTION

Myxomycetes are eukaryotic protists (supergroup Amoebozoa), living predatory on other micro-organisms. In contrast to most other groups of protists, fruit bodies with airborne spores of typical 7–12 µm size (Schnittler & Tesmer 2008) should make them strong colonizers (Kamono et al. 2009), even if their vegetative life cycle can be completed within a small section of a substrate, typically on decaying plant matter with a high density of saprophytic microbes (Schnittler et al. 2012). These high dispersal capabilities make them nearly ubiquitous inhabitants of all terrestrial ecosystems (Stephenson et al. 2008); and a given species seems to be able to reach, at least within geological time, all inhabitable regions on earth (Schnittler et al. 2000). However, populations within a species may show geographic differentiation, as tracked with molecular markers (Aguilar et al. 2013); in other cases the pattern is less clear (Feng & Schnittler 2015). This raises the question to which extent myxomycetes can develop endemic taxa. Some classical surveys conducted on rather remote areas like the Galapagos Islands (Eliasson 1971, Eliasson & Nannenga-Bremekamp 1983) or the Hawaiian archipelago (Eliasson 1991) did not recover endemic morphospecies. On the other side, there are numerous taxa known from a restricted region only. The work of Rammeloo (1981, 1983, 1984, 1986) surveying the high African mountains is a good example, resulting in several species new to science. This was the incentive for this survey, focusing on the Ethiopian highlands.

In contrast to other regions of the world, only a few studies on myxomycete diversity have been carried out in Africa. Nevertheless, a comprehensive myxomycete inventory by Ndiritu et al. (2009a) reports a total of 294 species represented by 49 genera from 31 African countries, mainly due to some extensive diversity assessments conducted in recent years. The arid regions of Tanzania

(Ukkola *et al.* 1996), Kenya (Ndiritu *et al.* 2009b) and Madagascar (Wrigley de Basanta *et al.* 2013) are relatively well studied. However, only anecdotal reports are known from most African countries, and Ethiopia is not an exception. A student field trip to this yet unexplored country gave an opportunity to conduct a first myxomycete survey, targeting the highlands around Lake Tana (Bahir), the Simien Mountain National Park (north of Gondar) and the rift valley around Metehara (Fig. 1).

Ethiopia is situated in the Horn of Africa and due to its rugged relief displays several high mountain ranges on both sides of the central Rift Valley and various vegetation types ranging from deserts to afroalpine vegetation (Friis *et al.* 2010). Several isolated mountain ranges have plateaus, often with steep escarpments, above 3 000 m a.s.l., with the highest peak, Ras Dashen, reaching up to 4 533 m. Annual precipitation shows a gradient from the western side of the country and the central mountains (1 200–2 200 mm) to the eastern dry lowlands (200–600 mm, with less than 200 mm in the Afar basin and the eastern tip of Ethiopia). This is reflected by the vegetation, with tropical dry forests (*Combretum-Terminalia* woodland, sometimes savannah-like, Friis *et al.* 2010) dominating in the northwest; dry evergreen afroalpine forests inhabit the central mountainous parts; and very dry, semideciduous *Acacia-Commiphora* woodlands, sometimes transient to semi-deserted grasslands, occur east of the central mountains. The central mountain range is sharply divided by the deep and dry Rift valley. A pronounced dry season with a short rainy season (June to mid-September, somewhat later in the south) is somewhat counterbalanced by the generally cooler climate in the afroalpine region above 3 000 m, where night temperatures can drop below zero.

Although the mountains should in general provide more favorable conditions for myxomycete growth, only a single myxomycete species, *Badhamia spinispora* (= *Physarum spinisporum*), is reported from Ethiopia, coming from a publication on coprophilous myxomycetes of Eliasson & Lundqvist (1979). Therefore, this study is intended to create a baseline of data of myxomycete diversity for Ethiopia, update the number of myxomycete records for the African region, and search for records of new and possibly endemic taxa.

MATERIALS AND METHODS

Study Area

Our first study region (Fig. 1) were the western Ethiopian highlands around Lake Tana (1 600–2 200 m, localities 1–17). The potential vegetation is tropical dry afroalpine forest with a rather high tree diversity (Friis 1992), but due to extremely intense agriculture and high population density only small forest pockets remained, namely as so-called Church forests around monasteries (Wassie *et al.* 2005, Eshete 2007). Although these forests preserve a considerable diversity of native plants and near-natural habitats, they are intensely searched for fuelwood (usually no woody debris exceeding 5 cm diameter can be found) and most of the understory shrubs are replaced by coffee (*Coffea arabica* L., Gole *et al.* 2008), using the natural forest cover as shade trees. Even here, some trees are selected over others, like *Mimosa kummel* Bruce ex DC. with edible fruits. Several church forests were studied (loc. 9, 10, 11 and 12), especially Dek Estefanos, a still nearly entirely wooded island in Lake Tana. The second study region was the northernmost of several high mountain ranges in the country, the Simien mountains, where all elevations from 3 200 to 4 000 m were studied (loc. 18–28). Technically, the area is a National Park, but due to high population density, the crop lands extend up to 3 000 m, to be replaced by intensely used pastures at most areas at higher elevations. Only at the cliffs of the escarpment or in steep ravines afroalpine forests remain. Below 3 000 m elevation these forests have a species-rich canopy, whereas at exposed places and at high elevation, *Erica arborea* L. that form trees up to 20 m in height, dominates the canopy. A giant lobelia (*Lobelia rhynchocephalum* Hemsl.), endemic to Simien and the Bale Mountains 700 km to the south, across the Rift Valley (Geleta & Bryngelsson 2012), still occurs abundantly above the tree line but its recruitment seems to be severely hampered by intense grazing of the ca. 12 000 people living permanently inside the park boundaries.

A short trip to the seasonal dry rift valley around Metehara provided only a few, rather anecdotal, records from substrata cultivated in moist chamber (950–1 200 m, loc. 29–30). The dominating vegetation is extremely dry semideciduous *Acacia-Commiphora* woodland with tall shrubs or low trees, which shed most or all leaves during the pronounced dry season. In addition, the valley of the Awash River provided gallery forests with taller trees like *Celtis africana* Burm. The following

list includes all localities where myxomycete fruitings or substrata for moist chamber studies were collected. Substrate samples came from a narrow (25–50 m) radius around the given locations; whereas for field samples the vegetation was searched in the radius given as coordinate precision. Locality numbers written in bold in the following brief descriptions refer to Fig. 1.

Lake Tana Highlands

1. Valley of the Blue Nile, near the cataracts, eastern side of the river, grazed savanna, scattered trees, former dry forest, 35 km ESE Bahir Dar, 1 634±25 m a.s.l., 37°35'39.7"E, 11°29'09.3"N±500 m
2. Valley of the Blue Nile, near the cataracts, eastern side of the river, grazed savanna with forest rudiments and *Cissus* scrub, former dry forest, 35 km ESE Bahir Dar, 1 684±25 m a.s.l., 37°35'34.3"E, 11°29'26.4"N±500 m
3. Valley of the Blue Nile, near the cataracts, eastern side of the river, grazed savanna, dry forest remnants, former dry forest, 35 km ESE Bahir Dar, 1 658±25 m a.s.l., 37°35'22.8"E, 11°29'23.0"N±500 m
4. Zege peninsula, trail from harbor to Ura Kidane Mihiret monastery, seasonal dry afro-montane forest with planted coffee shrubs, 11 km NNE Bahir Dar, 1 807±40 m a.s.l., 37°20'47.6"E, 11°41'24.7"N±500 m
5. Around Ura Kidane Mihiret monastery, ca 2 km E of Zege settlement, seasonal dry afro-montane forest with planted coffee shrubs, 11 km NNE Bahir Dar, 1 840±40 m a.s.l., 37°20'28.7"E, 11°41'42.4"N±500 m
6. Terraces near Lake Tana, near Gelda peninsula, tef fields, 17 km NNE Bahir Dar, 1 830±25 m a.s.l., 37°26'21.1"E, 11°44'54.6"N±500 m
7. Near the shore of Lake Tana at the Gelda peninsula, short grassy pastures with scattered trees, 17 km NNE Bahir Dar, 1 810±10 m a.s.l., 37°26'21.1"E, 11°44'54.6"N±500 m
8. Near the shore of Lake Tana, ca. 1.5 km from the tip of Gelda river delta, flooded pastures and fallow fields, 15.5 km NNE Bahir Dar, 1 797±10 m a.s.l., 37°25'40.8"E, 11°44'07.2"N±500 m
9. Dek Estefanos island, lower parts of the island near the harbor, fields and pastures, 33 km NNW Bahir Dar, 1 799±25 m a.s.l., 37°18'46.5"E, 11°53'48"N±500 m
10. Dek Estefanos island, lower parts of the island near the harbor, seasonal dry afro-montane forest with planted coffee shrubs, 33.4 km NNW Bahir Dar, 1 799±25 m a.s.l., 37°18'46.5"E, 11°53'48"N±500 m
11. Dek Estefanos island, lower parts of the island near the harbor, gardens, fields and mango plantations, 33.4 km NNW Bahir Dar, 1 799±25 m a.s.l., 37°17'55.5"E, 11°54'25.7"N±500 m
12. Dek Estefanos island, church hill, near the summit, seasonal dry afro-montane forest with planted coffee shrubs, 33 km NNW Bahir Dar, 1 810±25 m a.s.l., 37°18'42.6"E, 11°53'43.7"N±500 m
13. Church forest Tara Gedam, along the road from Addis Zemen to Gondar, ca. 70 km SSE Gondar, afro-montane moist forest, 15 km NW Addis Zemen, 2 286±35 m a.s.l., 37°44'31.5"E, 12°08'41.7"N±500 m
14. Church forest Tara Gedam, along the road from Addis Zemen to Gondar, ca. 70 km SSE Gondar, degraded, shrubby afro-montane moist forest, 15 km NW Addis Zemen, 2 373±40 m a.s.l., 37°45'01.4"E, 12°08'55.7"N±500 m [Loc_15]
15. Valley towards the Wada Mt., near the road from Addis Zemen to Gondar, degraded, shrubby afro-montane moist forest, 20 km NW Addis Zemen, 2 102±40 m a.s.l., 37°46'09.4"E, 12°08'10.2"N±750 m [Loc_16]
16. Valley towards the Wada Mt., near the road from Addis Zemen to Gondar, shrubby, severely degraded forest, 20 km NW Addis Zemen, 2 193±40 m a.s.l., 37°46'16.9"E, 12°08'23.8"N±750 m
17. Along the road from Addis Zemen to Gondar, pastures with shrub islands, 30 km NW Addis Zemen, 2 203±40 m a.s.l., 37°46'22.3"E, 12°08'08.6"N±750 m

Simien Mts.

18. High plain of the Simien mountains, grazed remnants of *Erica arborea* afroalpine forests, 3 215±50 m a.s.l., 38°00'54.7"E, 13°13'28.5"N±25 m
19. Escarpments of the Simien mountains, small valley just below Sankavar Camp, closed *Erica arborea* afro-montane forest, 3 254±50 m a.s.l., 38°02'26.1"E, 13°13'50.6"N±100 m
20. Escarpments of the Simien mountains, shallow NW-exp. slope, Sankavar Camp, shrubby *Erica arborea* afro-montane forest, 3 254±50 m a.s.l., 38°02'26.1"E, 13°13'50.6"N±25 m
21. Escarpments of the Simien mountains, viewpoint near Sankavar Camp, open rocks with scattered vegetation, 3 271±50 m a.s.l., 38°02'37.1"E, 13°14'09.6"N±25 m
22. Escarpments of the Simien mountains, side valley, trail from Sankavar to Gich Camp, montane grassland, pastures and small fields, 6 km SW Gich Camp, 3211±50 m a.s.l. 38°05'19.9"E, 13°14'45.9"N±25 m
23. Simien mountains, high plains of Gich Camp, montane grassland and pastures, 26 km NNW Debark, 3 619±50 m a.s.l., 38°06'29.2"E, 13°16'06.7"N±25 m
24. Simien mountains, margin of the escarpment, trail from Gich Camp to Chenek Camp, *Erica arborea* afro-montane forest, 4.5 km NE Gich Camp, 3 713±75 m a.s.l., 38°08'30.2"E, 13°16'43.1"N±25 m
25. Simien mountains, small valley with giant *Lobelia*s and *Erica* around Chenek Camp, montane grassland and pastures, 3 640±40 m a.s.l., 38°11'41.0"E, 13°15'39.2"N±25 m

26. Simien mountains, upper part of Wazla Wen forest, open montane meadows, moderately grazed, 6.8 km SW Gich Camp, 3 015±40 m a.s.l., 38°03'24.2"E, 13°14'02.6"N±25 m
27. Simien mountains, lower part of Wazla Wen forest, rich and dense afromontane forest, lower part of a river valley, 7.3 km SW Gich Camp, 2 848±40 m a.s.l., 38°03'13.4"E, 13°13'47.6"N±25 m
28. Simien mountains, lower part of Wazla Wen forest, rich and dense afromontane forest, lower part of a river valley, 7.3 km SW Gich Camp, 2 760±40 m a.s.l., 38°02'57.8"E, 13°13'35.5"N±25 m

Rift Valley

29. Heavily grazed shrubland, shrubby *Acacia-Commiphora* woodland, 46 km WSW Metehara, 1 245 ±25 m a.s.l., 39°32'31.3"E, 8°42'14.5"N±100 m
30. Valley of the Awash river, along the river, *Celtis*-dominated gallery forest, 15 km ENE Metehara, 956±25 m a.s.l., 40°01'12.9"E, 8°50'53.7"N±250 m
-

Specimen collection

A combined opportunistic sampling as described by Cannon & Sutton (2004), including moist chamber cultures (mc) set up according to Stephenson & Stempen (1994) were used to collect myxomycete specimens. Material for moist chamber cultures were sampled from leaf litter, barks of living trees and dung of herbivorous animals. A single mc was prepared for each sample to avoid pseudoreplicates. Material was spread on clean Petri dishes over three layers of white toilet paper and allowed to soak with distilled water over night. After 24h excess water was poured off and pH values were determined at three different points of the wet substrate surface using an Orion model 610 pH meter with a touch down probe. All cultures were incubated under ambient light at room temperature (ca 20–24 °C) for up to 90 days and were checked regularly (days 4, 9, 13, 25, 45, 60, 90) for the growth of plasmodia and/or fruiting bodies using a dissecting microscope. To maintain moisture, distilled water was added over the first six weeks of the incubation period. Mature sporocarps were directly transferred to herbarium boxes, with one record defined as a colony of fructifications from one taxon developing in one culture.

Myxomycete determination

Specimens were determined morphologically using web-based identification keys (Eumycetozoa Project: <http://slimemold.uark.edu/>) and monographs (Neubert *et al.*, 1995–2000, Poulain *et al.*, 2011). Selected air-dried sporocarps were further studied with a Zeiss Axio Imager A1 light microscope with differential interface contrast (DIC), a Stemi 2000 dissection microscope and a JSM-6390 LA scanning electron microscope at the Komarov Botanical Institute RAS (St. Petersburg). For microscopy, sporocarps were mounted as permanent slides in lactophenol or Hoyer's medium to preserve structures containing lime and studied with bright field and Nomarski contrast using a Leica DFC450 microscope. The freeware program CombineZ was used to create stacked images under a Stemi 2000 dissection microscope. Microscopic measurements were made with the program Axio Vision 4.8.0.0 (Carl Zeiss Imaging Solutions GmbH, free license). Spore features (diameter and spore ornamentation) were determined for 25–30 spores per certain specimen. Selected specimens were also subjected for electron microscopy by mounting them on copper stubs using double sided sticky film and sputter-coated with gold. Colors are given according to the NBS/ISCC standard (Anonymous 2012). Nomenclature follows Lado (2005–2015); authorities are cited according to Kirk and Ansell (1992). Voucher specimens are deposited in the private collection of the last author at the Botanical State Collection Munich (M).

For references to barcoding studies, for selected specimens of this survey partial sequences of the small-subunit ribosomal RNA (SSU) gene (see Novozhilov *et al.* 2013 for methodology) were obtained and documented in Genbank (KT731233–40), using the primer pair S1 (aacctggtgatcctgcc, Fiore-Donno *et al.* 2008) and SU19R (cggttaaagttgtgcggtta, all written as 5'–3') for dark-spored myxomycetes. This gene is currently the most promising barcode marker for myxomycetes (see discussion in Feng & Schnittler 2016).

Species accumulation curve

To estimate the extent to which the survey was exhaustive, individual-based species accumulation curves were constructed for the whole survey according to the rarefaction formula using the program EstimateS (Version 9.1, Colwell 2013, 100 randomizations), which computes as well

some estimators of species richness. In accordance with Unterseher *et al.* (2008), the Chao2 estimator (Chao *et al.* 2005) was chosen and calculated using the “classical settings” of EstimateS. As a second approach, a fit with the hyperbolic function $y = ax/b+x$ was attempted, with a yielding an estimate for species richness.

RESULTS

Annotated species list

The following annotated species list includes 39 taxa belonging to 14 different genera. Well-developed specimens that cannot be assigned to a known species without doubt are denoted as “cf.” (confer), scanty or weathered specimens not allowing safe determination are indicated by a question mark “?”. After each name, an estimation of abundance (Stephenson *et al.* 1993) is given in brackets, followed by the total number of records. This is based upon the proportion of a species to the total number of determinable fruiting body records for each group: **R** – rare (< 0.5 %), **O** – occasional (> 0.5–1.5 %), **C** – common (> 1.5–3 %), **A** – abundant (> 3 %). With the denominators **fc** (field collections), **mc** (collection from moist chamber) / **llae** (aerial litter), **llgr** (ground litter), **b** (bark), **lw** (lianas), **d** (dung) and **w** (wood, all field collections) the number of records made by different components of the survey and from different substrate types are indicated. For all species that developed from moist chamber cultures, the development time and substrate pH (for more than one collections, mean±SEM, minimum–maximum value) is given in parentheses. All species in the following annotated list are new for Ethiopia; one asterisk (*) marks the species reported as new only for the African continent, which excludes survey from nearby islands (La Reunion, Adamonyte *et al.* 2011; Madagascar, Wrigley de Basanta *et al.* 2013; Canary Islands, Beltran *et al.* 2004). For eight specimens partial SSU sequences were obtained; submission numbers for Genbank are given in parentheses.

Arcyria cinerea (Bull.) Pers. [C, 4] fc: 1 mc: 3 / b: 2 w: 1 llae: 1 (48.3 days, pH 6.96±0.19, 6.75–7.52)

Arcyria minuta Buchet [O, 1] fc: 1 / w: 1

Badhamia gracilis var. *melanospora* (Speg.) Castillo, Moreno & Illana [O, 1] fc: 1 / b: 1

Comatricha nigra (Pers. ex J.F. Gmel.) Schroet. [O, 1] fc: 1 / w: 1

Comatricha pulchella (C. Bab. & Berk.) Rostaf. [O, 2] fc: 2 / w: 2

Comatricha tenerrima (M.A. Curtis) G. Lister [O, 1] mc: 1 / lw: 1 (41.0 days, pH 6.75)

Cribraria cancellata (Batsch) Nann.-Bremek. [O, 1] fc: 1 / w: 1

Diderma cor-rubrum T. Macbr. (KT731233=sc28783, Fig. 7) [O, 2] fc: 2 / llgr: 2 Sporocarps crowded, sometimes sessile. Sporotheca globose to subglobose, white (263) or light grey (264). Peridium two-layered, outer layer with prominent depressed polygonal plates with elevated margins that are divided by white fissures. Inner layer membranous, iridescent; outer layer calcareous, brittle, fragile. Columella club-shaped, stout, brownish grey (64). Capillitium scanty, long, branched, deep brown (56). Spores in mass black (267), irregularly warted, lemon-shaped, blackish purple (230), (10.5–)11.0–12.5(–13.0) in diam.

Comment: The specimen we found deviates from Martin & Alexopolous (1969) description of its peridium of having polished, rugose, reddish purple within however our descriptions matched the taxonomic keys of Poulain *et al.* (2011).

Diderma crustaceum Peck [C, 3] fc: 3 / llgr: 3

Diderma hemisphaericum (Bull.) Hornem. [O, 1] mc: 1 / llae: 1 (57.0 days, pH 7.38)

**Diderma* cf. *miniaturum* Nann.-Bremek. (KT731234=sc28836, Fig. 4) [C, 3] fc: 3 / llgr: 3 Sporocarps scattered, about 1.5–2.0 mm tall. Sporotheca globose to subglobose, vivid reddish orange (34) to strong orange (50). Peridium smooth, resembling that of *Leocarpus*, with three layers. Inner layer membranous, smooth thin; the middle layer limy, thick and fragile, and the outer layer again membranous. Stalk conical but clearly separated from the sporotheca, about 0.5–1.0 mm long, filled with crystal lime, white (263). Columella present, subglobose, tapering at the center of the sporotheca.

Capillitium dense and reticulate, brownish orange (54) smaller threads. Spores in mass deep brown (56), irregularly warted, 9.7–11.9(–13.0) μm in diam.

Comment: In comparison to the typical form, our specimens strikingly differ by the orange color of the sporocarps.

Didymium difforme (Pers.) S.F. Gray [A, 28] fc:17 mc:11 / b: 2 w: 17 llae: 6 llgr: 1 d: 2 (57.4 days, pH 7.16 \pm 1.39, 5.73–7.92)

****Didymium* cf. *flexuosum*** Yamashiro (KT731235=sc28802, Figs. 19–21) [O, 1] fc: 1 / w: 1 Sessile, flat plasmodiocarps that are branched and anastomosed, light grey (264). Hypothallus expanding, colorless, inconspicuous. Peridium membranous, fragile, iridescent, covered with scattered lime crystals. Columella conspicuous, elongated along the midline of the plasmodiocarp. Capillitium abundant, consisting of slender threads, strong yellowish brown (74). Spores in mass purplish black (235), 9.8–13.2(–14.3) μm in diam.

Comment: Unlike the spore description given in the description of *D. flexuosum* that mentions scattered spines, our specimen shows a regular reticulum in both SEM and light microscopy. Especially conspicuous are the broad compound ridges (Fig. 21). This record may constitute a new species.

Didymium iridis (Ditmar) Fr. [A, 17] fc: 13 mc: 4 / b: 2 w: 13 llae: 2 (31.5 days, pH 7.02 \pm 3.13, 5.73–7.86)

****Didymium* cf. *pseudodecipiens*** ad. int. [O, 1] fc: 1 / w: 1 Expanded, flat plasmodiocarps. Peridium densely covered with lime crystals. Capillitium flexuous, branched, smooth, grayish yellowish brown (80). Spores in mass black (267), with evenly distributed prominent spines, purplish black (235), (11.6–)12.2–15.2(–15.8) μm in diam.

Didymium saturnus H.W. Keller (KT731236=sc28914, Figs. 17, 18) [O, 1] mc: 1 / d: 1 (110.0 days, pH 6.30)

Didymium squamulosum (Alb. & Schwein.) Fr. [O, 1] mc: 1 / llae: 1 (23.0 days pH 5.73)

Hemitrichia calyculata (Speg.) M.L. Farr [A, 6] fc: 6 / w: 6

Hemitrichia serpula (Scop.) Rostaf. [C, 5] fc: 2 mc: 3 / b: 3 w: 2 (83.8 days, pH 5.92 \pm 1.68, 5.80–6.03)

Lycogala exiguum Morgan [O, 1] fc: 1 / w: 1

Metatrichia floripara (Rammeloo) Rammeloo (Fig. 3) [O, 1] mc: 1 / w: 1 (110.0 days, pH 6.83) Sporocarps stalked, about 2 mm tall, scattered. Sporotheca spherical, blackish purple (230), dehiscing with triangular lobes, truncated. Stalk erect or slightly curved, about 1.0–1.5 mm long, reddish black (24). Capillitium consisting of short unbranched elaters. Spore mass dark red (16), spores ornamented with fine, evenly distributed warts, (9.2–)10.4–11.7(–12.2) μm in diam.

Perichaena areolata Rammeloo (Figs. 5, 10, 11, 12) [O, 1] mc: 1 / llgr: 1 (56.0 days, pH 7.26) Sporocarps sessile, clustered. Sporotheca ovoid with areolate patches, vivid yellow (88), about 0.3–0.6 mm in diam. Peridium with double layers that adhere closely to each other. Inner layer thin, membranous, translucent, ornamented with papillae arranged in a very regular way. Outer layer thicker, brownish orange (54) composed of spherical to elliptical granules. Capillitium absent. Spores in mass pale yellow (89); spores spherical, brilliant yellow (83), 10.7–12.8(–13.5) μm in diam., ornamented with regularly distributed verrucae.

Comment: A very rare specimen reported in the African region. In contrast to the original description most features matches except that the few small sporocarps obtained by us from moist chamber were not isolated, not sessile and seem to lack a capillitium. The taxonomic key of Lado *et al.* (2009) for *Perichaena* species also matches the SEM of the spore ornamentation having well distributed spine like excrescences.

Perichaena chrysosperma (Currey) Lister [A, 14] mc: 14 / b: 5 llae: 3 llgr: 5 lw: 1 (71.1 days, pH 6.99 \pm 0.11, 6.10–7.46)

Perichaena depressa Libert [A, 18] mc: 18 / b: 10 llae: 5 llgr: 3 (62.7 days, pH 7.02 \pm 0.10, 6.16–7.87)

Perichaena dictyonema Rammeloo [C, 3] mc: 3 / llgr: 3 (57.3 days, pH 6.61 \pm 0.73, 5.80–7.23)

Perichaena pedata (Lister & G. Lister) G. Lister (Fig. 9) [O, 1] mc: 1 / llgr: 1 (71.0 days, pH 7.26)

Perichaena quadrata T. Macbr. [A, 9] mc: 9 / b: 2 llae: 3 lw: 2 d: 2 (68.5 days, pH 7.31±0.19, 6.39–8.22)

**Perichaena tessellata* G. Lister [O, 1] mc: 1 / b: 1 (71.0 days, pH: 6.79)

Perichaena vermicularis (Schwein.) Rostaf. [O, 1] mc: 1 / llae: 1 (9.0 days, pH 7.07)

Physarina echinospora K.S. Thind & Manocha (KT731237=sc28915, Figs. 6, 16) [O, 2] mc: 2 / llgr: 2 (66.5 days, pH 6.74±0.29, 6.45–7.02) Sporocarps stalked, gregarious, about 1 mm tall. Sporotheca globose or subglobose, 0.5–0.7 mm in diam., light grey (264) to white (263), densely covered with distinct pale grey subcylindrical outgrowths about 50 µm in length. Peridium brittle, thin. Stalk short, calcareous, stout, light grey (264). Columella filled with amorphous lime, sometimes with nearly crystalline lime. Capillitial threads strong brown (55), paler towards their ends. Spores black in mass (267), resembling in shape a lemon with prominent ridges, spinulose, (10.0–)11.5–14.0(–14.7) µm in diam.

Physarum bivalve Pers. [O, 2] fc: 2 / w: 2

Physarum compressum Alb. & Schwein. [O, 2] mc: 2 / b: 2 (68.7 days, pH 6.56±0.53, 6.03–7.08)

Physarum diderma Rostaf. (KT731238=sc28785, Fig. 2) [A, 6] fc: 6 / w: 3 llgr: 2 lw: 1 Sporocarps sessile, densely crowded, with irregular dehiscence. Sporotheca globose to ovoid, white (263). Peridium two-layered, the inner layer membranous, the outer layer composed of a solid layer of amorphous lime. Capillitium showing white, elongated and large white lime nodes which are sometimes clustered, resembling a pseudocolumella. Spores in mass dark brown (59), spinulose, (8.2–)10.1–13.0(–13.5) µm in diam.

Physarum didermoides (Pers.) Rostaf. (KT731239=sc28904, Figs. 14, 15) [C, 4] mc: 4 / b: 4 (110.0 days, pH 7.05±0.26, 6.55–7.43)

**Physarum* cf. *murinum* Lister (KT731240=sc28835, Fig. 8) [O, 1] fc: 1 / l: 1 Sporocarps stalked, peridium covered with light brown (57) lime granules. Sporotheca globose, 0.4–0.6 mm diam. Peridium covered with lime granules and packed into scattered irregular rounded scales. Stalk stout, cylindrical, short, brownish orange (54). Spores in mass black (267), irregularly warted, (8.5–)9.0–11.0(–12.2) µm in diam.

Physarum pusillum (Berk. & M.A. Curtis) G. Lister [O, 1] fc: 1 / w: 1

Physarum stellatum (Masse) G.W. Martin [O, 1] fc: 1 / lw: 1

Stemonitis fusca Roth (Fig. 13) [O, 1] mc: 1 / llae: 1 (57 days, pH: 6.16)

Stemonitopsis cf. *microspora* (Lister) Nann.-Bremek. [O, 1] fc: 1 / w: 1 Sporocarps short stalked, gregarious, dark, 2–3 mm tall, elongated and cylindrical which are rounded at the ends, dark brown (59). Stalk hollow, about 1 mm long, black (267). Columella present, reaching up to the top of the sporotheca. Capillitium deep brown (56), flexuous, with irregular meshes, surface net absent. Spores in mass dark brown (59), with spines connected by thin ridges to a reticulum, subglobose, grayish olive (110), (2.5–)3.0–5.0(–5.5) µm in diam.

The survey resulted in 187 records from 68 field collections (fc) and 155 moist chamber cultures (mc) yielding 119 records. Of these records, 151 could be determined to species, 5 were malformed fruiting bodies, and 31 remained in plasmodial state. Hence, 151 determinable records (67 fc, 84 mc) were used to generate the annotated list for this survey. The individual-based species accumulation curves from the field and from moist chamber cultures displayed that the extent of sampling was still insufficient, as the confidence intervals for the Chao 2 estimator did not clearly converge (Fig. 22). However, for the area as a whole, 48% of the theoretical number of species that can be anticipated according to the Chao2 estimator (82.8±28.2) were found. The hyperbolic curve fit results in a somewhat lower figure (60.9±0.7; $R^2=0.995$). Both components of the survey, field collections and moist chambers, yielded the same amount of taxa (21), making up for about half of the taxa to expect (field collections: Chao2 35.3±12.3, hyperbolic function 34.7±0.5, $R^2=0.997$; moist chambers: Chao2 45.7±24.0, hyperbolic function 29.8±0.3, $R^2=0.995$). Approximately 66% (99 of 151) moist chambers were positive for myxomycetes.

Field records (67) in this survey were made only occasionally in the forest remnants of the Tana Lake highlands (23); most came from the Simien Mountains, especially from decaying trunks of *Lobelia rhynchopetalum* (44). The extremely dry shrub of the rift valley did not produce any field

records. For moist chamber cultures, the number of determinable records (84) was more evenly distributed (50 records / 104 mc for the lake Tana highlands, 32 / 39 mc for the Simien Mts., and only 2 / 12 mc for the rift valley).

DISCUSSION

The survey presented herein is to our knowledge the first fairly comprehensive report on myxomycetes for Ethiopia, therefore all species reported in this paper are new records for the country. Nevertheless, we were not able to survey even the more intensely studied Simien mountains completely, as it is indicated by the high difference between the numbers of taxa expected (82.8) and recorded (39). This difference is higher for moist chamber component (21 observed / 45.7 expected) than for the field collections (21/40). The low total number of determinable records (151) affected as well our reported estimation of abundance. Using the scale introduced by Stephenson et al. (1993), all species with only one record were already assigned to the category “occasional” since their relative abundance exceeds 0.5%.

As to expect, numerous myxomycete species known to be abundant and widely distributed in the Paleotropics, like *Arcyria cinerea*, *Didymium squamulosum*, *Hemitrichia calyculata*, *H. serpula*, *Perichaena depressa*, *P. chrysosperma* and *Physarum bivalve* were found. However, the survey produced as well records of several rare species. Remarkable is the otherwise Neotropical species *Diderma* cf. *miniatum*. Our specimens deviate from typical forms by the bright orange color of the sporocarps and may represent a new species, but material from the Neotropics is still needed to verify this. The taxon found in this survey is at least closely related to *D. miniatum*, and would, if assigned to this species, constitute the first record for the Paleotropics. Other examples of world-wide rare species are *Diderma cor-rubrum*, *Metatrichia floripara*, *Perichaena areolata*, and *Physarina echinospora*. According to the Eumycetozoon database in Discover Life, these species were previously collected by J. Rammeloo in Congo and Rwanda. These observations hint to a somewhat distinct myxomycete assemblage of the higher African mountains, similar to the unique afro-montane flora. However, the number of species collected in this survey (39) does not allow any sound conclusions. The comparison to surveys dedicated solely to myxomycete diversity, like Madagascar (124 taxa, Wrigley de Basanta et al. 2013) or Kenya (51, taxa, Ndiritu et al. 2009b) underpins, that only a small part of the total myxomycete diversity was discovered.

In addition, we must assume that many myxomycete species fruiting on coarse woody debris are now much more rarely found than in the natural vegetation. The ultimate reason for this is the extreme intensity of land use due to high human population densities, especially in the Lake Tana highlands (population growth rate 2.2%, with 85% of all people relying on agriculture, USAID 2008). Most of the land is now intensely used as farmland; or in the high mountains and in extremely arid areas as pastures. Therefore, on every remaining pocket of forests lasts an immense pressure from collecting fuelwood, since the main staple foods (tef and increasingly, rice) have to be boiled. This causes most forests literally to lack any piece of woody debris exceeding 5 cm in diam. Moreover, the understorey of most forests is converted into coffee plantations.

Especially interesting was the myxomycete assemblage on giant lobelia (*Lobelia rhynchopetalum*), found in the highest areas of the Simien mountains (above 3 600 m). These tree-like, up to 5 m tall plants are monocarpic and decay after developing a dense 2–3 m tall racemose inflorescence. The hollow trunk works as a kind of natural moist chamber, mitigating the extreme daily temperature differences (in 4 000 m elevation we recorded maximum daily temperatures of 25–27 °C but hoarfrost at night). Although the absence of permanent snow should exclude the occurrence of typical nivicolous species, we found with *D. cf. pseudodecapiens* at least one species that typically occurs on snowbanks of mountains in temperate regions.

Still, the number of myxomycetes recorded for the Simien mountains (localities 18–28, 78 records, 21 taxa) is considerably lower in comparison to other tropical mountain ranges. The cloud forest of Maquipucuna in Ecuador (1 200–2 700 m, Tropical Moist to Tropical Lower Montane Rain Forest, Holdridge et al. 1971) had 77 taxa (Schnittler et al. 2002); 89 taxa were altogether reported by Rojas et al. (2011) from the Northern Neotropics (3 100–4 200 m elevation, Tropical Montane Rain Forest). Beside the much higher precipitation, both Neotropical areas possess still vast stretches of undisturbed forest. In the Simien mountains, only isolated forest pockets remained in ravines and steep cliffs in the escarpment. The lower degree of sampling within the trip to the Simien mountains, lasting

only five days, does not allow a direct comparison, but we assume that the pattern of decreasing myxomycete diversity with increasing elevation (Schnittler & Stephenson 2000) applies as well for the African Paleotropics. As such, this report is intended to fill a one of the many gaps for our knowledge on diversity and distribution of myxomycetes in Africa, where major areas still lack baseline information on this group of organisms.

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Figures

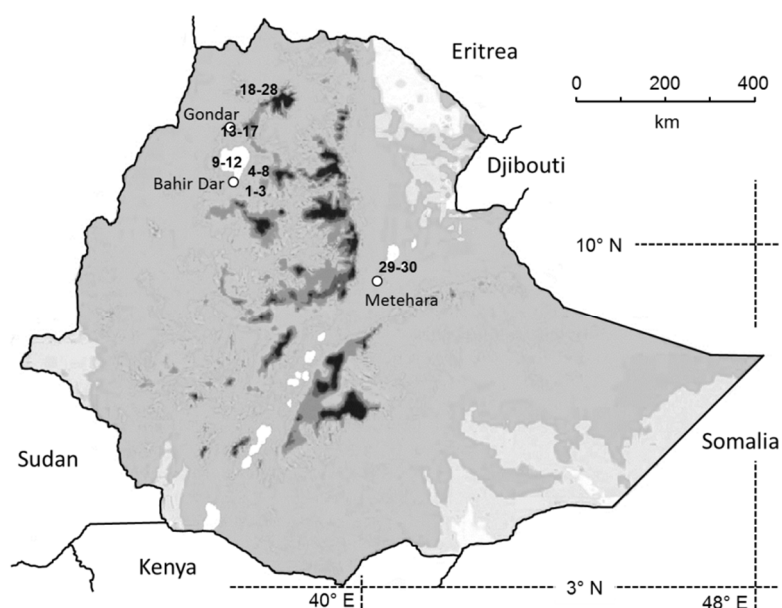
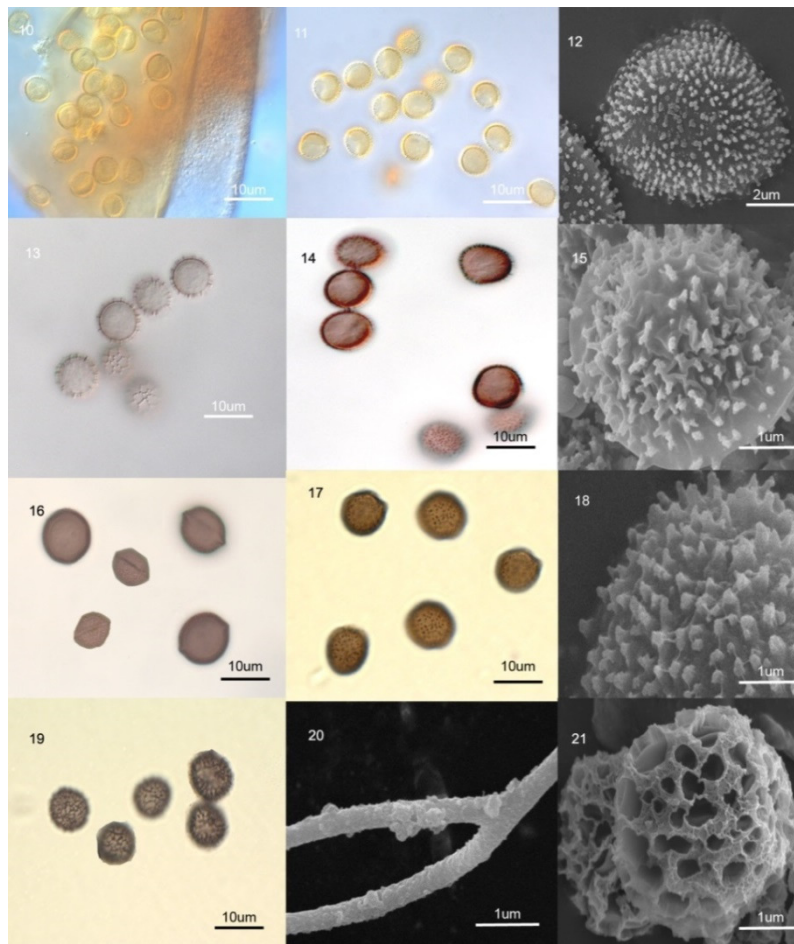


Fig. 1. Schematic map of Ethiopia, showing mountain ranges above 3 000 m (black) and the collecting localities for this study.



Figs. 2–9. Fruiting bodies of selected myxomycete species seen with the dissecting microscope. **2.** *Physarum diderma*, sc28785. **3.** *Metatrachia* cf. *floripara*, sc28906. **4.** *Diderma* cf. *miniatum*, sc28836. **5.** *Perichaena areolata*, sc28883. **6.** *Physarina echinospora*, sc28908. **7.** *Diderma cor-rubrum*, sc28783. **8.** *Physarum* cf. *murinum*, sc28835. **9.** *Perichaena pedata*, sc28883a. Stacked images: M. Hoffmann.



Figs. 10–21. Spore morphology of selected myxomycete species. **10–12.** *Perichaena areolata*, sc28883. 10. Double-layered peridium showing the translucent papillae. 11. Spores in transmitted light. 12. SEM micrograph showing irregularly distributed verrucae. **13.** *Stemonitis fusca*, sc28913. **14–15.** *Physarum didermoides*, sc28916. 14. Spores in transmitted light. 15. SEM micrograph, showing elevated warts on the spore surface. **16.** *Physarina echinospora*, sc28908, spores in transmitted light, showing the lemon-like shape and the central ridge. **17–18.** *Didymium saturnus*, sc28914. 17. Spores in transmitted light. 18. SEM micrograph, spore surface densely and irregularly warted. **19–21.** *Didymium* cf. *flexuosum*, sc28802. 19. Spores in transmitted light. 20–21. SEM micrographs of capillitium and coarsely reticulated spores, note the broad compound ridges.

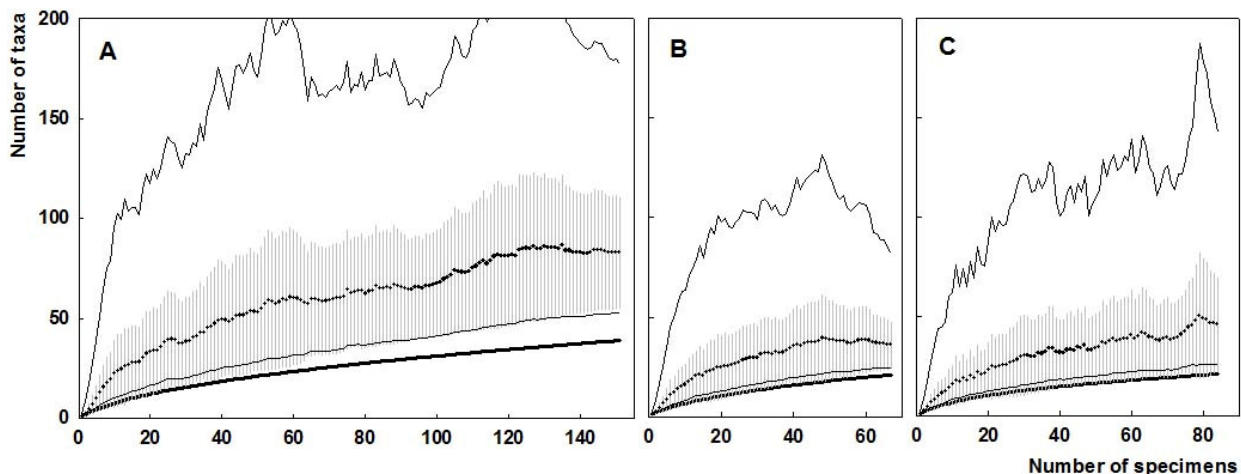


Fig. 22. Species accumulation curves (solid thick lines) for the survey as a whole (A), the field collections (B) and the collections obtained from moist chamber cultures (C). Shown is the Chao2 estimator (solid dotted line) \pm SD (grey bars) and its 5/95% confidence intervals (solid thin lines).

Biogeographical assessment of myxomycete assemblages across the Tropics

Nikki Heherson A. Dagamac ^{a,*} • Yuri K. Novozhilov ^{b,c} • Steven L. Stephenson ^d • Carlos Rojas ^e •
Thomas Edison E. dela Cruz ^f • Martin Unterseher ^a • Martin Schnittler ^a

^a Institute of Botany and Landscape Ecology, Ernst Moritz Arndt University Greifswald
Soldmannstr. 15, D-17487 Greifswald, Germany

^b Komarov Botanical Institute of the Russian Academy of Sciences, Laboratory of Systematics and
Geography of Fungi, Prof. Popov Street 2, 197376 St. Petersburg, Russia

^c Joint Russian-Vietnamese Tropical Research and Technological Center, Ho Chi Minh City, Vietnam

^d Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701, USA

^e Engineering Research Institute and Department of Agricultural Engineering, University of Costa Rica.
San Pedro de Montes de Oca 11501, Costa Rica.

^f Department of Biological Sciences, University of Santo Tomas, España 1015 Manila, Philippines

*Correspondence: Nikki Heherson A. Dagamac, Institute of Botany and Landscape Ecology, Ernst Moritz Arndt
University Greifswald, Soldmannstr. 15, D-17487 Greifswald, Germany. e-mail: nhadagamac@gmail.com

ABSTRACT

Aim:

Lowland / highland and Neotropic / Paleotropic assemblages of myxomycetes are compared to assess if differences in species composition between the two regions are influenced by geographic separation and/or elevational gradients.

Location:

Four pairs (lowland / highland) of comprehensive regional surveys including ca. 7 500 specimens were compared, representing the Neotropics (Yasuni / Maquipucuna in Ecuador; Guanacaste / Monteverde in Costa Rica) and the Paleotropics (Cat Tien / Bi Dup Nui Ba in Vietnam; Chiang Mai in Thailand / South Luzon in the Philippines).

Methods:

Each survey was carried out in an area with fairly homogenous vegetation, including natural or near-natural forests, and consists of field collecting and a moist chamber culture component where all observed fructifications were recorded. Analysis of diversity (i.e. richness) and community composition were performed using EstimateS and R.

Results:

Between 400 and 2 500 records per survey were obtained. Species accumulation curves and richness estimations indicated moderate (70% of expected richness) to exhaustive (94% of expected richness) completeness. Multivariate analyses suggest that geographical separation (Neotropic vs. Paleotropic) explains best the observed differences in composition of myxomycetes assemblages. Our dataset revealed some morphospecies to be restricted to a specific region.

Main Conclusion:

This study provides evidence that both at the species and community levels, myxomycetes tend to follow the moderate endemism hypothesis of protist biogeography.

Keywords: community ecology, dispersal barriers, distribution, endemism, morphospecies, plasmodial slime molds, protists

INTRODUCTION

Myxomycetes (plasmodial slime molds) are one of the few protistean groups that can be studied in the field, based on their above-ground macroscopic fructifications that release airborne spores (Schnittler *et al.*, 2012). Most of our knowledge relating to myxomycete assemblages was derived from numerous surveys recording fructifications directly in the field or from moist chamber cultures (Stephenson *et al.*, 2008). Most studies have been carried out in temperate regions of the northern hemisphere, including alpine and subalpine mountains (Novozhilov *et al.*, 2013; Ronikier & Ronikier, 2009), tundra (Stephenson *et al.*, 2000; Novozhilov *et al.*, 1999), winter-cold deserts (Novozhilov & Schnittler, 2008; Schnittler, 2001; Schnittler & Novozhilov, 2000) and temperate grassland (Rollins & Stephenson, 2013). The vast majority of species of myxomycetes display cross-continental distribution patterns (Stephenson *et al.*, 2008), and even remote archipelagos do not seem to harbor endemic taxa, as found for the Galapagos Islands (Eliasson, 1971; Eliasson & Nannenga-Bremekamp, 1983), the Hawaiian archipelago (Eliasson, 1991), or Macquarie island (Stephenson *et al.*, 2007).

However, numerous species described within the last few decades (Schnittler & Mitchell, 2000) are known from only one or a few localities. Whether this is due to a lack of information or the fact that myxomycetes indeed display, as do other protistean groups such as ciliates, “moderate endemism” (Foissner, 2006) is still a question under debate. Recent studies in arid zones seem to provide evidence for the moderate endemism hypothesis: deserts seem to harbor taxa with typical morphological features (thus often causing descriptions of a species new to science) which are common in the respective survey but rare to absent elsewhere. Examples include *Physarum pseudonotabile* in Central Asia (Novozhilov *et al.*, 2013; Novozhilov & Schnittler 2008), *Perichaena calongei* in Argentina (Lado *et al.*, 2009) and *Didymium infundibuliforme* in South America (Wrigley de Basanta *et al.*, 2009).

Unquestionably, at least the fructification patterns of some species display a preference for specific vegetation types (Schnittler & Stephenson, 2002), substrates (Tucker *et al.*, 2011; Wrigley de Basanta *et al.*, 2008) or seasonal conditions (Dagamac *et al.*, 2012; Rojas & Stephenson, 2007). The unresolved question is whether such differences are due to substrate preferences only (wherever a suitable microhabitat exists the respective species will occur, as suggested for *Barbeyella minutissima*, Schnittler *et al.*, 2000), or if distribution barriers exist, at least between continents. Like in true fungi (e.g. Hallenberg & Küffer, 2001), long-distance dispersal has been detected for myxomycetes (Kamono *et al.*, 2009), but no one knows if the frequency of gene flow mediated by spores is sufficient to prevent allopatric speciation between transcontinental populations. As shown for the much better known ferns (Karst *et al.*, 2005) and fern allies (which usually possess much larger spores than myxomycetes), species may show transcontinental distribution patterns (Europe and America, e.g., *Equisetum telmateia* or *Botrychium virginianum*) or can be restricted to one continent only (Europe: *E. sylvaticum* or *B. lanceolatum*). In contrast, molecular divergence of the gene coding for the largest subunit of DNA-dependent RNA polymerase II (RPB1) indicates that populations of *Tylopilus ballouii* (Boletales), with spores smaller than those of myxomycetes, have been isolated for long periods of time (Halling *et al.*, 2008).

An adequate “field laboratory” to address this question would be a major ecosystem that is stable for a long period of time and that is present around the globe – in other words, tropical regions covered naturally by rainforests. Moreover, tropical regions play a significant role in understanding biodiversity distribution patterns but are threatened by rapid habitat degradation and biodiversity loss (Tittensor *et al.*, 2010). However, even with a history of fructification-based myxomycete studies spanning more than 200 years (Stephenson *et al.*, 2008), there are still many areas that have not been sufficiently well surveyed. This is particularly true for the tropics, where the major portion of the biodiversity of the planet, at least for macroscopic organisms, is concentrated (Davis *et al.*, 1997). For myxomycetes, most of the diversity assessments have been carried out in Neotropical forest ecosystems. These range from pure checklists for different countries (El Salvador, Rojas *et al.*, 2013; Colombia, Rojas *et al.*, 2012a; Costa Rica, Rojas *et al.*, 2010) to abundance-based diversity assessments in natural habitats (Rojas & Stephenson, 2012; Lado *et al.*, 2003; Schnittler *et al.*, 2002). Half of the ca. 1 000 known morphospecies of myxomycetes (Lado, 2005–2016) have been recorded from the Neotropics (Lado & Wrigley de Basanta, 2008). More limited is our knowledge on myxomycete diversity of the Paleotropics, where fewer than 200 taxa are known, with no comprehensive list yet available. However, in more recent years, several investigations have been

carried out in lowland and montane vegetation types of Southeast Asia, including Thailand (KoKo *et al.*, 2010; Tran *et al.*, 2008), Singapore (Rosing *et al.*, 2011), Laos (KoKo *et al.*, 2012), Vietnam (Novozhilov *et al.*, 2014; Tran *et al.*, 2014) and the Philippines (Dagamac *et al.*, 2011, 2012, 2015a,b,c; Macabago *et al.*, 2016).

For these reasons, the authors of this study have focused on the Paleotropics with the goal of comparing assemblages of myxomycetes between Neo- and Paleotropic regions. We tried to answer the following main questions: (i) Are their differences in (a) species composition and/or (b) species abundances between the Paleotropical (Thailand, Philippines and Vietnam) and Neotropical (Costa Rica and Ecuador) ecoregions of the world? (ii) Are there species apparently restricted to only one of the regions? (iii) Will the data compiled from several data sets of regional surveys support the moderate endemism hypothesis (Foissner, 2006) for eukaryotic protists, providing evidence of limited gene flow for myxomycete spores over very large distances?

MATERIALS AND METHODS

Compilation of regional data sets

A double-paired design (four Neotropical and four Paleotropical/four lowland and four highland areas, Table 1, Fig S3 in Appendix 1) was chosen to be able to differentiate between differences in species composition caused by geography and elevation (which causes different forest types, Holdridge *et al.*, 1971). For the selection of the eight data sets used in this study, five criteria were applied: (1) *Area*: Each survey was limited to a certain area which is homogenous for a major forest type. (2) *Components*: Each survey included both a field and a moist chamber culture component; the first comprising at least 100 records from several collecting sites and the second involving at least 100 records representing all major types of substrates (bark, aerial and ground litter). (3) *Myxomycete records*: For both components, all myxomycete fructifications, even those of very common species, were recorded to allow abundance data to be compared. This excludes many studies providing pure species lists without abundance data. (4) *Exhaustiveness*: The Chao2 estimator for an individual-based accumulation curve (including all records) from a survey should converge and indicates a completeness $\geq 70\%$. (5) *Habitat*: Each area includes at least pockets of natural or near-natural forests, to reflect the natural species inventory of the region to a significant degree.

Moist chamber cultures and specimen determination

All moist chamber cultures were prepared in 9 cm Petri dishes using standard procedures (e.g., Schnittler, 2001), incubated under ambient light at room temperature (ca 20–24 °C) for up to 90 days and were checked regularly on at least five occasions. To maintain moisture, distilled water was added over the first six weeks of the incubation period. Mature fructifications were directly transferred to herbarium boxes, with one record defined as a colony of fructifications from one taxon developing in one culture. Myxomycete taxa were determined according to the prevailing morphological species concept, using standard monographs of the group (Martin & Alexopoulos, 1969; Farr, 1976; Neubert *et al.*, 1995–2000; Nannenga-Bremekamp, 1967, 1968; Poulain *et al.*, 2011) and public repositories (Eumycetozoa Project: <http://slimemold.uark.edu/>; Discover Life: <http://www.discoverlife.org>). The taxonomic concepts and nomenclature of the data base NomenMyx (Lado, 2005–2016) was applied; authorities are cited according to Kirk and Ansell (1992). Voucher specimens are deposited at the University of Arkansas (UARK), the private collection of the senior author at the Botanical State Collection Munich (M), the herbarium of the University of Costa Rica at San Jose (USJ), the collection of the Madrid Botanical Gardens at CSIC (MA-fungi), the myxomycete collections of the University of Santo Tomas, Manila and the Komarov Botanical Institute RAS (LE).

Data analysis

To estimate sampling intensity, individual-based species accumulation curves were constructed for each of the study areas with EstimateS (Version 9.1, Colwell 2013, 100 randomizations), which computes as well some estimators of species richness. In accordance with Unterseher *et al.* (2008), first the Chao2 estimator (Chao *et al.*, 2005) was chosen and calculated using the “default settings” of EstimateS; second a fit of the species accumulation curve with the hyperbolic function $y = ax/b+x$ was attempted, with a yielding an estimate for species richness. Myxomycete abundance was classified according to the ACOR scale of Stephenson *et al.* (1993), based upon the

proportion of a species to the total number of records for each survey: R – rare (<0.5%), O – occasional (>0.5–1.5%), C – common (>1.5–3%), A – abundant (>3%). A list of species and their abundances was generated for each area.

Abundance-based datasets from each of the eight study areas and environmental variables (geographic region and elevation) were used to perform the following analyses in R (R Core Team, 2015). Diversity between geographical regions (Neotropics vs. Paleotropics) and elevation (lowland vs. highland) were assessed using the classical richness index Fisher's alpha, the Shannon index and the first three numbers of Hill's diversity series (Hill, 1973; Morris *et al.*, 2014). In addition, the most probable abundance distribution model was determined from rank-abundance plots (Whittaker, 1965) testing five models following Wilson (1991): Null (fits the broken stick model), Preemption (fits the geometric series or Motomura model), log-Normal, Zipf and Mandelbrot. Both diversity indices and distribution models were calculated in the 'vegan' package of R, using the functions *renyi* and *radfit*, respectively. In addition, a cluster analysis, non-metric multidimensional scaling (NMDS), and PERMANOVA was performed to test for the influence of region vs. elevation, using the functions *hclust*, *metaMDS* and *adonis* of R (see Appendix S3 for input files).

RESULTS

Basic data and sampling intensity

Altogether, the surveys from eight areas (Table 1) included a total of 7 585 myxomycete records; 2 844 for the Neotropics and 4 741 for the Paleotropics. A total of 239 taxa (species and subspecies) were recorded, with the Neotropics displaying a lower richness (150 taxa) than the Paleotropics (196 taxa, see Appendix S2 for a collated list). This difference remains if the rarefied values (based on the lower number of 2 844 records) from an analysis of the species accumulation curves are considered: 150.0 taxa for the Neotropics vs. 172.8 for the Paleotropics.

In both parts of the world numerous taxa were found as singletons only (Neotropics: 40 taxa, 27% of all taxa; Paleotropics: 43 taxa, 22%). The eight surveys were between 70 and 88% complete according to the Chao2 estimator, and between 76 and 94% according to a hyperbolic regression (Table 2, Fig. S1 in Appendix 1). The relationship between the number of records per survey and its exhaustiveness can be approximated by a hyperbolic function (Fig. 1). If the degree of exhaustiveness was estimated by a hyperbolic function the fit was better ($R=0.885$) than for the final values of the Chao2 estimator ($R=0.716$). Even for the Philippine South Luzon area that has the highest number of records, the survey is only up to 91% (Chao2) and 94% (hyperbolic regression) complete.

According to the ACOR scale, 72% of all taxa in the four Neotropical surveys (pooled to a common species list, see Appendix 2 and Fig S2 in Appendix 1) were rare, 17% are occasional, 7% are common and 5% are abundant. In spite of being represented by 1.7 times more records, 80% of all taxa from the Paleotropical surveys were rare, 7% are occasional, 11% are common and only 3% abundant. The 5 most abundant species in the Neotropics were *Didymium iridis* (211 records), *Didymium squamulosum* (194), *Physarum compressum* (190), the dwarf form of *Arcyria cinerea* (172, described in Schnittler, 2000), and *Hemitrichia calyculata* (119). In the Paleotropics, the list is headed by *Arcyria cinerea* (933), *Didymium squamulosum* (194), *Perichaena depressa* (155), *Cribraria microcarpa* (149) and *Diderma hemisphaericum* (144).

Comparison of species composition

NMDS ordinations display contrasting signals of species turnover for geographical (Neotropics vs. Paleotropics) and elevational (lowland vs. highland) differences. Species composition from the Neotropics was significantly different from that of the Paleotropics ($R^2=0.278$, $p<0.01$) as the two assemblages did not overlap (Fig. 2A). Cluster analysis using Bray Curtis dissimilarity (Fig. 2C) confirmed this bipartition. In contrast, a large fraction of shared species was observed between lowland and highland samples, leading to significantly overlapping sample groups in species space (Fig. 2B, $R^2=0.117$, $p>0.01$).

Species abundance distribution and diversity

The rank-abundance plots tested for different abundance distribution models between the Neotropics and Paleotropics showed relatively similar graphs. For the pooled datasets from the two geographic regions the lognormal is the best fitting abundance distribution model; for the comparison

lowland vs. highlands the Mandelbrot model provided a slightly better fit for the latter (Fig. 3). The five different species diversity indices used to compare the assemblages in the Neotropics and Paleotropics showed no clear trend (Fig. 4A). But, a trend emerged when myxomycete assemblages between lowland and highland areas were compared. All indices indicated that lowland areas had a more diverse assemblage than highland areas (Fig. 4B). However, species richness alone did not fit into this trend: rarefied means were 73.5 ± 11.2 for lowland and 76.9 ± 13.4 for highland areas (values from species accumulation curves based on the lowest number of 441 records for a survey, Table 2). This was caused by the high taxon number for the survey from northern Thailand (133 taxa, rarefied value 96.4).

DISCUSSION

Within the last years, abundance-based myxomycete data from the Paleotropics became available, which allows a comparison with previous Neotropical inventories. Virtually all myxomycete surveys reveal a high proportion of rare taxa, often found only once in an area (e.g., Schnittler *et al.*, 2002). From this reason, only those surveys that include a large number of records appear appropriate to look for patterns behind the statistical noise caused by the many rare species. We focused on four large data sets from each part of the world to test the *prima facie* null hypothesis that neither diversity nor species composition differs between Neotropical and Paleotropical myxomycete assemblages. This is what one would expect for the case of efficient long-distance dispersal by spores and the assumption that macroecological conditions are roughly comparable between both regions.

For a regional survey, there is always some degree of inhomogeneity in targeted habitats and/or substrates. Even for datasets with a high number of records, as the one from South Luzon, the number of species recorded will always be lower than the number of expected species. This seems to hold true even for infinitely large surveys, estimating final percentages of 92% (analysis of species accumulation curves based on the Chao 2 estimator) and 97% (hyperbolic regression, Fig. 1). This reflects the unavoidable degree of inhomogeneity for a survey covering a larger area. Nevertheless, the analysis of the species accumulation curves generated from the eight study areas suggest that the sampling effort, using the combined results from moist chamber cultures and field collections, were exhaustive enough to recover most of the species that are realistically possible to recover. As to expect, a higher sampling effort will reduce the number of rare species: looking at the eight surveys, the number of records per survey is inversely correlated with the number of singleton species ($R^2=0.457$).

In all four pairs of surveys, species richness was higher in the lowlands than in the respective highland survey (Table 2). The only deviating pair is South Luzon (Philippines, lowland) vs. Chiang Mai (Thailand, highland), and this is as well the survey pair with the largest geographical distance. In addition to the number of species (richness), differences in numbers of records per species (evenness) affects some of the five indices calculated by us. Such differences in the abundance distributions are mainly known to be associated with different ecological niches of certain biological groups (McGill *et al.*, 2007). Nevertheless, this finding is in congruence with the hypothesis that myxomycete diversity in the tropics decreases with increasing elevation (Schnittler & Stephenson, 2000; Stephenson *et al.*, 2004; Rojas & Stephenson, 2008). However, this may apply only to comparisons across vegetational units located in different elevational floors suggesting that elevation *per se* is not the main driver for differences in the abundance of fruiting bodies (Rojas *et al.*, unpublished data). It is important to note that diversity indices showed no trends in the comparison Neotropics vs. Paleotropics, but higher diversity in lowland vs. highland areas (Fig. 4B). The only exception is the survey in northern Thailand (Chiang Mai), and this was the only data set compiled from individual surveys of several different researchers who determined the specimen independent from each other. For this area, we cannot rule out the possibility that in a few cases the same taxon was assigned to different names, causing an overestimation of species numbers. An indication is that this data set has the highest proportion of rare species (69.2%). In addition, the number of singleton species (33) is higher than to expect according to the overall trend between number of records per survey and number of singleton species (23.5).

Interestingly, when we consider species composition, a statistical significant difference was observed among myxomycete assemblages in relation to geography (Neotropics vs. Paleotropics) than the elevational factor (lowland vs. highland). Similar to what is acknowledged for some fungal endophytes (Langenfeld *et al.*, 2013; Peay *et al.*, 2010), and soil protist assemblages (Bates *et al.*,

2013; Foissner, 2007), marine ciliates (Stock *et al.*, 2013) and foraminiferans (Weiner *et al.*, 2014), geographical location explains best the differences in assemblage composition. A biogeographical study of myxomycetes that focused only on tropical highlands found a similar pattern: a cluster analysis separated the myxomycete assemblages recorded from Thailand from those of the Americas (Rojas *et al.*, 2012b). Moreover, despite the much smaller dataset generated in a single sampling effort from Doi Inthanon (a mountain National Park in northern Thailand) in that paper, relatively higher species richness and clear difference of species composition was observed for that study area when it was compared with all the other areas surveyed in Costa Rica. Remarkable is that the dataset from the Paleotropics includes myxomycete taxa known to be common in temperate zones, like *Barbeyella minutissima*, *Echinostelium brooksii*, *E. colliculosum*, *Lamproderma columbinum*, *Licea kleistobolus*, *Lindbladia tubulina*, *Paradiacheopsis rigida*, and *Trichia persimilis*. Somewhat surprising is the high overlap between assemblages of lowland and highland areas. Although elevation determines habitat availability (Dagamac *et al.*, 2014; Rojas *et al.*, 2010; Stephenson *et al.*, 2004) and forest types (Dagamac *et al.*, 2015c; Rojas *et al.*, 2011; Novozhilov *et al.*, 2000), myxomycetes seem to find in both elevations suitable microhabitats for fruiting. These are not necessarily the same for a given species. For instance, due to the higher moisture, myxomycetes seem to utilize aerial litter, which dries out faster, more readily at higher elevations (Schnittler & Stephenson, 2000).

In spite of the principal possibility of long distance dispersal (Kamono *et al.*, 2009), geographic barriers seem to affect the composition of myxomycete assemblages. Most often this is not obvious when looking at distribution ranges of morphospecies (see examples in Stephenson *et al.*, 2008): many morphospecies appear to have cosmopolitan ranges. However, at this point we have to consider the low taxonomic resolution provided by a morphospecies concept: all studies that entered the biospecies level via cultivation and compatibility experiments (e.g., Clark & Stephenson, 1990, 2003) or via molecular markers (e.g., Feng & Schnittler, 2016) found that a given morphospecies comprises several putative biospecies. Therefore, it is likely that a cosmopolitan morphospecies like *Arcyria cinerea*, which almost certainly consists of several biospecies (Clark *et al.*, 2002) will likely “split” if studied by molecular methods, and the putative biospecies may be more restricted in distribution.

However, due to the lack of molecular data, this study analyzed transcontinental distribution patterns of myxomycetes exclusively at the morphological level, where the number of described taxa remains slightly below 1 000 (Lado, 2005–2016). Only at this level, a sufficient number of large datasets is currently available, since molecular tools to analyze myxomycete diversity in environmental samples are still in its beginnings (Kamono *et al.*, 2013, Clissmann *et al.*, 2015, Fiore-Donno *et al.*, 2016). The first workable approach may be a barcoding with partial sequences of the nuclear small subunit ribosomal RNA gene (SSU; Feng & Schnittler, 2016). All morphospecies investigated so far with this molecular marker seem to consist of several taxonomic entities. These “phylopecies”, which can be seen as candidates for real biospecies, may (like those of the *Tubifera ferruginosa*-complex; Leontyev *et al.*, 2015) or may not (*Trichia varia*; Feng & Schnittler, 2015) be distinguishable by morphological characters. A first local survey with a full barcoding component estimated the relationship between morphospecies and phylopecies to be 1: 2–10 (Feng & Schnittler, 2016). The “moderate endemism” of a region is likely to become more pronounced if the morphospecies are studied by molecular markers, as shown for *Badhamia melanospora* (Aguilar *et al.*, 2014).

Therefore, with a morphospecies-based approach employed for this study we can only hope to see the “tip of the iceberg”, hoping that morphologically defined taxonomic entities that are sufficiently close to the real evolutionary significant units (i.e. biospecies). Similar to the approach displayed on finding biogeographical patterns of pyrenomycetous fungi (Vasilyeva & Stephenson, 2014), we should expect at least some biospecies to be restricted to certain regions. A real obstacle for such considerations is the apparent rarity of the fructifications in many species: about 50% of the myxomycete morphospecies have been reported from only a single type locality worldwide (Stephenson, 2011). From this reason, only very distinctive and/or more common species in fructification-based surveys can be recognized as regionally endemic. Examples are the myxomycetes *Comatrichia spinispora*, *Cribraria tecta*, *Diderma pseudostestaceum*, *D. cattense*, and *Perichaena echinolophospora*, all reported occasionally in both lowland and highland surveys from Vietnam (Van Hooff, 2009; Novozhilov & Mitchell, 2014; Novozhilov *et al.*, 2014; Novozhilov & Stephenson, 2015). Neotropical counterparts may be *Craterium paraguayense* and *Lamproderma muscorum*, reported occasionally from Costa Rican highlands (Monteverde). Another Neotropical myxomycete,

Diachea silvaepluvialis, was found exclusively but several times in Ecuador (Yasuni). These cases may represent the “tip of the iceberg” and fit best the moderate endemism hypothesis. Estrada-Torres *et al.* (2013) who considered all known environmental factors found that historical geography explains the distribution of myxomycete assemblages in the Americas. These authors similarly concluded that myxomycetes do not follow the cosmopolitanism theory but rather their distribution patterns support the idea that there are some species that are really restricted to particular geographical areas, and some of these may indeed be endemic to a continent, or parts of a continent.

In addition, first studies employing environmental PCR show a high proportion of sequences not assignable to any known species (dark-spored nivicolous myxomycetes: Kamono *et al.*, 2013; bright-spored wood-inhabiting myxomycetes: Clissmann *et al.*, 2015; dark-spored myxomycetes in temperate grasslands: Fiore-Donno *et al.*, 2016). This may, at least in part, be attributed to sequencing errors and the lack of comparison sequences from myxomycete fructifications. However, it cannot be ruled out that non-fruited strains of myxomycetes exist in nature, since a loss of functionality in a single gene in the complex ontogenesis of fructifications may halt spore formation and thus severely impede dispersal abilities, similar to the loss of a stalk in the sessile *Semimorula liquescens*, where molecular data suggest a close relationship to *Echinostelium*, a genus forming long stalked fructifications (Fiore-Donno *et al.*, 2009). There is no reason to assume that such non-fruited strains cannot persist indefinitely as amoebal populations in soil, even if they lost the ability for long-distance dispersal via spores. With classical methods, large densities of amoebae in soil have been found to exist (Madelin, 1984), and molecular methods have confirmed that they form a major fraction of soil protists (Hoppe & Schnittler, 2015; Urich *et al.*, 2008).

Similar to molecular phylogeographic studies in basidiomycetes (Agarics: Oda *et al.*, 2004; Boletes: Halling *et al.*, 2008) and ascomycetes (Earth Tongue fungi: Ge *et al.*, 2014), we can therefore expect that the geographic differences found in this study will become much more prominent if large data sets on molecular diversity will become available for myxomycetes. In addition, such datasets are critically needed to validate the results on myxomycete ecology obtained in ca. 200 years of fruit body-based surveys.

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SUPPORTING INFORMATION

Appendix S1: Supplementary figures.

Appendix S2: Collated species database for the eight study areas.

Appendix S3: Input files for the ecological analyses carried out in R.

BIOSKETCH

Nikki Heherson A. Dagamac is broadly interested in biogeography and biodiversity of tropical myxomycetes. He started this study as part of his doctoral dissertation in the working group of **Martin Schnittler** at the University of Greifswald who specialized in systematics, genetics, and reproductive biology of myxomycetes using molecular methods. **Yuri K. Novozhilov**, **Steven L. Stephenson** and **Carlos Rojas** worked for the past decades about the global distribution patterns, taxonomy and ecology of myxomycetes. **Thomas Edison E. dela Cruz** and **Martin Unterseher** are mycologists, the former mostly working on Philippine fungi and the latter concentrating in community ecology and next generation sequencing of fungal endophytes.

Authors' contributions: MS conceptualized and designed the present study. Data from the Neotropic region were taken from surveys of MS, YKN and SLS in Ecuador and MS, SLS and CR in Costa Rica. Data from the Paleotropic region originated from field surveys of NHAD, TEDC and MS in the Philippines, NHAD, SLS and MU in Thailand and YKN in Vietnam. NHAD compiled all data from the eight surveys, assembled the initial species lists and assembled the collated dataset. NHAD and MS constructed the species accumulation curves and carried out hyperbolic regressions. NHAD and MU performed the ecological analysis of the dataset in the R software. NHAD, MU and MS interpreted the results. NHAD and MS prepared the manuscript, which was approved by all authors and improved by YKN and CR. SLS did the English correction of the text.

Tables

Table 1. Characteristics of the eight investigated areas; naming the investigated area, literature references, elevation and geographical coordinates, the number of moist chamber cultures prepared and a short description of the vegetation types, applying the Holdridge et al. (1971) classification of tropical forests. Surveys are listed in the sequence Neotropical / Paleotropical (N/P) and lowland/highland (L/H) for each region. Exact locality data for each investigated site within an area are found in the supplementary databases 1–8

Region	Reference	Elevation (m a.s.l.)	Location	Moist chamber cultures	Vegetation type
NL: Costa Rica, Guanacaste	Schnittler & Stephenson, 2000	10–300	08°47'–10°58'N 82°49'–85°56'W	476	Tropical dry forest (excluding volcano Cacao)
NL: Ecuador, Yasuni	Schnittler <i>et al.</i> , unpubl.	200–275	04°00'–04°04'N 76°22'–76°25'W	225	Tropical moist forest, Amazonian lowland forest
NH: Costa Rica, Monteverde	Rojas <i>et al.</i> , 2010; Schnittler & Stephenson, 2000	1000–1500	10°16'–10°19'N 84°44'–84°48'W	ca.700	Tropical montane wet forest
NH: Ecuador, Maquipucuna	Schnittler <i>et al.</i> , 2002; Stephenson <i>et al.</i> , 2004	1250–2720	03°15'–07°28'N 78°34'–78°28'W	475	Tropical montane wet forest

PL: Philippines, South Luzon	Dagamac <i>et al.</i> , 2015b; Dagamac <i>et al.</i> , 2015c	10–524	12°75'–14°10'N 120°54'–124°09'E	1500	Tropical moist forest
PL: Vietnam, Cat Tien	Novozhilov <i>et al.</i> , in prep.	72–247	11°16'–11°27'N 107°03'–107°26'E	954	Tropical montane wet forest
PH: Thailand, Chiang Mai	Dagamac <i>et al.</i> , unpubl.; Rojas <i>et al.</i> , 2012; KoKo <i>et al.</i> , 2010	900–2500	18°48'–20°10'N 98°29'–99°72'E	ca. 1000	Tropical montane wet forest
PH: Vietnam, Bi Dup Nui Ba	Novozhilov <i>et al.</i> , in prep.	1375–1747	12°07'–12°11'N 108°38'–108°42'E	922	Tropical montane wet forest

Table 2. Statistical analysis of individual-based species accumulation curves for the eight regional surveys (giving the area by name and classified as L=lowland, H=highland), showing numbers of records (Rec), species (Sp), rarified species (SpR), and values for numbers of species to expect according to the Chao2 estimator (mean±SD) and a fit with a hyperbolic function $y=ax / (b+x)$, with a as the estimator for the number of species to be expected. The per cent values denote the proportion of species found on the number of species to expect

Survey	Found			Expected: Chao2			Expected: Hyperbolic			
Area	Rec	Sp	SpR	Chao2	SD	%	a	SD	R	%
L: Guanacaste	441	80	80.0	104.9	13.0	76.2	101.2	0.3	0.999	79.0
L: Yasuni	924	92	75.8	126.5	19.3	72.8	103.2	0.2	0.996	89.2
L: South Luzon	2045	80	57.1	86.0	4.7	93.0	85.6	0.1	0.994	93.5
L: CatTien	1105	105	81.2	120.3	8.3	87.3	120.4	0.2	0.996	87.2
H: Monteverde	522	76	71.2	108.3	16.0	70.2	99.7	0.4	0.997	76.3
H: Maquipucuna	957	85	66.1	105.8	10.9	80.3	97.7	0.3	0.992	87.0
H: Chiang Mai	1147	133	96.4	151.2	8.4	88.0	162.5	0.3	0.997	81.8
H: Bi Dup Nui Ba	444	74	73.8	94.0	10.5	78.8	100.2	0.3	0.998	73.8

Figures

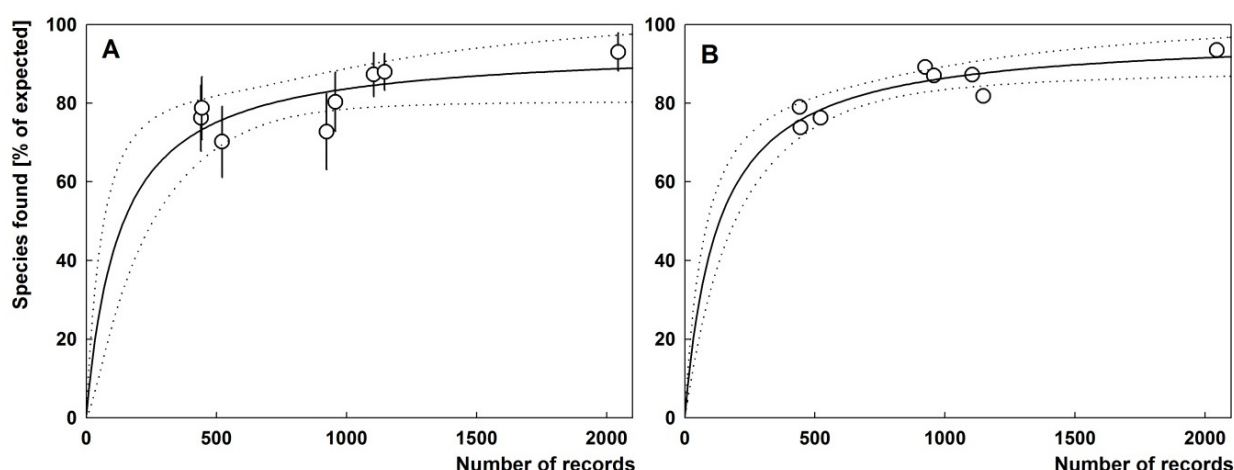


Fig. 1. Relationship between the number of records and exhaustiveness of a survey (number of species found as proportion on the number of species to expect) for eight surveys according to the Chao2 estimator \pm SD (**A**) and a hyperbolic regression (**B**). The values for eight regional surveys were in turn fitted with a hyperbolic function $y = ax / (b+x)$, indicated as solid line with 95% confidence intervals as dotted lines. This fit results in estimates of the maximum exhaustiveness for an infinite number of records of 94.2% (Chao2, $R=0.716$) and 97.3% (hyperbolic regression, $R=0.885$).

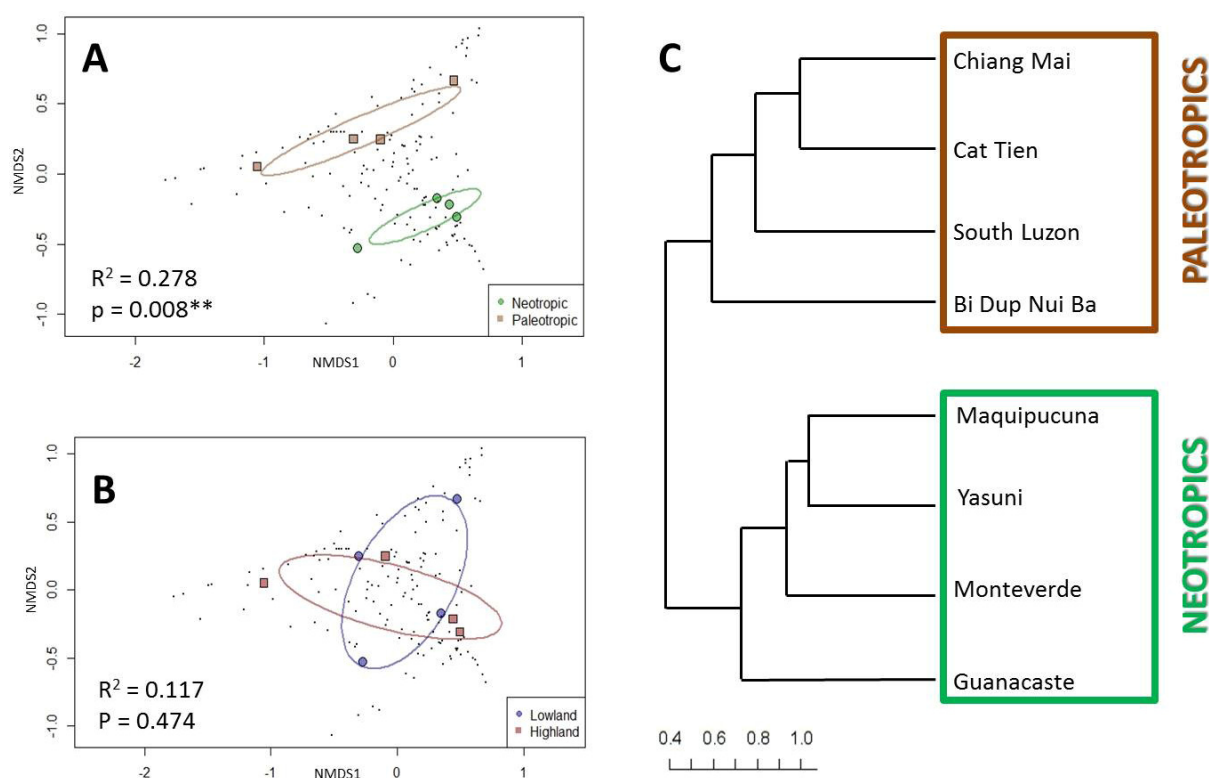


Fig. 2. Non-metric multidimensional scaling (NMDS) of species occurrences for the eight surveys based on **A** geographic location and **B** elevational gradient. Black dots represents the position of myxomycete species in the ordination space. Colored circles and squares represent the regions / areas; colored ellipses denote dispersion based on standard deviation of point scores. **C**. Clustering analysis using Bray-Curtis dissimilarity distance based on species composition (species and abundances).

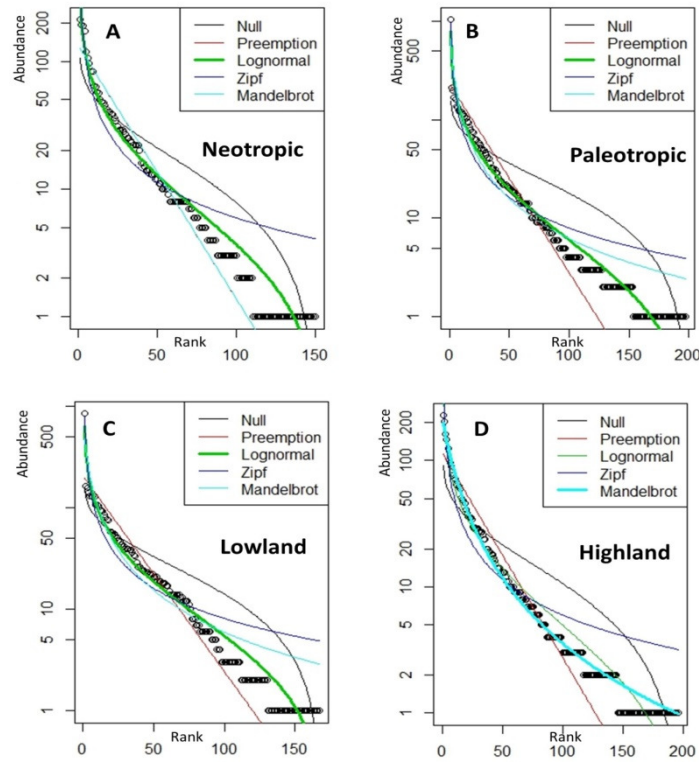


Fig. 3. Rank abundance plots based on the abundance of species for the (A-B) geographical (Neotropics vs. Paleotropics) and (C-D) elevational (Lowland vs. Highland) gradient fitted with five species distribution models. All records from region or elevation (four surveys each) were pooled. The model showing the best fit is highlighted by a thick line.

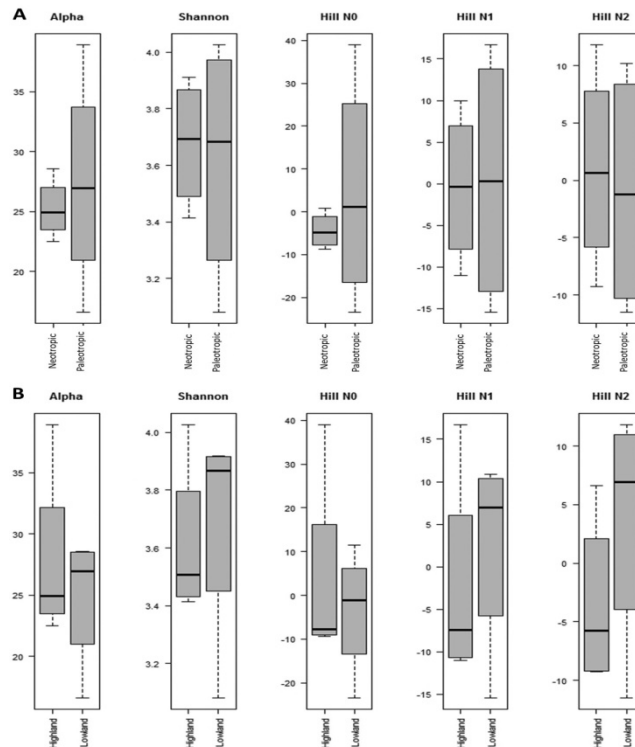


Fig. 4: Box plot showing the comparison of five different diversity indices (Alpha = Fisher's alpha; Shannon = Shannon's H index; N0 = species richness; N1= exponent of Shannon diversity and N2 = inverse of Simpson) in relations to (A) geography and (B) elevation. Data from four study areas were pooled according to A geography (Neo- vs. Paleotropics) and B elevation (lowland vs. highland regions).

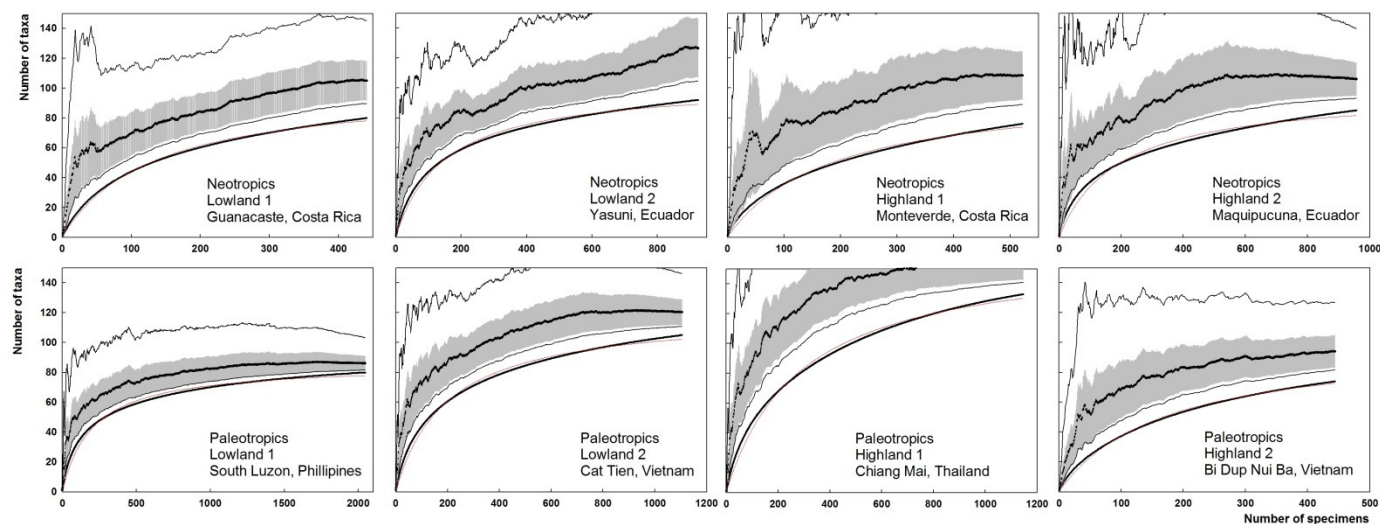


Fig. S1. Species accumulation curves (solid dark lines) for the eight regions investigated for this study; underlying thin grey lines indicate the fit with a hyperbolic function $y = ax / (b+x)$. The jagged solid dark lines depict the value of the Chao2 estimator with standard deviation (shaded in grey) and its 5/95% intervals (upper and lower jagged grey lines).

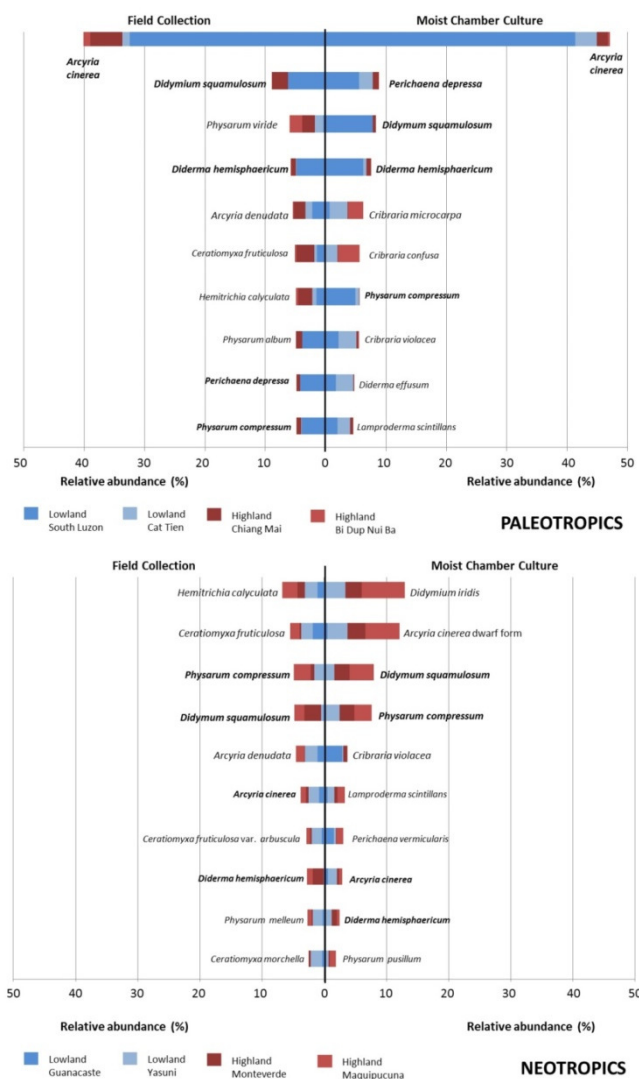


Fig. S2. Stacked bar graph of the ten most abundant species (based on their relative abundance) in field collections *vis-à-vis* moist chamber cultures in the Neotropics and Paleotropics. Colors in each bar denote the proportion of species coming from each of the four surveys for a region.



CHAPTER 3.3

molecular diversity of myxomycetes population from the tropics

CONTENTS

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Speciation in progress? A phylogeographic study among populations of *Hemitrichia serpula* (Myxomycetes)

Nikki Heherson A. Dagamac^{1*} • Carlos Rojas² • Yuri K. Novozhilov³ • Gabriel H. Moreno⁴ • Rabea Schlüter⁵ • Martin Schnittler^{1*}

¹ Institute of Botany and Landscape Ecology, Ernst-Moritz-Arndt, Ernst Moritz Arndt Universität Greifswald, Soldmannstr. 15, D-17487 Greifswald, Germany

² Engineering Research Institute and Department of Agricultural Engineering, University of Costa Rica, San Pedro de Montes de Oca 11501, Costa Rica

³ Komarov Botanical Institute of the Russian Academy of Sciences, Laboratory of Systematics and Geography of Fungi, Prof. Popov Street 2, 197376 St. Petersburg, Russia

⁴ Departamento de Biología Vegetal, Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, Spain

⁵ Laboratory of Electron Microscopy, Institute of Microbiology, Ernst-Moritz-Arndt, Ernst Moritz Arndt Universität Greifswald, Friedrich-Ludwig-Jahnstr. 15, D-17487 Greifswald, Germany

Corresponding email: nhadagamac@gmail.com / martin.schnittler@uni-greifswald.de

ABSTRACT

Myxomycetes (plasmodial slime molds, Amoebozoa) are often perceived as widely distributed, confounding to the “everything is everywhere” hypothesis. To test if gene flow within a species is restricted by geographical barriers we chose the widespread but morphologically unmistakable species *Hemitrichia serpula* for a biogeographic study. Partial sequences from nuclear ribosomal RNA genes (SSU) revealed 40 ribotypes among 135 specimens, belonging to three major clades. Each clade is dominated by specimens from a certain region and from one of two morphological varieties which can be differentiated by SEM micrographs. Partial sequences of the protein elongation factor 1 alpha (EF1A) showed each clade to possess a unique combination of SSU and EF1A genotypes. This pattern is best explained assuming the existence of several putative biospecies dominating in a particular region. However, occasional mismatches between molecular data and morphological characters, but as well some heterogeneous SSU and heterozygous EF1A sequences, point to ongoing speciation. Environmental niche models suggest that the putative biospecies are rather restricted by geographical barriers than by macroecological conditions. Like other protists, myxomycetes seem to follow the moderate endemism hypothesis and are in active speciation, which is most likely shaped by limited gene flow and reproductive isolation.

Keywords: biogeography, geographic barriers, genotyping, Myxogastria, protists, speciation

INTRODUCTION

For the last decades, molecular sequence data were successfully used to disentangle cryptic and emerging species among populations of different groups of organisms. Some examples include green algae (Skaloud & Rindi 2013), marine diatoms (Degerlund et al. 2012), fungi (Halling et al. 2008; Zhang et al. 2015), oomycetes (Lara & Belbahri 2011) and diptera (Anderson et al. 2013). Molecular data have provided a better understanding of the evolution and deep relationships of eukaryotic species, particularly eukaryotic microorganisms commonly recognized as protists (Pawlowski 2013). Within protists, myxomycetes are a special case. On one hand, they form visible fructifications, which have been studied for more than 200 years (Stephenson et al. 2008). Therefore, their morphological diversity is better known than for most other groups of protist. On the other hand, myxomycetes are neither pathogenic nor economically important. From this reason the molecular era of myxomycetes started quite late (Stephenson 2011), with the first phylogenies constructed only during the last decade (Fiore-Donno et al. 2005, 2010, 2011, 2012, 2013).

Myxomycetes constitute a monophyletic taxon in the supergroup Amoebozoa (Pawlowski & Burki 2009; Fiore-Donno et al. 2010) and are the most species-rich of several groups of protists that, often within independent lineages, disperse by airborne spores released from fruiting bodies (Schnittler et al. 2012). This permitted the development of a morphological species concept, which is applied in all hitherto published monographs of the group, beginning with the first descriptions of myxomycete species by Linnaeus (1753), the nomenclatural starting date for the group. Early studies on a few cultivable species, mostly of the Physarales, developed an alternative

species concept based on groups of compatible strains (biospecies, Collins 1981). The few morphospecies with numerous strains investigated often split into groups of compatible strains, called biospecies (Clark & Haskins 2013), which casts doubts to which extent the morphospecies concept is able to mirror adequately speciation processes in myxomycetes (Clark 2000). Recent molecular studies, using mostly the first 600 bp of the ribosomal small subunit rRNA gene (SSU) which is a useful barcoding marker in other groups of protists (Pawlowski et al. 2012; Adl et al. 2014), revealed a high ribotype diversity for many morphospecies. A barcoding study of some nivicolous species (all dark-spored myxomycetes) from the Caucasus Mountains, estimated an average of 1.7 ribotypes per morphospecies (Novozhilov et al. 2013); a survey focusing on bright-spored myxomycetes (the second major group of myxomycetes) found a figure of 2.9 (Feng & Schnittler 2016). Similar figures for the number of ribotypes per morphospecies have been found singular morphospecies with multiple accessions screened: *Lamproderma puncticulatum* (3), *L. columbinum* (7, both Fiore-Donno et al. 2011), two morphological differentiable groups within *Badhamia melanospora* (14, 23, Aguilar et al. 2014), *Tubifera ferruginosa* s. str. (24, some ribotype groups are morphologically differentiable, Leontyev et al. 2014, 2015), and *Trichia varia* (18, Feng & Schnittler 2016). In the cases of *Trichia varia* (Feng & Schnittler 2015) and *Meriderma* spp. (formerly *Lamproderma atrosporum*, Feng et al. 2016) studies with multiple markers found groups of ribotypes that seem to be reproductively isolated from each other, constituting putative biospecies. If these relationships are the rule, the number of nearly 1000 species described worldwide at a morphospecies level (Lado 2005–2016) would inflate by a factor between 2 and 10 at the biospecies level. To look if such putative biospecies differ as well in their ecological niches, the morphologically clear cut species *Hemitrichia serpula* (Scop.) Rost. (Trichiaceae) was chosen for this world-wide biogeographic study.

Hemitrichia serpula (Fig. 1) is a widespread myxomycete characterized by plasmodiocarps forming an unmistakable golden yellow reticulum with a spinulose capillitium and coarsely banded-reticulated spores. In spite of this distinct habit, morphological characters vary to a certain extent within the morphospecies, which led to the description of several varieties. As for virtually all myxomycetes, the mechanisms behind such variability (truly genetic or a caused by phenotypic plasticity) are still unknown (Schnittler & Mitchell 2000). Nannenga-Bremekamp & Yamamoto (1990) described var. *tubiglabra* from dead mossy wood found on Nepal, based on a more robust capillitium (8–9 µm in diam.) with smooth spirals and spores with a coarser reticulum. Cavalcanti & Mobin (2001) named var. *piaueinsis* from Brazil, noting the more scattered and shorter spines of the capillitium and a finer and more regular reticulation of the spores. Finally, Lizarraga et al. (1999) published var. *parviverrucospora* based on a faint reticulation visible in SEM that covers the usually smooth areas between the reticulum of the spores. The later variety was later elevated to species rank (Lizarraga et al. 2001). This triggered the first question this study intends to answer: Do these morphological differences hint towards the existence of cryptic species within *Hemitrichia serpula*? Second, does morphology coincide with molecular sequence data?

Finally, the study was set up to evaluate the biogeographic hypotheses “everything is everywhere” against the concept of “moderate endemism”, combining a ribotype phylogeny with environmental niche modeling (ENM). Based on morphological data, many species of myxomycetes seem to be cosmopolitan (e.g., *Barbeyella minutissima*, Schnittler et al. 2000). If restrictions appear, temperature seems to be a factor (e.g. *Ceratomyxa morchella*, Stephenson et al. 2008). Not surprisingly, long-distance dispersal of myxomycete spores was demonstrated by Kamono et al. (2009). The potentially effective dispersal combined with the life style (suitable habitat conditions have to be realized only for limited periods of time in small spatial niches) let myxomycetes appear as a prominent example for the ubiquity hypothesis of protist biogeography that “everything is everywhere” (EiE). However, the environment selects, and therefore patchy distribution patterns are not only due to insufficient knowledge but can be expected, as demonstrated for *Barbeyella minutissima* (Schnittler et al. 2000). Myxomycete spores are typically between 8 and 12 µm in diameter; their terminal velocity seems to follow Stokes law (Tesmer & Schnittler 2007). Therefore, leptokurtic dispersal curves can be expected, as found for fungal spores (Rieux et al. 2014) or pollen grains (Saro et al. 2014). Hence geographic barriers (like oceans) can be expected to restrict gene flow between populations of a widely distributed myxomycete species. This may cause allopatric speciation and is more in accordance with the “moderate endemism” model. Supporters of the ubiquity hypothesis assume a lower global diversity among free living protists since their distribution is cosmopolitan (Fenchel & Finlay 2004; Finlay 2002) but the proponents of the moderate endemism hypothesis assume a higher global diversity and thus a significant amount of hidden diversity (Foissner et al. 2008; Foissner 1999). The differences found between ribotypes of *Badhamia melanospora* in the Old and New World (Aguilar et al. 2014) seem to advocate the latter hypothesis, assuming a moderate level of endemism for protists (Foissner 2006, Cotterill et al. 2008). However, speciation processes in myxomycetes are virtually not studied. *B. melanospora* may be a special case, as this species is specialized on decaying tissues of succulent plants and should thus be limited to arid regions, whereas *Hemitrichia serpula* grows on all kinds of litter and is well known for its global distribution (www.discoverlife.org). With its conspicuous fructifications, it is a flagship morphospecies within myxomycetes and seems to be well suited to test both hypotheses.

Environmental niche models (ENM) may help to decide if “phylogroups”, which may constitute cryptic species within a morphospecies, are limited by ecology (environmental constraints) or by geography (dispersal barriers). These models rely on numerical tools that combine observations of species occurrence or abundance

with environmental parameters to calculate potential distribution ranges (Elith & Leathwick 2009). Rojas et al. 2015 used this tool to predict the distribution probability of five common myxomycete species in Costa Rica. For this study we used the tool to investigate to which extent phylogroups in *H. serpula* may be restricted by dispersal barriers or by environmental constraints.

MATERIALS AND METHODS

Specimen acquisition

Lowland forests from four major land masses (Asia, Europe, North and South America) were surveyed for *Hemitrichia serpula* between 2012 and 2014. Additionally, specimens from other myxomycete collectors were obtained, resulting in a total of 135 herbarium specimens coming from 43 localities that were used in this paper. The holotype specimen (AH24440 from Mexico) and an isotype specimen (AH43993 from Argentina) of *H. serpula* var. *parviverrucospora* were obtained from the herbarium collection of G. Moreno. Localities and determination results are listed in supplementary file S1.

Morphological evaluation

Specimens of *H. serpula* were analysed independently using scanning electron microscopy (SEM), focusing especially on the internal structures within the spore mesh. According to the location of the collections, three different instruments were used (i: DSM-950 [Carl Zeiss Microscopy GmbH], critical point drying, University of Alcalà de Henares, Spain, 11 specimens; ii: JSM-6390 LA, Komarov Botanical Institute RAS, St. Petersburg, 22 specimens; iii: EVO LS1 [Carl Zeiss Microscopy GmbH], Imaging Center of the Department of Biology of the University of Greifswald, 100 specimens).

DNA extraction, amplification and sequencing

A part of a plasmodiocarp equivalent to 10–15 separate sporocarps of *Trichia varia* was transferred into a sterile 2 ml safe-lock Eppendorf tube with coarse sea sand, cooled to -80°C for 30 minutes, and homogenized manually using a plastic pestil. DNA was extracted with the E.Z.N.A. Plant DNA Kit (Omega Bio-Tek, Georgia, USA), following the manufacturers protocol.

Amplification of the first part of the nuclear small subunit ribosomal RNA gene (SSU) was conducted for 135 samples of *H. serpula* using two newly designed primers for this study, named S1A (CTGGTTGATCCTGCCAGAAT) and SRHem1 (gggggttaaaggtccccc, all primer sequences written in 5'-3' direction). A total volume of 25 μl , adding 5 μl colored reaction buffer (5x Mango-TaqTM), 1.7 μl MgCl_2 (Bioline, 50 mM), 0.5 μl dNTPs, 0.625 μl of each primer, 0.2 μl (1U / μl) of Mango-TaqTM DNA Polymerase, and 4 μl of template DNA was used and adjusted with ddH₂O. PCR included 2 min at 95°C , 39 cycles (30s at 95°C , 30s at 52°C , 1 min at 72°C) and 5 min at 72°C .

Three specimens (sc28076, sc28090 sc28128, all from Costa Rica) displayed heterogeneous SSU sequences. To ascertain these results, the PCR was repeated. In all cases, the two overlaid sequences could be disentangled, since they matched already existing ribotypes. An exception was the second sequence of specimen sc28076, which represented a new, slightly deviating ribotype. For all further analyses, the heterogeneous sequences were coded like two alleles, and both were included in phylogenetic analyses.

Partial sequences of the protein elongation factor 1 alpha (EF1A) were obtained from the last part of the gene (downstream of the spliceosomal intron, covering amino acids 141–393 (243 triplets) referring to the sequence from *Physarum polycephalum*, GenBank AF016243, Baldauf & Doolittle 1997). We amplified sequences from 30 samples using the newly designed primer pair EMyxF4 (CTYGGTGTGAARCARATGATYGT) and EMyxSF1rmod (cctccaagcccatgtgygt) with modified concentrations (5 μl colored reaction buffer (5x Mango-TaqTM), 0.7 μl MgCl_2 (Bioline, 50 mM), 0.5 μl dNTPs, 1.5 μl of each primer, 0.2 μl (1U / μl) of Mango Taq DNA Polymerase, and 4 μl of template DNA, adjusted to a total volume of 25 μl with ddH₂O); cycle conditions were 2 min at 94°C , 45 x (30s at 94°C , 30s at 52°C , 1 min at 72°C) and 5 min at 72°C . All products were purified with the SureClean kit (Bioline) and analyzed with an ABI 3730 sequencer.

Sequence Alignment and Phylogenetic Analyses

The generated sequences were initially aligned automatically using the default settings of the software MUSCLE (Edgar 2004) executed in Mega 6.0 (Hall 2007) and, if necessary, corrected manually. Alignments including 453 sites (SSU, see supplementary file S2) and 729 sites (EF1A, see supplementary file S3) were used for further phylogenetic analyses. All 135 investigated specimens were sequenced for SSU, and a total of 30 specimens representing each major clade in the SSU phylogeny were selected to generate EF1A sequences. Phylogenetic trees were constructed using Bayesian Interference (BI), MrBayes 3.2.5 (Ronquist and Huelsenbeck 2003) with the GTR + I + γ model of substitution, the gamma distribution being approximated by eight categories. The MCMC search was run with 4 chains for 1 million generations with sampling every 100 generations. Maximum Likelihood (ML) trees were calculated with RAXML (Stamatakis 2006) using the model GTR+GAMMA for nucleotide substitution, the rapid hill-climbing ($-\text{fd}$) option and the rapid bootstrap algorithm

(-fa) with 1 000 bootstrap replicates. The resulting “.tre” output file was edited using FigTree v. 1.4.2 (Rambaut 2009). A sequence from a specimen of *Trichia varia* (GenBank JX481344) was used as outgroup in the phylogenetic analyses. To calculate a parsimonious network for ribotypes with a 95% of coherence according with a set of possible outcomes based on coalescent theory (Hudson, 1989), a gene genealogy of all unique ribotypes separated by mutational steps was created using the software TCS v.1.21 (Clement et al. 2000). TCS calculates the probability that pairs of ribotypes are similar for all combinations of ribotypes and then joins the most similar ribotypes together into a network where their combined probability is >95%. Therefore, the resulting network will remove divergent ribotypes whose true genealogy may be concealed by homoplastic characters (Templeton et al. 1992).

Population genetic analyses

To estimate the exhaustiveness of the survey, a ribotype accumulation curve was constructed using EstimateS (Version 9.1, Colwell 2013, 100 randomizations). In accordance with Unterseher et al. (2008), first the Chao1 estimator (Chao et al. 2005) was chosen and calculated using the “default settings” of EstimateS. Significance of the correlation between genetic and geographic distances was tested by a Mantel test. For a first matrix, pairwise genetic distances from the 453 bp multiple sequence alignment of only the specimens with homogenous SSU sequences were computed using the maximum composite likelihood model implemented in MEGA 6.0. The geographic distances between the collection sites for the specimens were computed with an Excel macro for the Vincenty formula (Dalglish 2015), obtaining a second matrix. Correlations and the Mantel test with 999 iterations were calculated with the ExtraStats function in PopTools v. 3.2.5 (Hood 2010). To detect differentiation of ribotypes among geographical groups obtained in this study, an analysis of molecular variance (AMOVA) was performed using Arlequin 3.5 (Excoffier et al. 2005) with a significance based on 10 000 permutations. An analysis of polymorphic sites that generated the diversity of the ribotypes, nucleotide diversity, and average number of nucleotide differences was performed using DnaSP v. 5.10 (Librado & Rozas 2009).

Historical Biogeography

An event-based ancestral area reconstruction suggesting an explicit model of processes that possibly explains the geographic distribution of our generated SSU tree was constructed by defining distribution areas based on the three major sampling regions: (A) Northern temperate zone (Far East of Russia, Europe and Eastern North America), (B) Paleotropics (Southeast Asia, Africa), and (C) Neotropics (Caribbean region, Central and South America). We ran S-DIVA analyses using RASP v.3.01 (Reconstruct Ancestral State in Phylogenies; Yu et al. 2015) to infer a probable biogeographic history of *H. serpula* ribotypes based on the phylogeny constructed from partial SSU data. In S-DIVA, the frequencies of an ancestral range at a node in the ancestral reconstructions are averaged over all trees, and each alternative ancestral range at a node is weighted by the frequency at which the node occurs or by some other measure of support for the node (Yu et al. 2010). The previously produced 10 001 trees in MrBayes 3.2.5 and the distribution matrix of each ribotypes were loaded into the software. The maximum number of ancestral areas at each node were limited at two, the maximum reconstruction was set to 100, and maximum reconstruction for the final tree was set to 1 000.

Species Distribution Modeling

For each of the three most important clades in the SSU phylogeny three different scenarios were modelled based on 1) current climatic conditions, 2) future conditions under the HadGEM (Hadley Centre Global Environmental Model), 4.5 scenarios for 2070 and 3) past conditions in the Holocene. All bioclimatic grid data (rasters) were downloaded from the BIOCLIM dataset (<http://www.worldclim.org/bioclim>). Initially, grid data for all 19 bioclimatic variables were tested for all three clades using the MaxEnt (Maximum entropy) v3.3.3k software (Schapire 2016) to choose the best combination of variables able to explain at least 70% of the variability in the model. According to this criterion bioclimatic variables 3, 7, 12 and 15 (Isothermality, Annual temperature range, Annual precipitation and Seasonality of precipitation) were chosen and all models were created using these variables only. In addition, elevation data were included in the model. Finally all models were ran on MaxEnt, using standard settings, applying a bootstrap replicated run type and 500 maximum iterations on 50 different runs per biotype. To assess the accuracy of the discriminatory capacity (sensitivity for true positive and specificity for true negative) of the generated niche models, each model was evaluated using the receiver operating characteristic (ROC) analysis that resulted to the area under the curve (AUC) values. The final models were also subjected to the analysis of variable contributions. For visualization the rasterized output data were imported into ArcMap v10.2, to reclassify values for probability of occurrence under a particular model into a heat map.

RESULTS

Morphological and molecular diversity

Fig. 1 shows the morphological structures for peridium, capillitium and spores of *Hemitrichia serpula*. All except two of the 135 specimens were examined by SEM, with 74 determined as var. *serpula*, 58 as var.

parviverrucospora, and 1 as var. *tubiglabra*; represented by 26, 14, and 1 ribotype, respectively. Most ribotypes could be uniformly attributed to either var. *serpula* (25 ribotypes from 70 specimens) or the var. *parviverrucospora* (13 ribotypes from 54 specimens). However, two ribotypes included specimens from both varieties: r3 (15 specimens var. *serpula* / 4 var. *parviverrucospora*) and r28 (4 var. *serpula* / 19 var. *parviverrucospora*). The ribotype of the single specimen of var. *tubiglabra* (r26) is shared with three other specimens determined as var. *serpula*. The alignment of partial SSU sequences comprised 453 positions; of these 96 were polymorphic, including 17 variable singleton sites and 79 parsimony informative sites. Counting the alleles of three heterogeneous ribotypes separately for each allele, 40 ribotypes were found for 138 sequences from 135 specimens. According to an accumulation curve constructed for the ribotypes, 52% (Chao 1 estimator) of all ribotypes to be expected were found.

Using *Trichia varia* as outgroup, Bayesian interference and Maximum likelihood analyses of partial SSU sequences resulted in identical tree topologies differentiating three major clades (Fig. 2), yet with different figures for bootstrap / posterior probability support. Clade 1 was composed of 41 sequences (36 came from areas in the temperates and 5 from the tropics) and 7 ribotypes. The more divergent Clade 2 included 30 sequences with 19 ribotypes, all originating from the old world tropics and temperate zones. Clade 3 splits into two prominent subclades (3a and 3b), altogether comprising 67 sequences with 14 ribotypes, all from tropical regions.

Clades 1 and 2 included var. *serpula* (66 of 70 specimens, spores with smooth intermesh areas, Fig. 1e); with the exception of four specimens from Costa Rica (sc28033, sc28034, sc28090 and sc28128). Most (57 of 63 specimens) of clade 3 fit the description of var. *parviverrucospora* (spores with faintly warted intermesh areas (Fig 1f). Specimens used to describe var. *parviverrucospora* (ribotypes r33, isotype AH43993 from Argentina and r39, holotype AH24440 from Mexico) grouped in subclades 3a and 3b, respectively. The three specimens showing heterogeneous SSU sequences, all from Costa Rica, represented a mixture of ribotypes r3+r35 (sc28090) and r3+r28 (sc28128); all these ribotypes were as well recorded in homogeneous state in the population. The remaining specimen sc28076 was composed of ribotype r39, which is the ribotype of the type specimen of var. *parviverrucospora* (AH24440 from Mexico) and a new ribotype r40, deviating in seven positions.

The second marker, EF1A, differentiated the same major clades within 30 selected specimens, yet with a somewhat different topology. Fig. 3 shows the mirrored trees of partial SSU and partial EF1A sequences. All clades showed exclusive combinations of EF1A genotypes and SSU ribotypes (clade 1: 3 and 3, clade 2: 4 and 5, subclade 3a: 3 and 2, subclade 3b: 7 and 2). All EF1A genotypes and SSU ribotypes were limited to a single clade/subclade, with the exception of the EF1A genotype 7, which showed a single clear heterozygosity (specimen sc28067), constituting a mute mutation in the third base of a triplet coding for Leucine.

The ribotype network (Fig. 4) was highly compatible with the constructed phylogenetic trees. Due to the large distance between the clades the software could not connect all sequences to a single network. Instead, every clade or subclade formed its own network. An exception was the most diverse clade 2, resulting in two separate networks. In addition, the statistical parsimony algorithm employed in TCS failed to include its two most distant ribotypes of clade 2 (r22, a singleton and r26, four specimens) into a network.

Ancestral area reconstruction

The S-DIVA analysis proposed 9 dispersal and 6 vicariance events to account for the present distribution of the ribotypes. According to the analysis the center of origin for *H. serpula* lies in the Tropics of the New World; subsequent dispersal events led to the separation of the three major clades. The ancestral area for clades 1 and 2 may be situated in the temperate zone and the New World Tropics, respectively; further dispersal and vicariance events caused the current distribution of the ribotypes within the clades.

Population structure

The world population of *H. serpula* is geographically structured: pairwise geographic and genetic distance matrices are correlated with each other ($R = 0.4667$). According to a Mantel test, this value is significant, since it falls clearly out of the 95% confidence interval (-0.0134 to 0.0211) calculated for 999 matrix permutations. Accordingly, one can expect that geographical separation explains a substantial proportion of the genetic variation among the world population of *H. serpula*.

However, the analysis of molecular variance among the proposed distribution areas (Table 1) showed higher variation within a region (temperate zone / Neotropics / Paleotropics) than among these regions. These figures reverse if molecular variance within / among the major clades 1–3 is analyzed: now two thirds of the variation is among clades.

Species distribution modeling

Niche modeling based on the recorded species occurrence was carried out separately for each major clade (subclades 3a and 3b were pooled) with the MaxEnt algorithm. If the area under the curve (AUC) is taken as a measure of the predictive value of the model, quality improved from clade 1 to clade 3 in all time scenarios (past: 0.757, 0.823, 0.917; current: 0.789, 0.888, 0.921; future: 0.753, 0.855, 0.928 for clades 1–3, respectively). Clade 2 apparently has a broader distribution range than clade 3. Considering a changing environmental scenario, the

model consistently showed that clade 3 ribotypes seems to be limited to tropical regions (Fig. S1). For all three clades, elevation was the most important variable. The second-most important variable changed with the time scenario: for current climatic conditions it was seasonality of precipitation, for the future scenario it was overall annual precipitation, for the past scenario it was annual temperature range.

DISCUSSION

The accurate circumscription of species is an essential prerequisite for any diversity assessments and biogeographical studies, which is most often based on morphological criteria. Applying a morphological species concept to protists like myxomycetes poses two obvious difficulties. First, myxomycete fructifications develop not out of a growth process but by rearrangement of the biomass of the plasmodium within a short period of time, typically hours to days (Schnittler et al. 2012). Therefore, extreme weather events during the short time of sporocarp development may easily cause aberrations in morphological characters (Schnittler & Mitchell 2000). Since sporocarps are not actively living but contain only spores as dormant stages, they can exhibit a high phenotypic plasticity without being penalized by a lower fitness. Second, the fructifications show only a limited number of morphological characters. Therefore, it cannot be expected that every geno- or ribotype will be recognizable by a unique combination of characters. In addition, none of the markers currently established for myxomycetes codes for any morphological character.

All studies investigating numerous accessions for a myxomycete species resulted in multiple ribotypes; an apparent exception was only *Hemitrichia calyculata* (one ribotype shared by 52 accessions, but these are all from one area (Feng & Schnittler 2016). Therefore, a nearly cosmopolitan species like *Hemitrichia serpula* is likely to consist of numerous different ribotypes, which can be used to test hypotheses of protist biogeography.

Ribotypes and morphological varieties in *Hemitrichia serpula*

Only two of the four described varieties of *Hemitrichia serpula* occur commonly, and the diagnostic character telling them apart is the absence (var. *serpula*) or presence (var. *parviverrucospora*) of the internal warts that can be seen inside the reticulum of ridges covering the spores (Fig. 1). Most but not all of the specimens grouping in clades 1 and 2 have the morphology of var. *serpula*, whereas most specimens of clade 3 were assigned to var. *parviverrucospora* (Fig. 2). The two other described varieties are very rare; they differ in capillitial characters (shorter spines, var. *piaueinsis* / rare to missing spines, var. *tubiglabra*) and spore ornamentation (finer / coarser reticulation). We were only able to obtain a single specimen from var. *tubiglabra*, and its ribotype (r26) is identical to this of three other specimens clearly assignable to var. *serpula* (clade 2). This indicates that the var. *tubiglabra* does not bear any taxonomic value. This situation may be not uncommon for rare morphotypes that differ from their common counterparts only by the lack or malformation of a character (like the capillitial spines), which is likely to be caused by environmental alterations during development (Schnittler & Mitchell 2000). An examination using molecular methods may help to avoid formal description of such morphotypes.

Cryptic speciation in *H. serpula*?

The phylogeny based on partial SSU sequences clearly revealed three distinct clades (Fig. 3) for *Hemitrichia serpula*, with clade 3 splitting again into two subclades. In myxomycetes, nuclear (18S) SSU genes reside with multiple copies on extrachromosomal units and show non-Mendelian inheritance (see discussion in Feng & Schnittler 2015). To confirm this topology, selected specimens were sequenced with an independent second marker. EF1A is a nuclear single copy gene located on regular chromosomes and is expected to show Mendelian inheritance. Each of the four phylogroups (clades 1, 2, and subclades 3a and 3b) was characterized by a unique combination of SSU and EF1A genotypes (Fig. 3). This pattern is similar to the results found for *Trichia varia* (Feng & Schnittler 2015), where three markers, SSU, EF1A and the mitochondrial cytochrome oxidase gene (COI) displayed as well clades with unique combinations of the genotypes. All these markers are independent from each other and should thus freely recombine in a sexual population. Therefore, the absence of recombination among the clades is explained best by the assumption of reproductively isolated units, i.e. biospecies in the sense of Clark & Haskins (2013). Feng et al. (2016) provide a discussion of the biospecies concept and possible reproductive modes in myxomycetes on the example of the genus *Meriderma*, where as well several reproductively isolated, putative biospecies were found with SSU and EF1A markers. In our case, the reproductive isolation is additionally underpinned by the distribution of the two varieties among clades (var. *serpula* dominates clades 1 and 2, var. *parviverrucospora* subclades 3a and 3b), with none of the phenotypic characters distinguishing the varieties coded by any of the investigated markers.

Ongoing speciation?

However, exceptions proof these rules and indicate that the reproductive isolation is not complete for *H. serpula*. First, four of seventeen specimens belonging to the common ribotype r3 (clade 1) showed the “wrong” phenotype (var. *parviverrucospora*, Fig. 2); likewise two ribotypes of subclade 3a belong to var. *serpula*. Second, the single heterozygous EF1A genotype (e7) occurred in two clades (1 and 3b). Third, three specimens from the most diverse population (Costa Rica) displayed heterogenous SSU sequences, representing apparent crosses

between representatives of different clades (1 x 3a, 1 x 3b, 3b x 3b). Such heterogeneous SSU sequences are rare since the crosses between two ribotypes are usually homogenized by gradual elimination of one ribotype during plasmodium formation (Ferris et al. 1983), but were found as well in *Trichia varia* (Feng & Schnittler 2015).

These inconsistencies may be explained best by recent dispersal events and subsequent hybridization between amoebae belonging to usually separated clades. As indicated by the geographic origin of ribotypes from different clades and by the results of a Mantel test, geographic separation within ribotypes of *H. serpula* occurs but is incomplete. This is corroborated by the AMOVA (Table 1) results for the three investigated regions (temperate zones, Neo-, and Paleotropics) which indicated higher variation within a region than among the regions. We thus hypothesize that we are witnessing ongoing, yet incomplete speciation. This may or may not lead to completely isolated species within *H. serpula*; and from this reason we propose to treat the two morphologically separated entities (var. *serpula* and var. *parviverrucospora*) as varieties, not as species. This would concur with the moderate endemicity model (Foisner 1999, 2006). A similar argument, assuming ongoing speciation, was put forward for a worldwide study using SSU genes in the planktonic foraminiferan *Globigerinella* sp., where incomplete separation of cryptic lineages was noted (Weiner et al. 2014). If our interpretation is valid, this would be the first evidence of ongoing speciation for myxomycetes.

The ancestral area reconstruction postulated *H. serpula* to originate in the Neotropic region. Several phylogeographic studies propose as well Neotropical origins for protists, such as *Leptomonas pyrrhocris* (Trypanosomatidae, Votycka et al. 2012), or *Badhamia melanospora* (Myxomycetes, Aguilar et al. 2014).

Geographic patterns or ecological constraints?

The few available studies about geographic separation in myxomycete populations using molecular markers showed mixed patterns. No geographic separation at all was observed in populations of the dark-spored species *Lamproderma columbinum* in a limited sampling area (<50 km diameter) in deep ravines of Saxony, Germany (Fiore-Donno et al. 2011), or even in a worldwide study using mtDNA for *Didymium difforme* (Winsett & Stephenson 2011), although the marker resolution may not be sufficient to reveal differentiation in the latter case. A study on the bright-spored myxomycete *Trichia varia* showed weak evidence for geographic separation of three reproductively isolated biospecies collected across Eurasia (Feng & Schnittler 2015); one of these occurred much more common in a forest pocket in Northeastern Germany (Feng & Schnittler 2016).

In our study, the correlation between genetic and geographic distance matrices (0.4667) indicated that geographical differentiation explains at least partly genetic variation in *Hemitrichia serpula*. For a widely distributed morphospecies like *H. serpula* geographic restriction may potentially lead to speciation, if mutations in mating-type genes governing amoebal compatibility become widespread in a regional population and cut it off from gene flow (see discussion in Feng et al. 2016). Such mutations should facilitate genotypic divergence, but with Kamono et al. (2009) we must still assume a limited amount of gene flow between continents, mediated by long-distance dispersal. Apparently, some regions can serve as transitional zones. A good example is the temperate broadleaved forest of the Russian Far East, harboring ribotypes from clades 1 and 2. In accordance, the environmental niche models showed as well a niche overlap between clades 1 and 2 in this region (Fig. 6).

The niche models clearly showed that all three major clades overlap in the tropical ecozone. However, the putative biospecies represented by these clades may have different ecological requirements, with ribotypes from clade 3 showing a more restricted distribution in the pantropics in comparison to ribotypes in clades 1 and 2, having a broader distribution range. Furthermore, the models suggested that under a changing environmental scenario (Fig. S1), ribotypes from clade 3 have an exclusive environmental niche only in the pantropical areas. Patterns like these of ribotypes in clade 3 (which showed 12 ribotypes exclusively occurring in Costa Rica and three ribotypes recorded only from Vietnam) and the fact that one ribotype from Clade 1 was found once in Poland and many times in Far East of Russia would be better explained by dispersal limitations than environmental constraints. Nevertheless, it is important to note that these models only generate a probability map for the likeliness of occurrence of the ribotypes in each clade based on our available data and thus should not be seen as a truly predictive approach. As usual for myxomycetes, we still lack information regarding the actual occurrence of *H. serpula* in many parts of the world (but see www.discoverlife.org). Similarly, Aguilar et al. (2014) assumed a weak dispersal scenario explaining the niche models for two ribotype groups among the *Badhamia* population (although in this case possible reproductive isolation could not be demonstrated, as only one marker was used).

Conclusions

This study demonstrates intraspecific variation in the morphologically clear-cut cosmopolitan myxomycete species *Hemitrichia serpula*. Distribution of ribotypes suggests limited gene flow between continents, which may lead to allopatric speciation caused by geographic barriers. The EF1A gene as a second marker indicates reproductive isolation between groups of ribotypes, which may be caused by incompatibilities in mating type genes, as demonstrated for Physarales in early cultivation experiments (Clark & Haskins 2013). This would enable sympatric speciation processes. In our case, we found additional morphological evidence for speciation within *H. serpula*: the two most common varieties (var. *parviverrucospora* and var. *serpula*) seem to

belong to different putative biospecies, whereas two rare varieties (var. *piauensis* and var. *tubiglabra*) are likely to represent maldeveloped specimens. However, heterogeneous (SSU) and heterozygous (EF1A) sequences together with incomplete separation of morphotypes among major clades indicates speciation to be incomplete.

What do these data tell about myxomycete biogeography? Myxomycetes are ubiquitous in the sense that they are able to reach every suitable habitat on earth, at least considering a geological time scale. Consequently, many morphospecies show wide, sometimes cosmopolitan, but local distribution patterns. Following this line of thought, the “everything is everywhere” hypothesis for protist biogeography is met, at least if the limitation “but the environment selects” is regarded as well. However, with a more differentiating species concept (biospecies) that looks deeper into the molecular diversity of a widely distributed species, it becomes apparent that the gene flow mediated by possible long-distance dispersal of spores, which enables myxomycetes to fill out their whole potential distribution range, is not strong enough to prevent differentiation in regional gene pools, which may be the first step towards speciation.

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AUTHORS' CONTRIBUTIONS

M.S. and N.H.A.D. conceptualized and designed the present study. Y.K.N. and G.H.M. provided specimens of *H. serpula* from their herbarium collections. SEM micrographs of all the specimens were independently executed by Y.K.N., G.H.M. and R.S. Molecular experiments and analyses were carried out by N.H.A.D. Environmental niche modeling was performed by C.R. Interpretation of results and manuscript preparation was done by N.H.A.D. and M.S. with the approval of all coauthors

Table and Figures

Table 1. Analysis of Molecular Variance generated from Arlequin comparing each population among regions and among each clades

<i>Regions (Temperate zone vs. Paleotropics vs. Neotropics)</i>				
Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Among regions	2	3833.826	44.96	37.79
Within region	126	9326.64	74.02	62.21
Total	128	13160.47	119.78	
F _{ST} Fixation Index	0.38			
<i>Clades (Clade 1 vs. Clade 2 vs. Clade 3)</i>				
Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Among clades	2	7109.5	85.19	63.95
Within clade	126	6050.96	48.02	36.05
Total	128	13160.96	133.11	
F _{ST} Fixation Index	0.64			

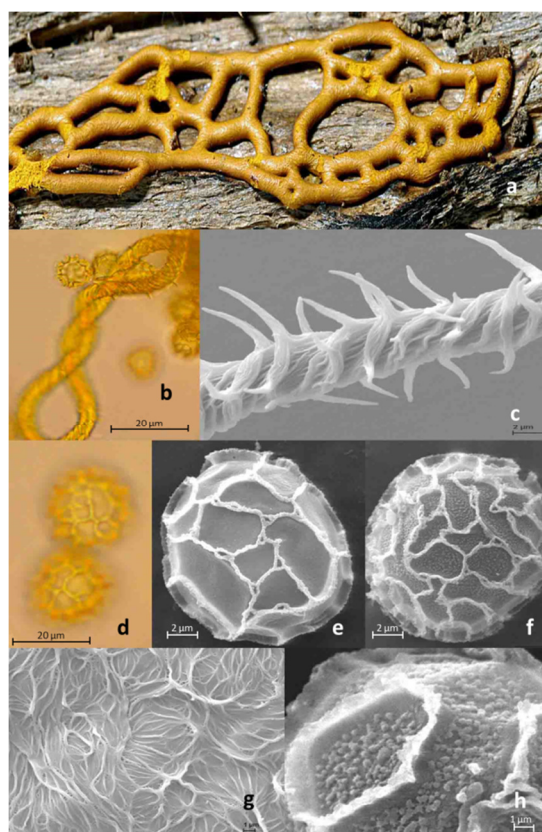


Fig1 *Hemitrichia serpula*: **a.** mature fruiting body, **b-c.** capillitium of var. *serpula* (specimen M754) as seen in (b) light microscope and (c) scanning electron microscope (SEM), **d.** spore morphology of var. *serpula*, **e-f.** SEM micrograph of spores for (e) var. *serpula* (LE297865) and (f) var. *parviverrucospora* (sc28101), **g.** SEM of the internal linings of the peridium, var. *parviverrucospora*, **h.** close-up, showing the internal warts between the reticulations of var. *parviverrucospora* (sc28065).

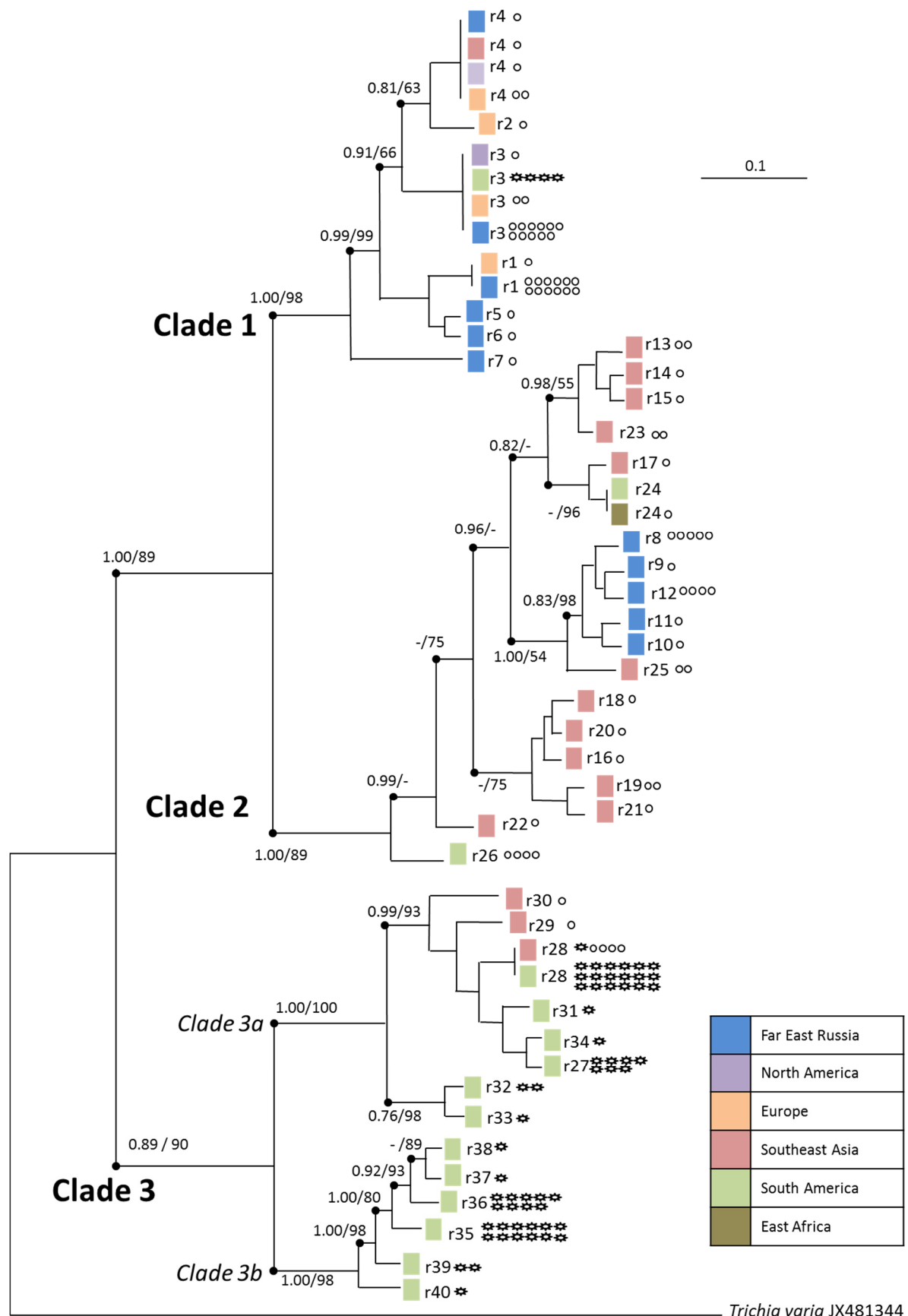


Fig2 Rooted consensus tree based on the 50% majority rule of Bayesian interference for partial SSU sequences for 40 ribotypes from 135 specimens of *Hemitrichia serpula*. Shown are Bayesian posterior probabilities >0.70 and support values >50 for a corresponding tree calculated with RAXML (all branches indicated by a dot). Colored squares indicate the origin of the specimens. The ribotype number and the morphology of the respective specimens are indicated by smooth (var. *serpula*) and spiny circles (var. *parviverrucospora*). Scale bars represent evolutionary distance as changes per site.

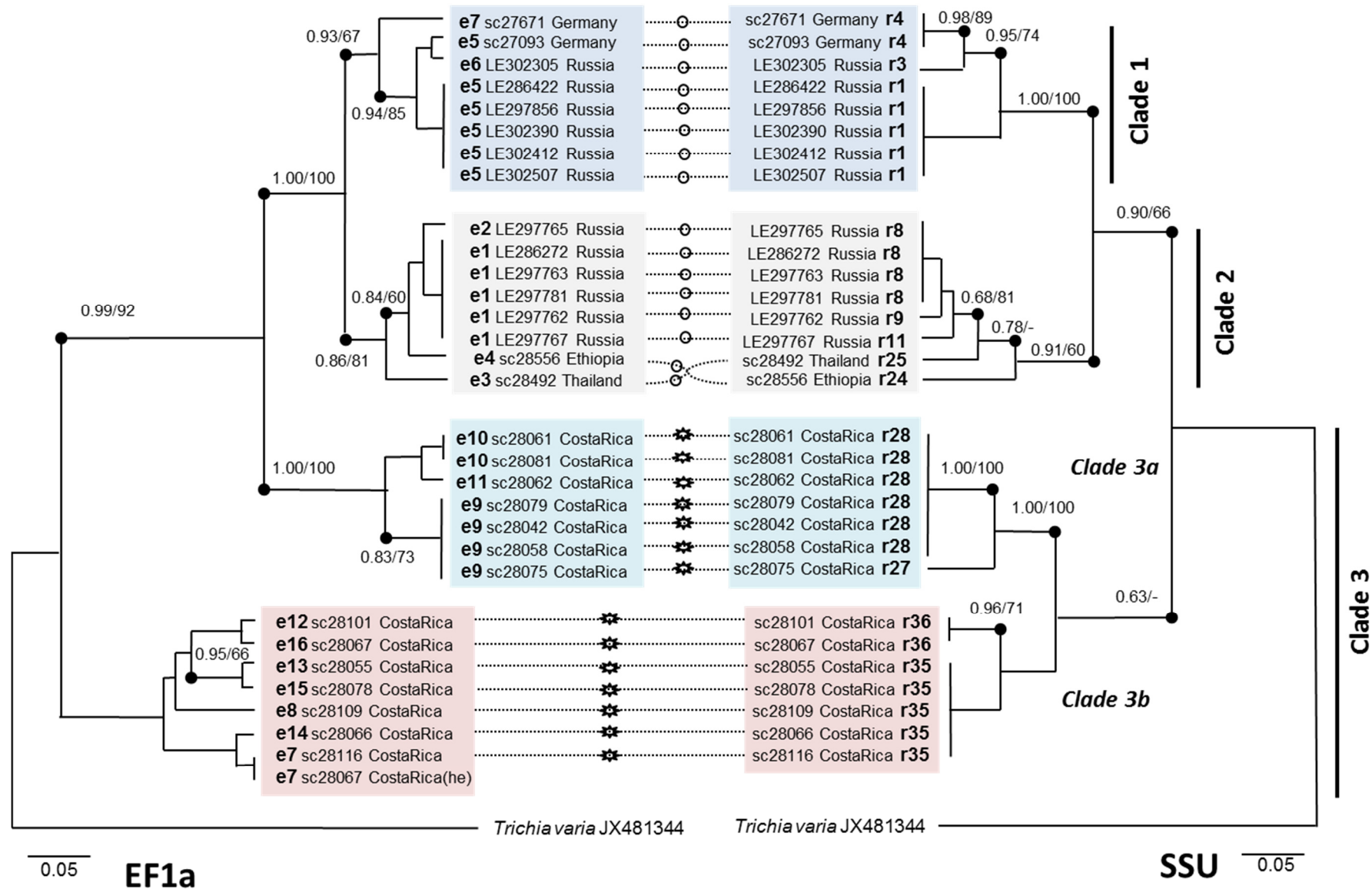


Fig3 Phylogenetic analysis showing a mirrored image comparing tree topologies for partial SSU and EF1A sequences for 30 specimens. Bayesian posterior probabilities >0.70 and RAxML support values >50 are indicated (nodes with dots). Scale bars represent evolutionary distance as changes per site. Ribotype (r) and EF1A genotype numbers (e) are shown for both markers. Dotted lines connect sequences from the same specimen, with symbols for var. *serpula* (smooth circles) and var. *parviverrucospora* (spiny circles) in the middle.

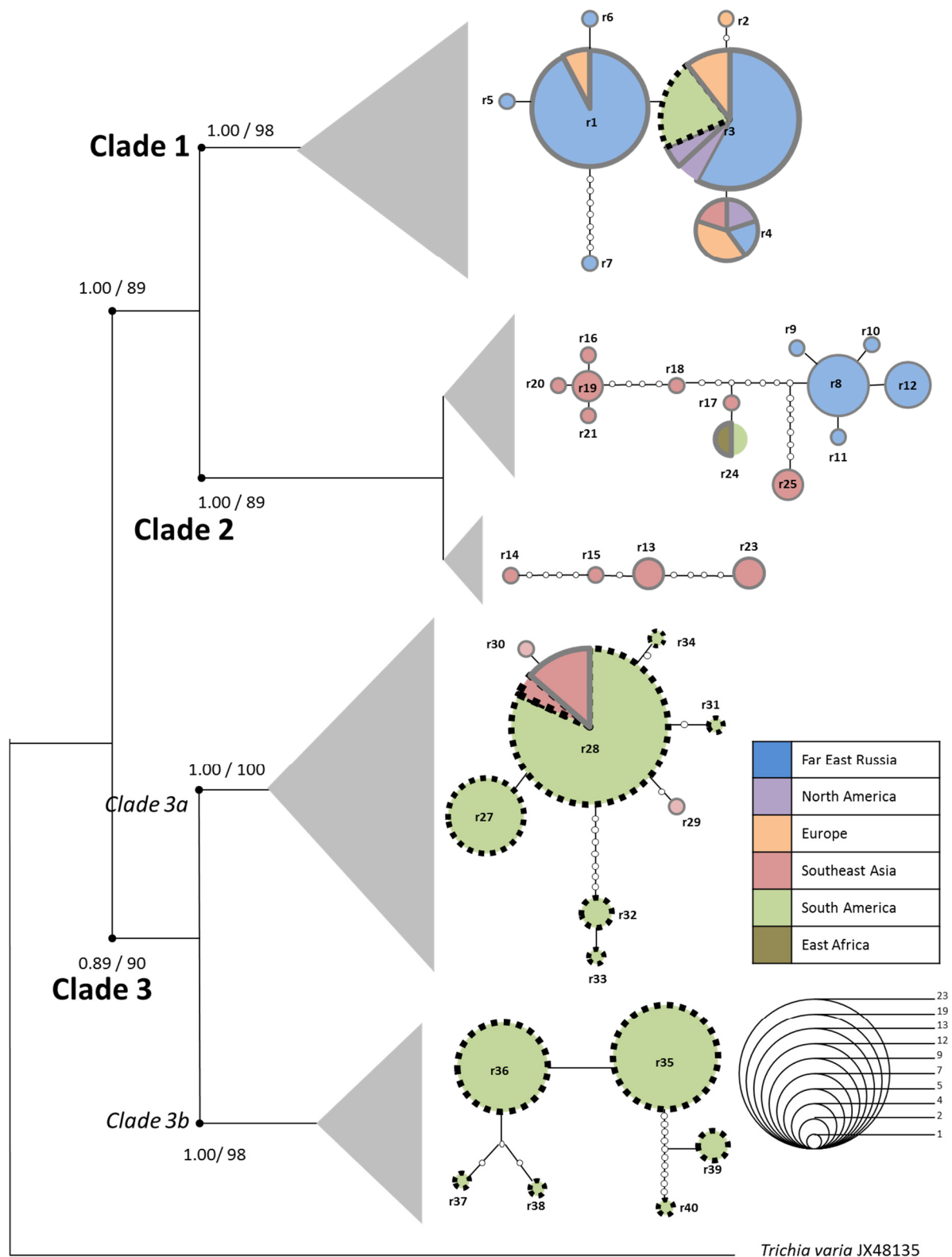


Fig4 Statistical parsimony ribotype network representing genealogical relationships among 40 ribotypes of the partial SSU estimated by TCS superimposed on a Bayesian interference tree. A grey triangle is sized relative to the number of specimens per network. Line segments represent mutational steps between alleles. Circles are scaled in proportion to the number of sequences represented by each ribotype. Small circles between ribotypes indicate hypothetical transitional ribotypes. Colors designate the origin of the specimen. Morphotypes displayed by specimens showing the respective ribotype are indicated by smooth (var. *serpula*) or broken lines (var. *parviverrucospora*).

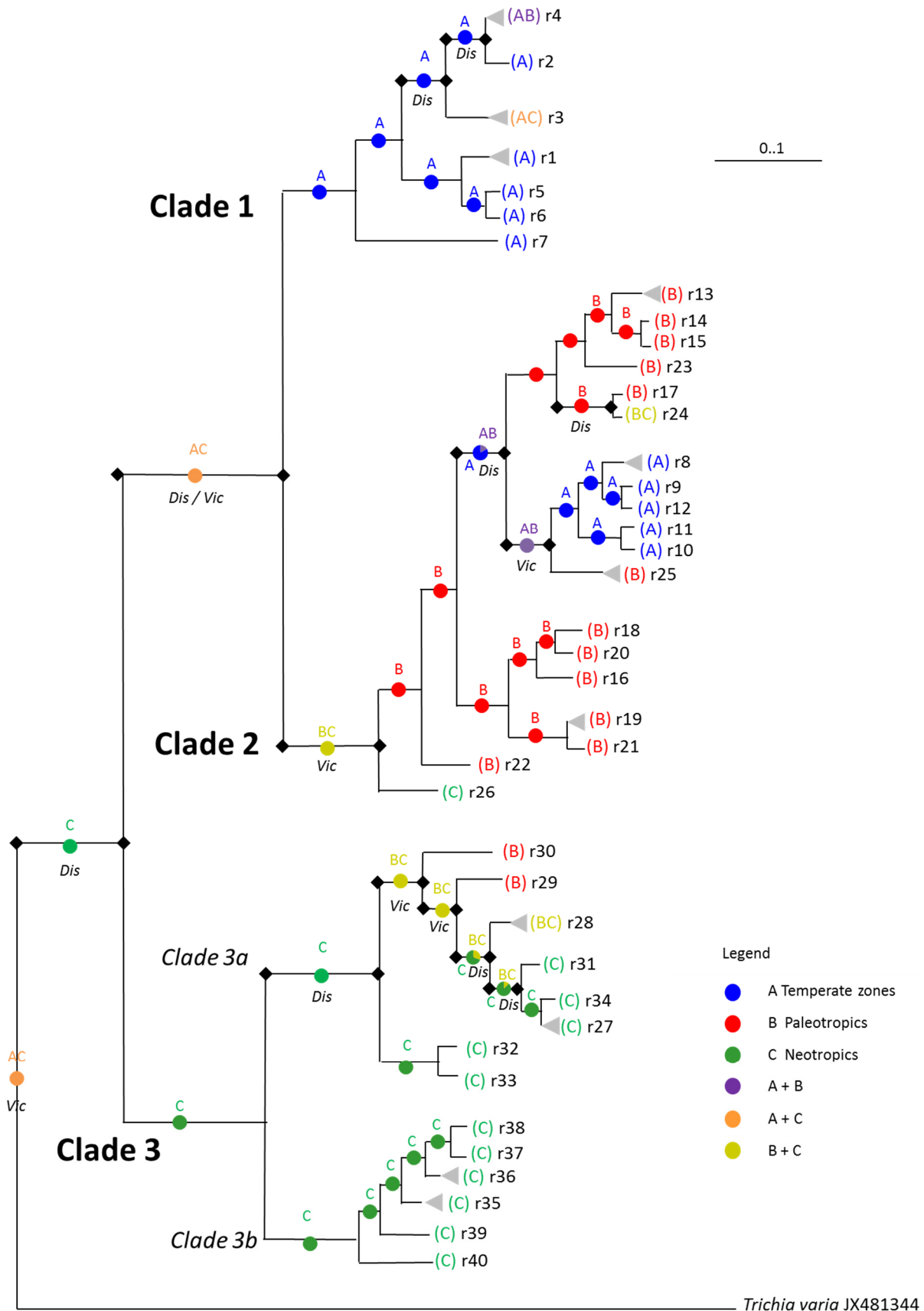


Fig5 Hypothesized event-based (Vic =vicariance; Dis= dispersal) ancestral area reconstruction of the *H. serpula* ribotypes as inferred by the S-DIVA analysis of RASP. Pie charts at the nodes give the relative frequencies of the ancestral-area reconstruction. Grey triangles indicate ribotypes represented by multiple specimens.

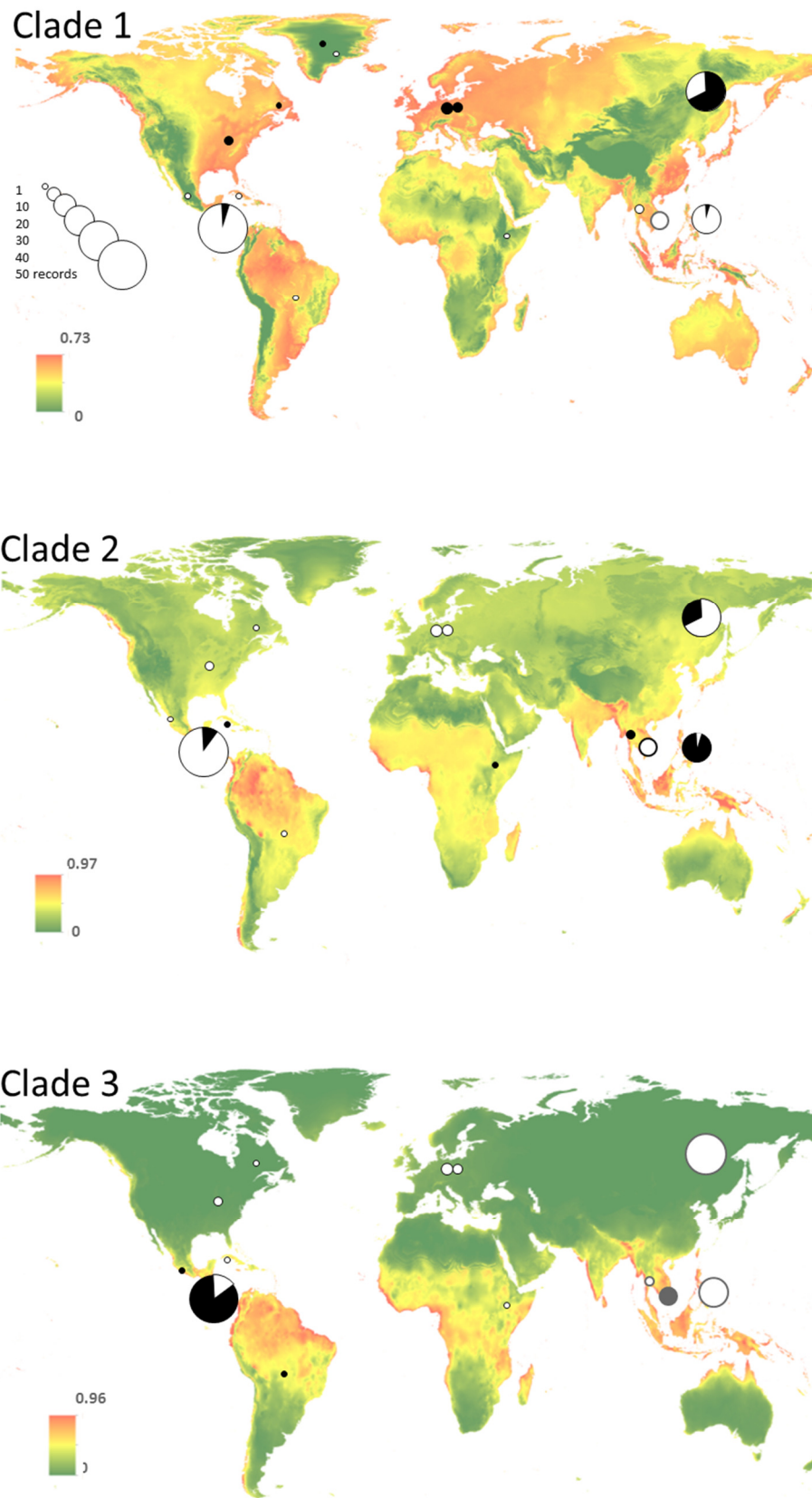


Fig6. Probability-based environmental niche models for the three major clades in a ribotype phylogeny of *Hemitrachia serpula* calculated with the MaxEnt algorithm. Circles are located over areas where specimens were collected; their size is scaled according to the number of specimens collected in an area. Black filling of the pie diagrams indicates proportion of specimens belonging to the respective clade. The underlying heat map shows the likelihood of occurrence for the respective clade.

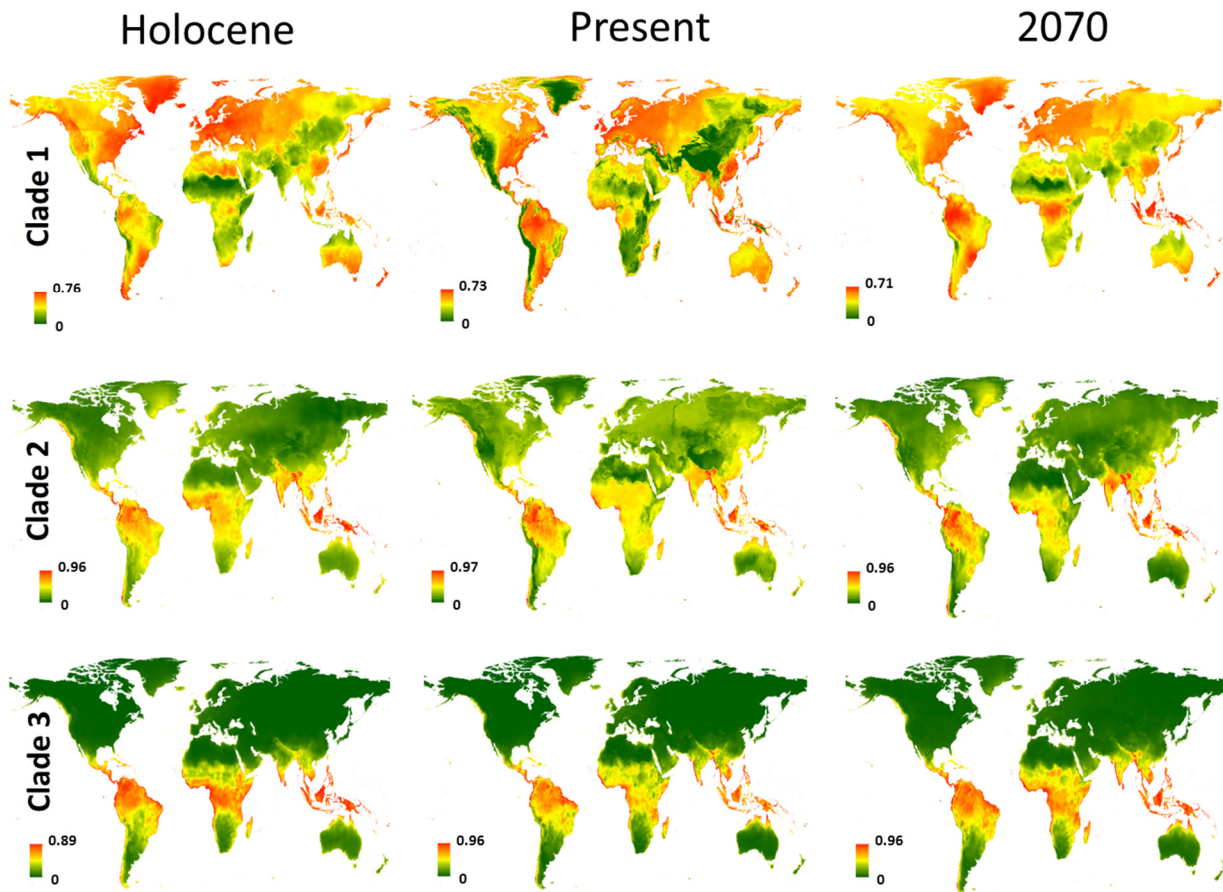


Fig. S1. Probability-based environmental niche models for the three clades of *Hemitrichia serpula* populations under different time scenarios calculated with the MaxEnt algorithm, based on climate models for the Holocene, present and the forecast (Hadley Centre Global Environmental Model, 4.5 scenarios) for 2070. The underlying heat map shows the likelihood of occurrence for the respective clade.

Supporting Information

Data S1(Microsoft Excel 2010). Database including collection number, geographic coordinates, SSU ribotype (for all 135 specimens), EF1 α genotype (for 30 selected specimens), and morphotype for the investigated accessions of *Hemitrichia serpula*.

Data S2 (fasta file). Alignment of partial SSU sequences for 135 specimens (making 138 sequences with three specimens being heterogeneous for SSU) used in this study.

Data S3 (fasta file). Alignment of partial EF1A sequences of 30 specimens (30 sequences) used in this study. Specimen sc28067 shows a heterozygous sequence.



CHAPTER 3.4

Other publications related to myxomycetes diversity

CONTENTS

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

JULIUS RAYNARD D. ALFARO, DONN LORENZ IVAN
M. ALCAYDE, JOEL B. AGBULOS, NIKKI HEHERSON A.
DAGAMAC*, THOMAS EDISON E. DELA CRUZ*
DEPARTMENT OF BIOLOGICAL SCIENCES,
COLLEGE OF SCIENCE, UNIVERSITY OF SANTO TOMAS,
ESPAÑA, MANILA, PHILIPPINES

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CORRESPONDING AUTHORS

*Nikki Heherson A. Dagamac
nhadagamac@gmail.com
Institut für Botanik und
Landschaftökologie
Ernst – Moritz – Arndt
Universität Greifswald,
Soldmannstrasse 15 D-17487
Greifswald, Germany

*Thomas Edison E. dela Cruz
tedelacruz@mn1.ust.edu.ph
Department of Biological
Sciences, College of Science,
and Fungal Biodiversity and
Systematics Group, Research
Center for the Natural and
Applied Sciences
University of Santo Tomas,
España 1015 Manila,
Philippines

KEYWORDS

- abundance
- amoeboid
- eukaryotes
- diversity
- fruiting
- body
- sclerotia

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 rain-forest 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 were glued in herbarium trays and placed inside matchbox-sized herbarium boxes for permanent storage.

MOIST CHAMBER PREPARATION 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. Eumycetozoa 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) = $-\sum_i (p_i \ln p_i)$, where p_i = the total number of individuals in the i th species.

Equation 2: Gleason Index (HG) = $N_p - 1/\ln N_i$, where N_p = the total number of species and N_i = the total number of individuals in the i th species.

Equation 3: Pielou's index of species evenness (E) $E = HS/H_{max}$ where HS = Shannon Index of Diversity and H_{max} = 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: $PS = \sum \min(A, B, \dots X)$
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,

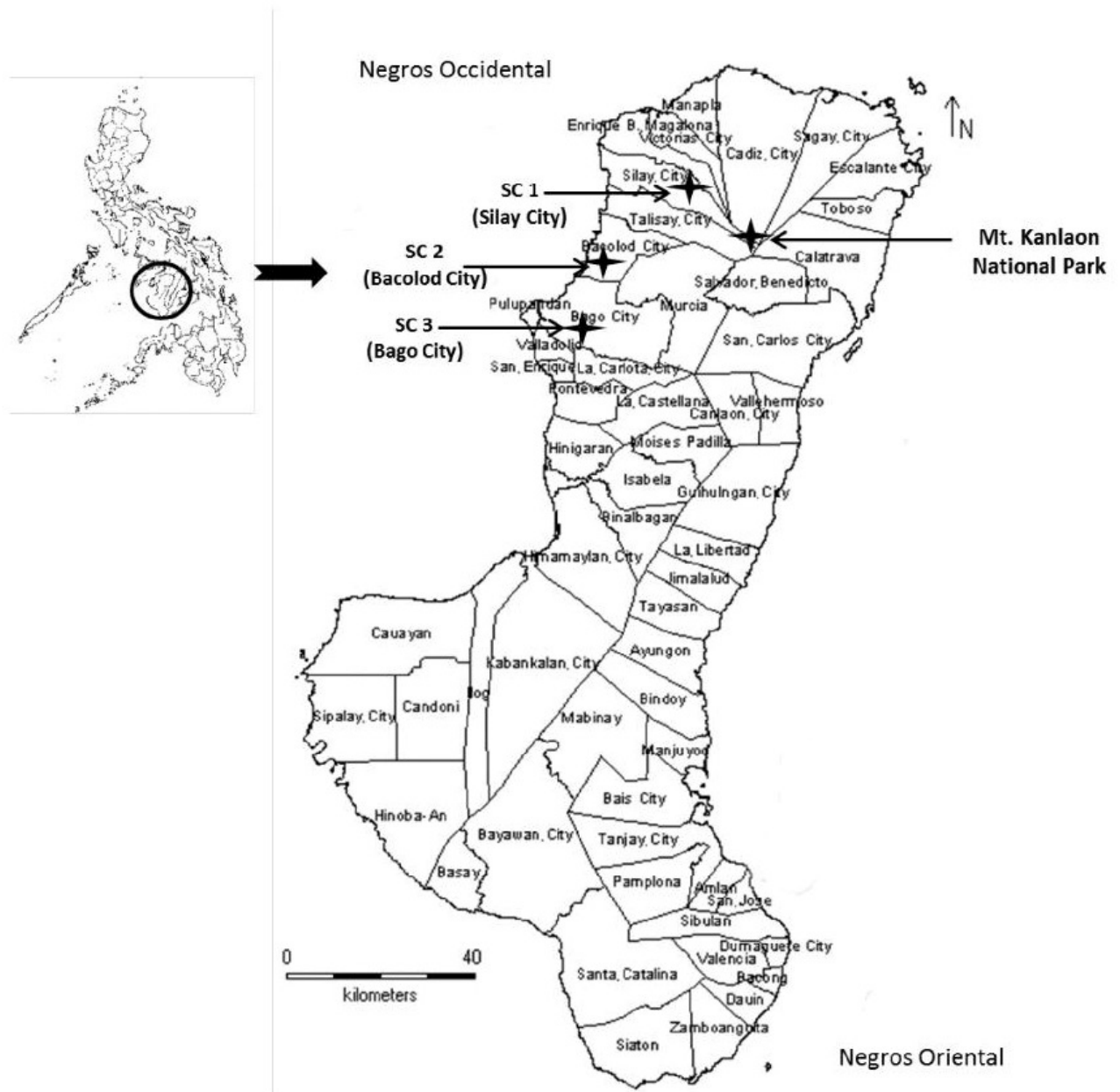


Fig. 1. Study sites: Mt. Kanlaon National Park (forested areas) and the agricultural plantation (SC) in Negros Occidental, Western Visayas, Philippines, May 2013.

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

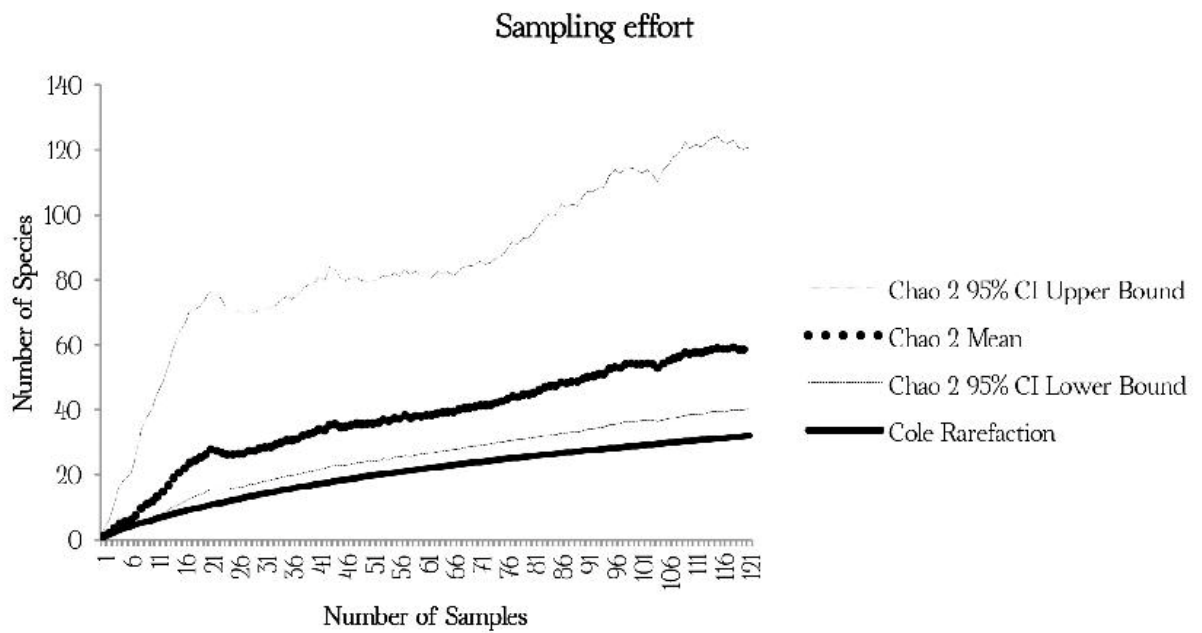


Fig. 2. Individual based species accumulation curve smoothed by Cole rarefaction for the myxomycetes collection in Negros Occidental.

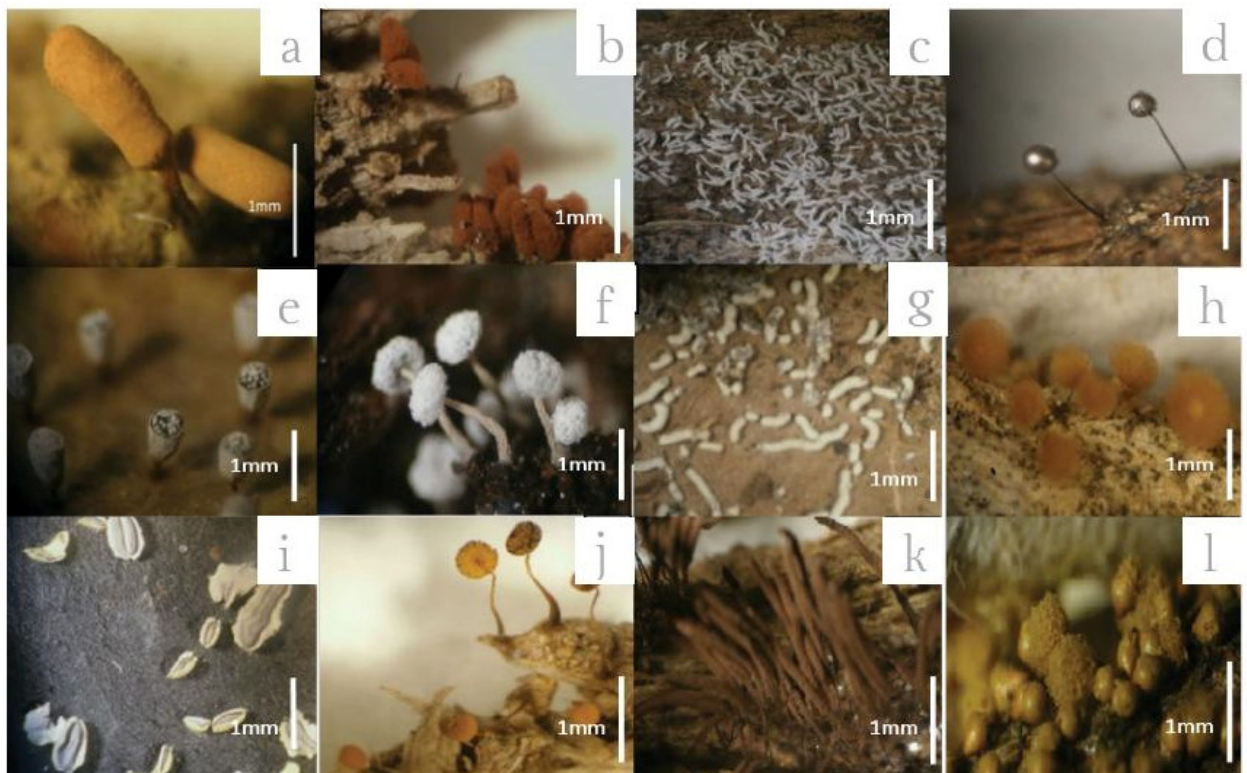


Fig. 3. Some representative myxomycetes collected in Negros Occidental: (a) *Arcyria cinerea*, (b) *Arcyria denudata*, (c) *Ceratiomyxa fruticulosa*, (d) *Collaria arcyrionema*, (e) *Craterium leucocephalum* var. *cylindricum*, (f) *Didymium squmolosum*, (g) *Diderma effusum*, (h) *Hemitricha calyculata*,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

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

Species	Number of Records							Total
	Field Collection	Moist Chamber Cultures						
		AL	GL	F	TW	VN	S	
Abundant								
<i>Arcyria cinerea</i> (Bulls.) Pers.	1	25	10	10	5		2	53
<i>Didymium nigripes</i> (Link) Fr.	4	2						6
Common								
<i>Arcyria denudata</i> (L.) Wetts.	4							4
<i>Ceratiomyxa fruticulosa</i> (Olf. Mull.) Macbr.	3				2			5
<i>Craterium leucophaeum</i> var. <i>cylindricum</i> (Pers ex J. F. Gmel)								
Ditmar	3							3
<i>Diderma effusum</i> (Schwein.) Morgan	2	2				1		5
<i>Hemitrichia calyculata</i> (Speg.) M.L. Farr.	4							4
<i>Lamproderma scintillans</i> (Berk& Broome.) Morgan.	1	1			2			4
<i>Physarum album</i> (Bull.) Chevall.	3	1						4
<i>Physarum viride</i> (Bull.) Pers.	2		2		1			5
<i>Stemonitis fusca</i> Roth	1	1			1			3
Occasional								
<i>Cribraria cancellata</i> (Batsch) Nann. – Bremck.	2							2
<i>Cribraria microcarpa</i> (Schrad) Pers.	2							2
<i>Didymium iridis</i> (Ditmar) Fr.	1	1						2
<i>Physarum melleum</i> (Berk. & Broome.) Massee.	2							2
Rare								
<i>Arcyria afroalpina</i> Rammeloo		1						1
<i>Collaria arcyronema</i> (Rostaf.) Nann. – Bremek. Ex Lado.					1			1
<i>Comatricha nigra</i> (Pers. Ex J. F. Gmel) J. Schroet	1							1
<i>Didymium squamosum</i> (Alb. & Schwein.) Fr.	1							1
<i>Hemitrichia serpula</i> (Scop.) Rostaf. Ex Lister.	1							1
<i>Physarum bogoriense</i> Racib.	1							1
<i>Physarum cinereum</i> (Batsch) Pers.						1		1
<i>Physarum compressum</i> Alb. & Schwein.	1							1
<i>Physarum echinosporum</i> Lister				1				1
<i>Physarum nucleatum</i> Rex	1							1
<i>Stemonitis axifera</i> (Bull.) T. Macbr.					1			1
<i>Trichia decepiens</i> (Pers.) T. Macbr.	1							1
<i>Tubifera ferruginosa</i> (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 ($H_s = 0.75$). 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
<u>Mountainous forest</u>								
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
<u>Agricultural plantation</u>								
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 fruiting 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 herbivorous 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 *Arctia cinerea* to be of cosmopolitan distribution worldwide, since it is widely known to be tolerant to many environments.

MYXOMYCETE DISTRIBUTION IN AGRICULTURAL LITTER 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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Comparative diversity and heavy metal biosorption of myxomycetes from forest patches on ultramafic and volcanic soils

M.A.D. Rea-Maminta^{a,b,c,*} , N.H.A. Dagamac^d , F.Z. Huyop^e, R.A. Wahab^f  and T.E.E. dela Cruz^{a,b,c}

^aThe Graduate School, University of Santo Tomas, Manila, Philippines; ^bDepartment of Biological Sciences, College of Science, University of Santo Tomas, Manila, Philippines; ^cFungal Biodiversity and Systematics Group, Research Center for the Natural and Applied Sciences, University of Santo Tomas, Manila, Philippines; ^dInstitute of Botany and Landscape Ecology, Ernst-Moritz-Arndt Universität Greifswald, Greifswald, Germany; ^eDepartment of Biotechnology and Medical Engineering, Faculty of Biosciences and Medical Engineering, Johor Bahru, Malaysia; ^fDepartment of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, Johor Bahru, Malaysia

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Ultramafic and volcanic soils are exploited for industrial activities such as mining due to their high metal content, thus it is important that species in these areas are documented before irreversible environmental damage sets in. In this study, aerial and ground leaf litter, dead vines and twigs from six forest patches on volcanic and ultramafic soils in the provinces of Bataan, Pangasinan and Zambales in the Philippines were cultured in moist chambers (MC) and assessed for myxomycete diversity. From the 77% positive MC for myxomycetes, a total of 40 species from 14 genera were identified. Despite the higher heavy metal content, forest patches on ultramafic soils had greater species diversity as compared to volcanic soils. In this study, 10 species were abundant in both forest patches, namely *Arcyria cinerea*, *Diachea leucopodia*, *Didymium effusum*, *D. hemisphaericum*, *Didymium ochroideum*, *Perichaena chrysosperma*, *P. corticalis*, *P. depressa*, *P. dictyonema* and *Physarum melleum*. Selected myxomycetes tested for Cr and Mn content had equal or higher heavy metal levels than that of their leaf substrate. The study hypothesised that the presence of Mn⁷⁺ in fruiting bodies of myxomycetes was due to the phagocytosis of food bacteria inhabiting the substrates on the forest soil laden with heavy metal.

Keywords: hexavalent chromium; manganese; mining; Philippines; slime molds; species diversity

1. Introduction

The Philippines, as a biodiversity hotspot, is endowed with a pristine environment and mineral-rich resources.[1] Mineral deposits found in volcanic (VC) and ultramafic (UM) soils of the Philippines produces a wide array of metallic minerals such as Cr, Cu, Fe, Au, Ni, Ag and Zn which are available as export commodities.[2] Thus, mining is classified as a high-profit business in the Philippines. However, this anthropogenic activity has caused alarming destruction by creating permanent damage to habitat leading to the loss of local fauna and

*Corresponding author. Email: mangelicare@gmail.com

flora [3,4,5] [3–5] as well as liberation of surplus heavy metal into the environment that affects microbial diversity and its role in nutrient recycling.[6,7] Nonetheless, tolerant plant species, such as *Pearsonia metalifera*, *Dicoma nicollifera* and *Alyssum murale*, and some microorganisms are still capable of growing in these metal-rich soils.[8,9] Previous study suggested utilising microorganisms such as bacteria and fungi in substrates with high heavy metal content [10–13] such as those found in mined-out areas. However, the use of both bacterial and fungal species as part of a microbial consortia results to a competitive interaction that leads to a predominance of bacteria in the cultures.[14–16] In myxomycetes, bacteria and fungi serve as food, however, later in the life cycle of myxomycetes, the engulfed bacteria or fungi establishes mutualism with myxomycetes.[17,18] Despite the research on microorganisms such as bacteria and fungi in clean-up technologies, very few have focused on the potential of protist [19] such as myxomycetes in the biosorption of heavy metals. Since myxomycetes ‘feed’ on bacteria and fungi by phagocytosis [20] and then later on establish a mutualistic interaction with the microorganisms,[18] clean-up technologies may utilise myxomycetes grown vis-à-vis bacterial and fungal cultures to easily harvest contaminants from the soils. Heavy metals such as Al, Ca, Cu, Fe, Hg and Zn were previously detected in the fruiting bodies of myxomycetes, however, no follow-up study was conducted in subsequent years.[21–24]

Since ecosystem reconstruction in post-mining sites uses metal-rich soil to cover a mined-out area,[25] myxomycetes could be used to take in heavy metals from this environment and their ability for biosorption of heavy metals as a single organism or as a member of microbial consortia could possibly be harnessed. At present, only plants are introduced on UM mined-out areas for the absorption of heavy metal, and are used for ecosystem reconstruction.[9] Plasmodial slime molds could possibly be used as an agent to absorb the heavy metal and then followed by extraction or disposal of the heavy metal to remove unwanted pollutants left by mining operations.[13,26] Hence, the study described here aims to (i) assess the ability of myxomycetes to absorb heavy metals in fruiting body and plasmodium state and (ii) compare the diversity and occurrence of these species in forest patches on UM and VC soils from the Philippines.

2. Materials and methods

2.1. Study sites

Six different sites within the Central Luzon, Philippines, were randomly chosen for our study (Table 1). These sites were either rehabilitated or disturbed forest patches located on VC and UM soils as indicated in the 2012 Mineral Map of the Philippines’ Mines and Geosciences Bureau (Figure 1).

2.2. Collection and test of soil samples for heavy metals

Approximately 10–15 g of soil per sampling point was collected at a depth of 0–15 cm, placed in labelled plastic bags and air-dried for seven days prior to detection of heavy metal. Air-dried soil samples were sieved in a 2 mm mesh to remove rocks, plant particles and other large materials. Soil samples taken near each other within a study site were pooled together and digested using wet ashing protocol published by Tüzén [27] prior to analysis in atomic absorption spectroscopy (AAS, Shimadzu AAS-7000, Kyoto, Japan). Analysis of metals, namely Cu, Fe, Mn and Zn was performed at the Bureau of Soils in Diliman, Quezon City, and Cr analysis was carried out at the Mines and Geosciences Bureau in Quezon City, Philippines. Soil pH was also measured using a pH meter (Hannah I98107), calibrated using Colourkey buffer solutions at pH 4, 7 and 10, respectively. (Table 1)

Table 1. Collection localities of the forest patches on ultramafic and volcanic soils of Central Luzon, Philippines.

Site ^a	pH	Metal concentration (mg L ⁻¹)	Coordinates	Habitat type
[VC1] Bataan Business and Leisure Park, Morong Bataan	4.4–6.0	Cr: 0.0036 Cu 7.87 Fe 47.06 Mn 56.5 Zn 1.51	N14°42'47.8" E120°17'20.2"; 98 metres above sea level (masl); ca. 365 ha	Volcanic soil with a wide variety of dipterocarp trees, some species of <i>Artocarpus heterophylla</i> , <i>Mangifera indica</i> L. and low-lying shrubs
[VC2] Pamulaklakin Forest Trail, Subic, Zambales	4.7–6.3	Cr 0.0022 Cu 11.49 Fe 90.7 Mn 18.56 Zn 6.98	N14°48'14.2" E120°19'48.8"; 67 masl; ca. 57 ha	Another volcanic soil with a dense lowland tropical rainforest dominated by dipterocarps
[UM1] Cabangan, Zambales	5.9–7.1	Cr 0.093 Cu 7.84 Fe 61.05 Mn 50.61 Zn 4.55	N15°08'48.7" E120°03'08.1"; 22 masl; ca. 15 ha	A road-side forested area in ultramafic soil dominated by <i>Tamarindus indica</i> L. and few patches of herbaceous and woody shrubs
[UM2] Barangay Mambog in Iba, Zambales	5.7–6.9	Cr 1.05 Cu 12.25 Fe 131.32 Mn 111.16 Zn 3.93	N15°20'13.2" E120°00'38.8"; 41 masl; 10 ha	An ultramafic forest site dominated by <i>Artocarpus heterophylla</i> , <i>Mangifera indica</i> L. and <i>Nauclea orientalis</i> L. which also has an active quarrying operation
[UM3] Candelaria-Masinloc	5.8–6.8	Cr 0.33 Cu 0 Fe 0 Mn 10.62 Zn 0	N15°33'55.9" E119°58'45.6"; 45 masl; ca. 900 ha	An ultramafic forest area with dipterocarp trees and shrubs near an active mining area
[UM4] Mantelug Spring Protected Landscape, Pangasinan	5.7–6.9	Cr 0.68 Cu 8.56 Fe 62.8 Mn 28.81 Zn 2.13	N15°42'14.0" E120°16'54.2"; 237 masl; ca. 1941 ha	An ultramafic forested area dominated by <i>Lagerstroemia speciosa</i> L., <i>Vitex parviflora</i> Juss. and <i>Leucaena leucocephala</i> Lam.

Source: Data from Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA).

^aSites have type I climate, i.e. wet from May to October; temperature range of 22–27°C; rainfall range of 56.0–136.9 mm. Sampling done during the wet season (May–June).

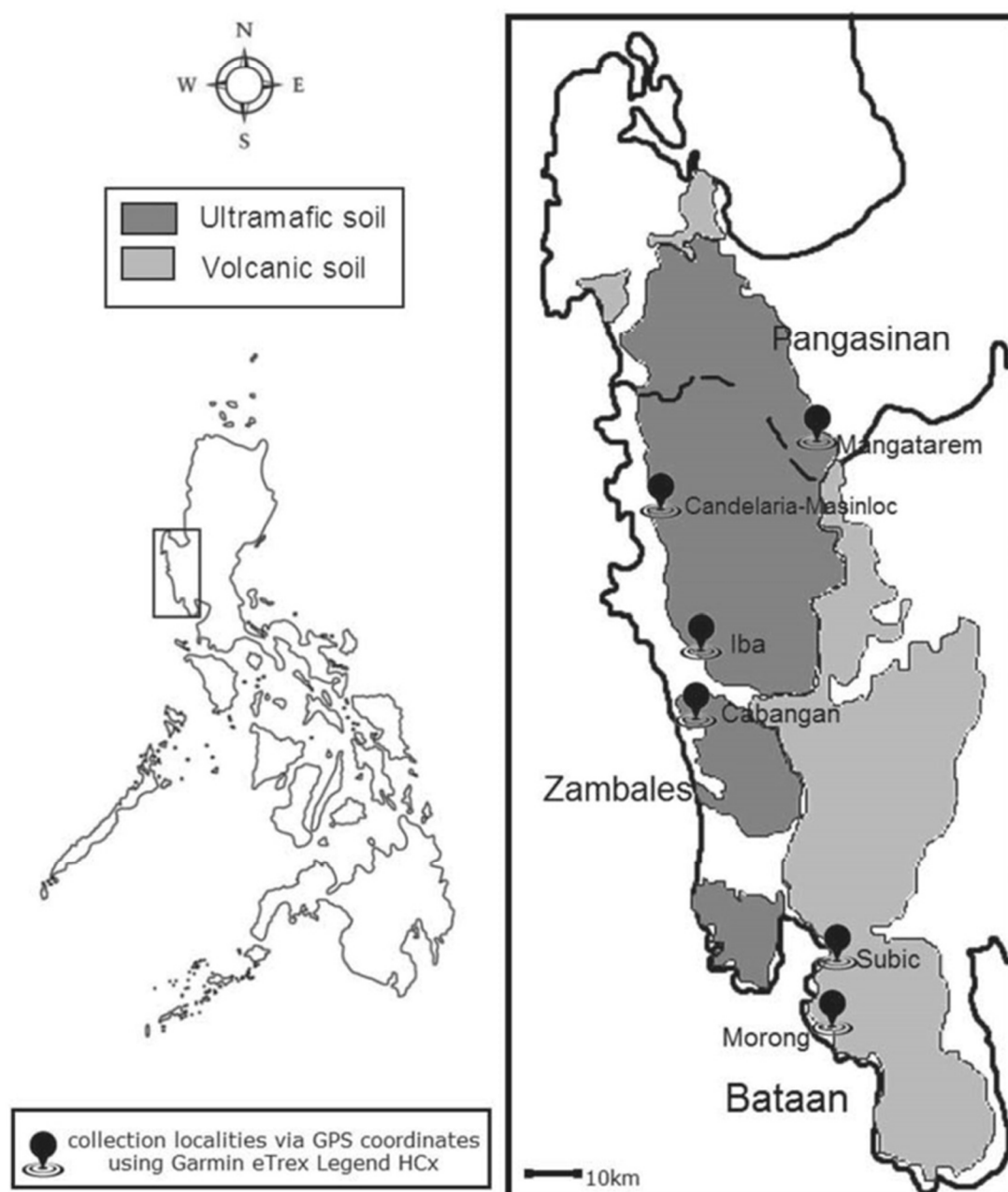


Figure 1. Site map of the forest patches on ultramafic and volcanic soils of Bataan, Zambales and Pangasinan, Philippines.

2.3. Collection and preparation of moist chambers and identification of myxomycetes

Aerial (AL) and ground (GL) leaf litter, dead twigs (TW) and woody vines (VN) were randomly collected every 200 metres within the forest patches of the six study sites in June 2013. Thirty samples each of AL, GL, TW and 10 samples of VN were placed in individual brown paper bags, air-dried and set up in moist chambers (MCs) following the protocol of Stephenson and Stempen.[28] A total of 100 substrates were collected in each forest patch and prepared one MC per sample to avoid pseudo-replication. Air-dried TW and VN samples were cut into 10–15 cm fragments and leaf litter (aerial and ground) was cut into postage stamp-sized pieces. These were then placed on standard petri dishes lined with clean filter paper. Next, distilled

water was poured into the MC and the substrate was soaked overnight. Then the pH of the substrates was checked with a pH meter and the excess water was drained. All MC were maintained under diffuse light at room temperature (22–25°C) for up to 12 weeks. The MC were checked regularly for the growth of plasmodia and/or fruiting bodies. Mature fruiting bodies in MC were immediately transferred and glued into herbarium boxes. Voucher specimens of the myxomycete collections were deposited at the Pure and Applied Microbiology Laboratory, Research Centre for the Natural and Applied Sciences, University of Santo Tomas in Manila, Philippines.

To characterise and determine the myxomycetes found in MC set-ups, fruiting bodies and spore morphologies of collected specimens of myxomycetes were observed under a dissecting (Olympus SZ61) and a light-compound (Olympus CX21) microscope. The following characteristics were documented: type, size, shape and colour of fruiting bodies, and internal structures such as capillitium and columella, appearance of the stalk, presence of lime, and the size shape and appearance of spores. Identification of the species was done using published literature and web-based identification keys (e.g. SYNKey [29] and the Eumycetozoa Project [30]). Valid names were based on the online nomenclatural information database for eumycetozoans.[31]

2.4. Ecological analysis

The per cent yield of the MC in each habitat type and substrate type were computed. The detailed procedure has been published by Dagamac et al.[32] The species accumulation curve (SAC) was also generated, using the web-based, free software EstimateS 9.0 [33] to assess adequacy of the sampling effort to represent the diversity of the UM and VC forest patches. The species–sample curve was used rather than the species–area curve, since myxomycete diversity and distribution was based on the microhabitat present rather than the area that was covered. The taxonomic diversity (S/G ratio) was then computed.[34] Relative abundance (RA) was also determined for each species by dividing the total number of collections for specific myxomycetes by the total collections. Here, an MC positive for a particular species of myxomycete is considered as one positive collection for that species. The RA was then assigned as abundant (A), common (C), occasionally occurring (O) and rare (R) as previously discussed in detail by Stephenson et al.[34] Based on the RA, species diversity was calculated using the Shannon Index of Diversity (H_S), Gleason Index of Species Richness (H_G) and Pielou's Index of Species Evenness (E). Further details about these theoretical constants is discussed by Stephenson.[35] To test for the similarities of communities of myxomycetes in the habitat type and the substrates, Sorensen's coefficient of community (CC) and the Percentage similarity (PS) indices were calculated.[35] A modified *t*-test was computed to test for significant differences between the different species diversity values.[36] A CC value close to one indicates that both communities have the presence of all species of myxomycetes and zero indicates that no species are present in either community to be compared. The PS index was then computed based on the relative abundance as well as their presence, for each collection site and substrate type. A higher PS value indicates that the communities were more similar in terms of composition and abundance.

2.5. Assay for biosorption of manganese and chromium by myxomycetes

Initially, spores from the fruiting bodies of abundant or commonly occurring species, i.e. *Arcyria cinerea*, *Diderma effusum*, *D. hemisphaericum*, *Didymium ochroideum*, *Physarum album*, *P. cinereum* and *P. melleum*, were separately mixed in sterilised distilled water to reconstitute the spores and then spread plated on water agar (WA) (15 g L⁻¹ agar). The plates were incubated in the dark at room temperature (27–30°C) and observed for spore germination and the presence of amoeboid or flagellated cells, and eventually for plasmodial growth. Sterile ground oat flakes

were occasionally placed near the plasmodium of these myxomycetes as a food source. Routine checks were done every two days for up to 14 days. Only *Physarum album*, *P. cinereum* and *P. melleum* exhibited plasmodial growth on WA, and hence were used in the succeeding experiments. The agar dilution method was used to test the tolerance of these three *Physarum* species to Cr (added as $K_2Cr_2O_7$) and Mn (added as $KMnO_4$) with concentration ranging from 3 mg L⁻¹ to 1000 mg L⁻¹. Since Cr⁶⁺ and Mn⁷⁺ are both highly toxic to various organisms, plasmodial growth on agar supplemented with these heavy metals would indicate that some species of *Physarum* can tolerate these toxic metals. Plasmodial agar blocks cut from WA cultures were placed at the centre of heavy metal plates and allowed to grow in the dark at room temperature for seven days.

2.6. Test for Cr and Mn content of myxomycetes and leaf litter

Following incubation, the plasmodia (0.6 g) were placed in clean, dry 1.5 mL microcentrifuge tubes and allowed to dry. Similarly, the fruiting bodies (0.6 g) of *Arcyria cinerea* and *Physarum album* + *P. pusillum* (pooled together) obtained from MC and the substrate (ground leaf litter) where the species were growing were also placed in clean, dry 1.5 mL microcentrifuge tubes. The Mn and Cr content of the leaf litter substrate and fruiting bodies were measured for later comparison and as a reference point of bioabsorption of metal. All samples (0.01 g) were individually soaked overnight in concentrated HNO₃ to prepare the samples for inductive coupled plasma-mass spectroscopy (ICP-MS, Perkin Elmer ELAN 6100, USA). The homogenates were then subjected to ICP-MS detection of Cr and Mn. The bioconcentration factor (BCF) was computed based on the published journal on plant systems,[37] with a slight modification to refer to the ratio of the metal concentration in myxomycetes fruiting bodies FB_m relative to metal concentration where the myxomycete was grown Sb_m whereby BCF was expressed as FB_m/Sb_m . The BCF from soil to leaf litter was also computed in this study. A higher BCF indicates higher heavy metal levels were obtained from the source. However, the translocation factor was not computed since the fruiting body was not separated into stalk and sporotheca.

3. Results

3.1. Per cent yield of the MCs

From a total of 600 MCs prepared, 77% were positive for myxomycetes (Table 2). Forest patches on UM soil had higher MC productivity (78%) than on VC soil (76%). Among the substrates used, AL had the highest per cent yield of myxomycetes (89%). The pH was also measured from UM soil (pH 5.7–7.1) which exhibited a characteristic near neutral pH. On the contrary, VC soils (pH 4.8–6.3) were found to be with slightly acidic pH in concurrence to a previous study by Proctor.[38]

3.2. Occurrence, diversity and community analysis of myxomycete assemblages

From 519 collections, 40 species of myxomycetes belong to 14 genera and four taxonomic orders. More species were reported from the forest patches on UM than on VC soils (Table 2). The smooth SAC of the forest patches on VC and UM soils indicated that the 200 and 400 collected samples were sufficient to reflect the species diversity in the area. However, more species may be recorded by increasing the number of collected samples (Figure 2). As for the assessment of the relative abundance, 10 species were reported to be abundant in both habitat types,

Table 2. Taxonomic and species diversity indices for myxomycetes collected at different habitat and substrate types.

	%MC Productivity ^a	No. of collection	No. of species	No. of genera	S/G ratio ^b	Hs ^c	H _G	E
Habitat^d								
VC	76	159	29	13	2.23	1.14	5.52	0.52
UM	78	360	37	13	2.85	1.20	6.12	0.47
UM + VC	77	519	40	14	2.86	1.20	6.24	0.44
Substrate^e								
VC								
AL	90	70	19	11	1.73	1.07	4.24	0.58
GL	58	28	8	6	1.33	0.78	2.10	0.54
TW	73	33	15	9	1.67	1.01	4.00	0.67
VN	95	28	9	6	1.50	0.63	2.40	0.43
UM								
AL	89	159	25	12	2.08	0.94	4.73	0.43
GL	64	68	19	8	2.38	1.07	4.27	0.58
TW	79	93	29	12	2.42	1.30*	6.18	0.66
VN	83	40	13	8	1.63	0.90	3.25	0.56

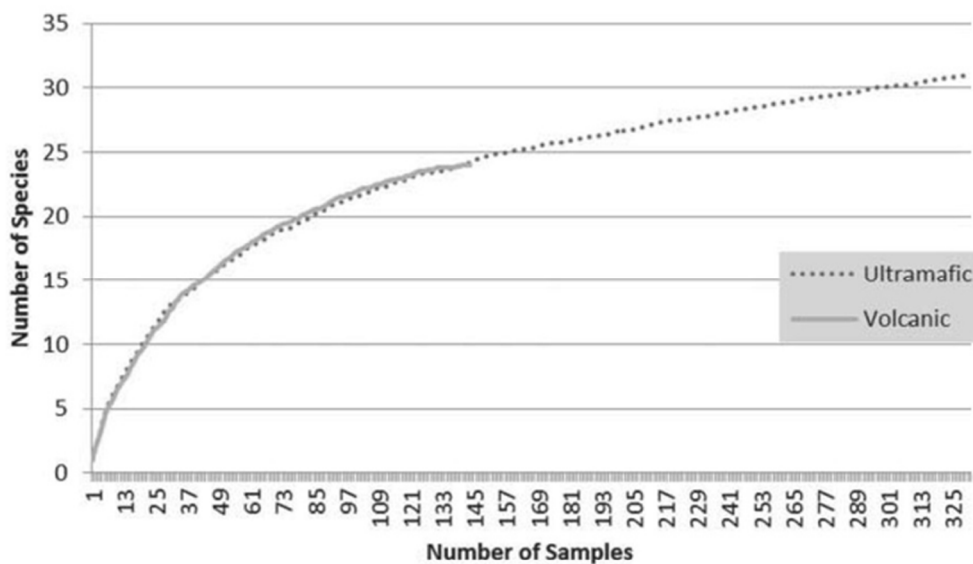
^aMC – most chamber.^bS/G – Species/Genera.^cH_s – Shannon Index of Diversity, H_G – Gleason Index of Species Richness, E – Pielou's Index of Species Evenness.^dSoil type: VC – Volcanic, UM – Ultramafic.^eSubstrates: AL – aerial leaf litter, GL – ground leaf litter, TW – twigs, VN – woody vines.*Statistical significance at $p < .05$.

Figure 2. Species accumulation curve of myxomycetes in forest patches on ultramafic and volcanic soils generated using EstimateS for the number of myxomycete species and substrates in the two forest patches.

i.e. being $> 3\%$ of the total collection (Table 3). Of the 37 species recorded from the UM site, 17 species were classified as rare and five were abundant. From a total of 29 species recorded on VC sites, two species were classified as abundant and 14 were found to be rare species. The matured fruiting bodies of representative species are shown in Supplemental data 1 A-I. In terms of substrate type, AL and TW had 17 species classified as rare (Table 3). Also, 12 species were exclusively recorded in UM, compared to only two species exclusive to VC.

Further analysis was done in terms of taxonomic and species diversity in relation to habitat types and substrates per habitat type. In this study, UM showed a lower taxonomic diversity but was higher in species diversity than that of VC, which comprises 37 species in UM and 29 species in VC both with 13 genera (Table 2). The reported higher value of taxonomic diversity in VC is only influenced by the number of genera and the number of species present, whereas species

Table 3. Relative abundance of myxomycetes in different habitat and substrate types.

Taxon	Habitat type		Substrate per habitat type							
	UM ^a	VC	UM				VC			
			AL ^b	GL	TW	VN	AL	GL	TW	VN
<i>Arcyria cinerea</i>	A ^c	A	A	C	A	C	C	C	C	A
<i>Arcyria denudata</i>	O	R	R		R					R
<i>Arcyria cf. oerstedioides</i>	R		R							
<i>Arcyria pomiformis</i>	R	R			R					R
<i>Collaria arcyrionema</i>	O	O	R		O	R	O			
<i>Comatricha laxa</i>	R	R	R		R		R		R	
<i>Comatricha nigra</i>	R				R	R				
<i>Comatricha pulchella</i>	R	R			R				R	
<i>Comatricha tenerima</i>	O	R			O	R			R	
<i>Cribraria microcarpa</i>	R					R				
<i>Cribraria violacea</i>	O	O		R	R	R	R		R	R
<i>Diachea leucopodia</i>	C	C	O	R	R		C	R		
<i>Diderma effusum</i>	A	A	A	C	R		C	C		R
<i>Diderma hemisphaericum</i>	A	C	A	C	O		O	O		
<i>Didymium decipiens</i>	O	R	R	R	R		R	R		
<i>Didymium minus</i>	R			R						
<i>Didymium nigripes</i>	R				R					
<i>Didymium ochroideum</i>	C	O	R		C	O			R	O
<i>Didymium squamulosum</i>	O	R	R	R			R			
<i>Hemitrichia calyculata</i>		R							R	
<i>Hemitrichia serpulata</i>	O	O	R		R		R		O	
<i>Lamproderma scintillans</i>	C	O	O	R	O		R		R	
<i>Licea cf. variabilis</i>	R		R							
<i>Perichaena chrysosperma</i>	C	O	R	R	O	O	R		R	R
<i>Perichaena corticalis</i>	C	O	O	O	R	O	R		R	
<i>Perichaena depressa</i>	C	C	R	O	O	R	O	R	O	
<i>Perichaena dictyonema</i>	A	R	O	O	O	O			R	
<i>Perichaena pedata</i>	R	R	R		R					R
<i>Physarum album</i>	R	O	R	R			O			
<i>Physarum bivalve</i>	R			R	R					
<i>Physarum cinereum</i>	O	R	R	R	O		R			R
<i>Physarum compressum</i>		R					R	R		
<i>Physarum echinosporum</i>	O	R	R	R	R		R			
<i>Physarum melleum</i>	A	O	C	R	O	R	O	R		
<i>Physarum pusillum</i>	R			R						
<i>Physarum roseum</i>	R				R					
<i>Stemonitis axifera</i>	R		R							
<i>Stemonitis fusca</i>	C	R	R		O	R			R	
<i>Stemonitis pallida</i>	R				R					
<i>Willkommia reticulata</i>		R							R	

^aSoil type: VC – Volcanic, UM – Ultramafic.

^bSubstrates: AL – aerial leaf litter, GL – ground leaf litter, TW – twigs, VN – woody vines.

^cAbundance indices: Abundant (A) = $\geq 3.0\%$ of the total collections; Common (C) = $\geq 1.5\%$ but $< 3\%$; Occasional (O) = $\geq 0.5\%$ but $< 1.5\%$; Rare (R) = $< 0.5\%$.

diversity takes into account factors such as species richness and evenness in a community.[35] In terms of substrate for each habitat type, the VN was calculated to have a higher taxonomic diversity than the TW and leaf litters (AL, GL). However, the species diversity was higher for the TW. In general, species diversity in UM showed a higher species richness but exhibited a lower species evenness than VC. A modified *t*-test indicated that the values are statistically significant ($p < .05$) across all substrates. The higher species diversity in UM than in VC may be due to the near neutral pH range of UM soil as compared to a slightly acidic pH of VC soil (Table 1).

In terms of community similarities of myxomycetes in forest patches on UM and VC soils, a relatively high CC value (.79) and PS (.72) were obtained (data not shown). Between substrates

Table 4. Sorensen's Coefficient of Community (lower left) and Percentage Similarity (upper right) values of the myxomycetes for substrate type in forest patches on ultramafic and volcanic soils

		UM ^a				VC			
		AL ^b	GL	TW	VN	AL	GL	TW	VN
UM	AL		0.64	0.40	0.61	0.66	0.71	0.41	0.40
	GL	0.67		0.46	0.39	0.68	0.58	0.30	0.23
	TW	0.73	0.63		0.62	0.47	0.44	0.56	0.43
	VN	0.46	0.44	0.57		0.34	0.36	0.57	0.51
VC	AL	0.76	0.84	0.67	0.44		0.61	0.33	0.26
	GL	0.41	0.59	0.38	0.29	0.59		0.36	0.32
	TW	0.50	0.42	0.60	0.67	0.48	0.18		0.41
	VN	0.40	0.29	0.47	0.36	0.36	0.24	0.35	

^aSoil type: VC – Volcanic, UM – Ultramafic.^bSubstrates: AL – aerial leaf litter; GL – ground leaf litter; TW – twigs; VN – woody vines.

per habitat type, the highest CC and PS values were calculated between AL of VC and GL of UM forest patches (Table 4).

3.3. Biosorption of manganese and chromium by myxomycetes

When the selected species of *Physarum* were cultured *in vitro* and the plasmodia were grown in the presence of Cr and Mn, *P. melleum* and *P. album* exhibited positive growth only in Mn at 3–1000 $\mu\text{g mL}^{-1}$ but no growth was observed from *P. cinereum* even at 3 $\mu\text{g mL}^{-1}$ Mn. No plasmodial growth was observed either in Cr plates in all concentration tested. The present study detected Mn in fruiting bodies of pooled *P. album* + *P. pusillum* which contained 0.33 $\mu\text{g mL}^{-1}$ of the metal as opposed to only 0.07 $\mu\text{g mL}^{-1}$ on its leaf substrate. Also, the soil sample for this UM site was tested to contain as much as 50.6 $\mu\text{g mL}^{-1}$ concentration of Mn. Remarkably, the ICP–MS results of the *in vitro* grown plasmodium of *P. album* (Supplemental data 1F) illustrated that the myxomycetes were only able to absorb .0001 $\mu\text{g mL}^{-1}$ of Mn from 300 $\mu\text{g mL}^{-1}$ of the metal present in the WA (data not shown). The BCF was then calculated from the soil to the leaf substrate to the fruiting body. In this study, a BCF greater than 1.0 was considered a good indicator of heavy metal absorption from the source to the organism. Interestingly, analysis revealed a BCF of Mn = 4.7 and Cr = 1.5 from the leaf substrate to the fruiting body of *P. album* and *P. pusillum* (pooled together) as opposed to Mn = 0.001 and Cr = 4.3×10^{-6} from the soil to the leaf litter. For the fruiting body of *A. cinerea*, a BCF of Mn = 1.37 and Cr = 0.17 was calculated from leaf to fruiting body, while parts of the leaf to the fruiting body has a BCF of Mn = 0.07 and Cr = 0.001, respectively. The computed BCF indicated that the biosorption by the fruiting body was equal to or higher than that of the substrate.

4. Discussion

According to the literary review, the presence of certain minerals in large amounts renders UM soils to be selective of the species growing in them.[38] The selective advantage of these plants on such soil condition may result in the efficient extraction of metal pollutants from a heavy metal-laden substrate. Some plant species were said to be pre-adapted to a high metal environment, which results in variation in the plant's phenotypic traits such as stunted growth, altered storage mechanisms and varied development time.[26,39] In this study, the higher value for species diversity and richness including a higher myxomycete yield in the UM forests than in

a non-UM (VC) forest were indications that the myxomycetes species were capable of thriving in a UM forest despite the heavy metal toxicity. As compared to a non-UM forest, the observable higher diversity and yield in a UM site may also be attributed to less canopy cover (Table 2), which consequently allowed a wide distribution of myxomycete spores across various microhabitats. This observation was confirmed in a previous study which reported that even a slight breeze can cause dispersal of myxomycete spores to kilometres away from the reference position.[40] Furthermore, the near neutral pH of UM soils near the collection site was within a better pH range for myxomycetes spores to thrive as compared to an acidic environment.[35,41]

The present study also found that twigs from the UM forest patches had the highest species diversity of all substrates (Table 2), hence more productive than other types of forests or woodland substrates. Such observation was in concurrence with reports of earlier studies.[35,42–44] This trend is due to the unique characteristics of woody substrate which has a high water-holding capacity as well as an advanced stage of decay that harbours more food bacteria,[40] thus, favourably promoting the growth of myxomycetes.

Assessment of myxomycete occurrence showed that 10 species were classified as abundant. Most of these abundant species have been known to be of cosmopolitan distribution.[43] Species such as *Arcyria cinerea*, *D. effusum* and *D. hemisphaericum* were observed as abundant in the Philippines, for example, in Mt Arayat National Park, Pampanga and Lubang Island, Occidental Mindoro.[32,45] This is also true for myxomycetes in the temperate region.[35,40] Species belonging to *Arcyria*, *Diderma* and *Perichaena* were reported to be abundant in different ecoregions.[40,42,46] Such similarity may be ascribed to the similar microenvironments in these regions, such as substrate moisture and diameter, height above the ground and canopy openness. In contrast, the species that were reported as rare in this study such as *Didymium squamulosum*, *Physarum cinereum*, *Stemonitis axifera* and *S. fusca* were previously classified as abundant in different habitat types.[44,46] Although 12 species were exclusive to UM and two species were exclusive to VC, these species were not considered as unique to the habitat type, since these were previously documented in other parts of the Philippines.[32,45,47] In terms of distribution, members of the Order Liceales, Stemonitales and Trichiales forms the 7%, 25% and 28% of the species assemblages, respectively, whereas the Physarales (40%) were represented by the greatest number of species. Such trend is typical in myxomycetes studies since majority of the species of myxomycetes were under Physarales.[34] Also, a close similarity in species assemblages from forest patches on UM and VC soils was observed due to the fact that more than 20 species were present in both habitat types. Perhaps, the similar microenvironmental conditions in the UM and VC forest patches resulted to the close similarity in terms of myxomycete composition.

In terms of the biosorption ability in this study, the computed BCF was used as a quantitative measure of the uptake of heavy metal from the leaf or soil substrate since there is no reference value with regard to the heavy metal biosorption of myxomycetes or the permissible limit for these organisms. Thus, the values were only expressed as BCF. The higher BCF observable from the leaf to the fruiting body of *A. cinerea* and the pooled samples of *P. album* + *P. pusillum* in comparison to the BCF from the soil to the leaf (Table 5) indicates that both intrinsic and extrinsic factors may influence the absorption of heavy metals in comparison to the leaf litter which already showed signs of decay at the time of collection. Such extrinsic factors may include metal concentration, pH and myxomycetes' fruiting body development.[42] Similarly, mushrooms were also reported to have high BCF in terms of accumulation of heavy metal such as Zn, Cu and Pb with the accumulation rate dependent on the species of mushroom, age of the fruiting body and the distance from source of contaminant. It was also previously reported that saprophytic mushrooms can accumulate higher heavy metal concentration than its mycorrhizal variants.[48–50] Such observations may also be applicable to the myxomycetes

Table 5. Concentration of heavy metal detected from myxomycetes fruiting body collected in moist chambers and leaf litter substrates.

Source	Cr ($\mu\text{g mL}^{-1}$)		Mn ($\mu\text{g mL}^{-1}$)	
	VC	UM	VC	UM
Soil	22.0	930	18.56	50.6
Ground leaf litter	0.03	0.004	1.30	0.07
Fruiting body				
<i>A. cinerea</i>	0.005	nd	1.78	nd
<i>P. album</i> + <i>P. pusillum</i>	nd*	0.006	nd	0.33
Bioconcentration factor				
Soil to leaf litter	0.001	4.3×10^{-6}	0.07	0.001
Leaf to fruiting body	0.17	1.5	1.37	4.78

*nd: not detected.

that also feed on decaying matter. In addition, the close association of myxomycetes with soil and decaying organic matter allows a good rate of exchange of material from the substrate to the organism.[20,50,51] The results of this study suggests that the mechanism of biosorption in myxomycetes may be more efficient in obtaining the heavy metal directly from the ground leaf litter and soil. However, the observed low biosorption from the agar plates to the plasmodium of myxomycetes in this study implied that the presence of heavy metal is not through direct biosorption of metals from the substrate but could have originated from biosorption of associated microorganisms [17,18] on the leaf that were also exposed to the heavy metals. The absence of bacteria or other microorganisms and suitable enzymes [18,52,53] on the heavy metal agar plates probably explains the low concentration of the heavy metal in the plasmodium of *in vitro* grown *P. album*. Since it has long been known that myxomycetes feed via phagocytosis [20] then myxomycetes may obtain the heavy metal from the associated microorganisms that were also exposed to heavy metal-laden substrates, for example, ground leaf litter. These may then possibly become concentrated in the food vacuoles of myxomycetes.[23,54,55]

Further work on myxomycetes must be done to elucidate the cellular mechanism and the direct role of this organism in the biosorption of heavy metals. Apart from utilising various culture methods to produce large plasmodia of myxomycetes [18,56,57] it is believed that utilisation of myxomycetes in combination with bacterial isolates for the purpose of bioremediation may be feasible. The screening for bacteria and myxomycetes species that may be used in a microbial cocktail to be tested in real contaminated sites also warrants further investigation.

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Disclosure statement

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Supplemental data

Supplemental data for this article can be accessed at [10.1080/02757540.2015.1091884](https://doi.org/10.1080/02757540.2015.1091884)/description of location.

ORCID

M.A.D. Rea-Maminta  <http://orcid.org/0000-0003-4818-0824>

N.H.A. Dagamac  <http://orcid.org/0000-0002-5155-5415>

R.A. Wahab  <http://orcid.org/0000-0002-9982-6587>

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Ecological factors limiting occurrence of corticolous myxomycetes – a case study from Alaska



M. Schnittler^{a,*}, N.H.A. Dagamac^a, M. Sauke^b, M. Wilmking^a, A. Buras^a, S. Ahlgrimm^a, P. Eusemann^{a,c}

^a Institute of Botany and Landscape Ecology, Ernst Moritz Arndt University Greifswald, Soldmannstr. 15, D-17487 Greifswald, Germany

^b Institute for Stochastics, TU Bergakademie Freiberg, D-09596 Freiberg, Germany

^c Johann Heinrich von Thünen-Institute of Forest Genetics, Eberswalder Chaussee 3a, D-15377 Waldsiedersdorf, Germany

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ABSTRACT

In search of decisive factors for the occurrence of corticolous myxomycetes we investigated a plot comprising 380 white spruce trees (*Picea glauca*) in the Denali National Park and Reserve, Alaska. Moist chambers were prepared from the bark of 260 trees and cultured for 77 d. Occurrence patterns of corticolous myxomycetes were related to tree position and eight environmental variables; among them are diameter at breast height (dbh), tree age and vitality, and bark pH. Tree age and dbh, but not vitality, are highly correlated with each other. Bark pH decreases with increasing diameter of a tree. Only two species of myxomycetes, *Leocarpus fragilis* (164 records) and *Paradiacheopsis solitaria* (72) were common. The first species fruits typically on litter; in the study it preferred small trees (low dbh) with a rather high bark pH and high vitality. The second species is known as truly corticolous and prefers thick (usually older) trees with a low pH and a lower vitality. The low and statistically insignificant Cole index for association between the two species (0.047) points to niche separation. For both species mark connection functions indicate a near-random spatial distribution among trees. *L. fragilis* could effectively colonize small trees (55% of all records occurred on trees below 15 cm dbh). *P. solitaria* seems to avoid small trees (92% of all records occurred on trees exceeding 15 cm dbh). Our data indicate that the occurrence of corticolous myxomycetes is not limited by dispersal capability but by habitat suitability.

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1. Introduction

Plasmodial slime molds (myxomycetes) are eukaryotic microorganisms (superclass Amoebozoa) developing visible fructifications. It is mainly for this reason that we know more about their diversity and biogeography than for most other protistean groups (Stephenson et al., 2008). Bark-inhabiting (corticolous) myxomycetes have been known since the pioneering study of Gilbert and Martin (1933), who were the first to apply the moist chamber culture technique revealing a whole microcosm with numerous species of myxomycetes forming minute fruit bodies 0.05–0.5 mm tall. This species-rich group of myxomycetes (Mitchell, 2004; Everhart and Keller, 2008) inhabits the bark of living trees, preying on microorganisms and algae which live on the outermost, decaying bark scales. Ecological hallmarks are the usually short

developing times and minute fruiting bodies which are often stalked, and this seems to be an ancestral character of myxomycetes (Fiore-Donno et al., 2013). Fructifications are hard to detect in the field, but occur in substratum cultures 5–20 d after the bark scales have been wetted and incubated under ambient temperature. Corticolous myxomycetes are an ecological guild rather than a taxonomic group since its members belong with roughly equal numbers of species to both the bright- and the dark-spored myxomycetes (Schnittler et al., 2012a). In addition, some myxobacteria (Reichenbach, 1993) and Acrasiales (Blanton, 1990; Brown et al., 2011) respond as well to the moist chamber culture method, developing fructifications within a few days. The typical (and most often investigated) habitat is the tree trunk, but fructifications develop also on thinner branches of living trees in the canopy (Schnittler et al., 2006). Corticolous myxomycetes seem to be adapted best to climates with fluctuating rainfalls. They are more abundant in tropical seasonal dry than in moist forests (Schnittler and Stephenson, 2000), but can also occur in extremely arid

* Corresponding author.

E-mail address: martin.schnittler@uni-greifswald.de (M. Schnittler).

climates with annual precipitation below 100 mm (Schnittler et al., 2012b).

Ecological studies of corticolous myxomycetes are still rare; most research activities have focused on species diversity. However, despite the time required to evaluate large series of cultures, the opportunity to standardize conditions makes the moist chamber culture technique very suitable for ecological investigations, e.g. as shown by data sets from Finnish boreal forests (Härkönen, 1977; Härkönen and Ukkola, 2000). For arctic and boreal North America, several studies about myxomycetes (Stephenson and Laursen, 1993, 1998; Novozhilov et al., 2007) and two other related groups (protostelids: Moore et al., 2000; dictyostelids: Cavender, 1978; Landolt et al., 1992) have led to a basic species inventory for this region. As expected for microorganisms that can bridge continents via spores, regional species inventories for boreal and arctic regions display a high degree of similarity in terms of myxomycete composition (Stephenson et al., 2000). From these studies, a total of 90 myxomycete species are known for Alaska (Stephenson and Laursen, 1998). Of these, approximately 20 species can be regarded as corticolous. The main tree species investigated with the moist chamber culture technique include *Populus balsamifera*, *Larix laricina* and *Picea glauca*.

A project investigating the growth of white spruce (*P. glauca*) in connection with climate changes in interior Alaska towards warmer and drier summers during the last 60 yr (Wilmking et al., 2004) gave the opportunity to establish a plot in a contiguous stand of white spruce. The investigated south-facing slope represented a natural, monospecific stand with trees of different size and age, and a set of parameters was recorded for each tree, including trunk diameter, age, vitality, or crown diameter. We carried out a systematic sampling of this stand to answer the following questions: which factors determine the occurrence of corticolous myxomycetes? Do trees of different size provide different niches which are inhabited by different species? Do myxomycetes show a random or patchy distribution within the plot (the latter could hint at limitations on spore dispersal)?

2. Materials and methods

2.1. Study region

Alaska's Denali National Park and Reserve is a part of the Alaska Range, the southernmost of the two large mountain ranges framing Interior Alaska. The investigated stand (DPFG) included 380 trees of *P. glauca* in a rectangular area of 1.12 ha (80 m east-west by 140 m north-south extension) and is situated at Rock Creek watershed, ca. 1.2 km north of the park access road (149°00'36" W, 63°43'29" N). The chosen south-facing slope (mean inclination ca. 7° degrees) supports a fairly dense (ca. 340 trees/ha) stand of white spruce, with the largest trees reaching 65 cm diameter at breast height (dbh) and up to 20 m in height. Except for two large, tree-like individuals of *Salix bebbiana*, white spruce was the only canopy tree in the stand. The understorey is characterized by a patchy shrub layer of uniform height (1–1.5 m, heavily pruned by browsing of moose). The main shrub species is *Betula nana*, occupying most of the open areas between trees, followed by several species of willow. The latter, being under especially high grazing pressure, rarely exceed 1.2 m in height; most common were *Salix glauca*, *S. bebbiana*, and *Salix pulchra*. Vascular plants were identified according to Hultén (1968) and Viereck and Little (2007).

2.2. Field work

All 380 trees of *P. glauca* in the selected plot were mapped with a differential GPS (Trimble R3) in July 2012, allowing a precision of

≤30 cm (floating mode and post processing). For this study, the investigated tree parameters were coded as eight environmental variables. Vitality of the trees (*vit*), was assessed using a five-division scale: 1 = all branches green, ending with freshly grown shoots; 2 = 5–15% dead branches, crown intact, fresh growth in about 70% of all outer branches; 3 = 15–50% dead branches, crown damaged but alive with at least half of all outer branches in fresh growth; 4 = >50% dead branches, crown damaged or broken off, <20% fresh growth, tree dying; 5 = tree dead. The pH of the bark surface (*pH*) was measured in the prepared moist chamber cultures for all 260 trees where bark was cultured. Diameter at breast height (*dbh*) was measured for each tree. Tree age (*age*), was determined using tree cores and ring counts of all trees allowing coring, altogether 175 trees. Tree height (*height*) was measured directly or determined with a clinometer. The area of the crown (*crown*) was estimated from two perpendicular measurements of crown diameter. An index of light competition (*comp*) was computed from crown radius (mean of two measurements perpendicular to each other), tree height and geographical position. The overlap of neighboring tree crowns was calculated as the overlap of two circles. Overlap was then inversely related to the height of the two trees in question, with the higher value assigned to the smaller tree. As such, this index estimates the degree to which one tree is shaded out by others – which should lead to a cooler and moister microclimate for this tree in comparison to the others. Per cent shrub cover (*shrub*) was estimated in a 3 m radius around each tree.

From 260 of the 380 *P. glauca* trees in the plot about 20–30 bark pieces around each trunk (to minimize possible differences related to different exposition of N- or S-facing sides of the trunk) were sampled between 1.2 and 1.5 m height; this height was chosen to exclude soil-inhabiting myxomycetes, where plasmodia often migrate to elevated points to fruit. Sampling included nearly all larger trees (*dbh*>4 cm); the majority of the 120 trees not sampled were below 0.5 cm dbh or were saplings not reaching sampling height. The outermost, dead bark and bases of small dead branches were carefully removed without injuring the living part of the tree. Bark samples were air dried in paper bags and transported back to the laboratory.

To obtain an overview of the assemblage of corticolous myxomycetes to expect, additional bark samples were collected and cultivated as described above. These included all common trees and shrubs occurring in a ca. 5 km radius around our plots: twigs of *P. glauca* (10 samples), and bark of the tree-forming willow *S. bebbiana* (10), *Populus tremuloides* (10), *Betula neoalaskana* (syn. *Betula papyrifera*, 10), *Alnus viridis* (10), and *B. nana* (16); altogether 66 samples.

2.3. Moist chamber cultures and specimen identification

The collected bark samples were cut into pieces of 1 × 1 cm and placed in a 9 cm Petri dish lined with three layers of absorbent paper towel in a way that most of the surface (ca. 60 cm²) was covered. Samples were completely soaked in distilled water overnight. After 24 h, excess water was removed, and the pH of three pieces was measured with an Orion 610 solid state probe. Moist chambers were incubated at room temperature under diffused light for in total 77 d and observed regularly (6, 11, 22, 33, 52, 64, and 77 d) for the presence of plasmodia and/or fruiting bodies. All fruiting bodies of myxomycetes that developed in the moist chamber cultures were harvested, air-dried and mounted in matchboxes. Furthermore, the number of fruiting bodies per culture was recorded to obtain abundance data. Nomenclature of myxomycetes followed Lado (2015–2014, Table 1). Voucher specimens were deposited in the Botanical State Collection Munich (M), with duplicates of selected specimens in the herbarium of the

Table 1

Recorded myxomycetes (24), myxobacteria (2) and acrasid amoebae (1) on *Picea glauca* (Pic) and bark of deciduous trees and shrubs (Dec). For all species found on bark of *P. glauca*, average and SD figures for pH, tree dbh and the day of the first appearance in a culture are given. A question mark (?) indicates species with extremely scanty or maldeveloped fructifications, the symbol cf. (confer) species where our determination must be regarded as uncertain.

Species	Pic	Dec	pH	dbh	day
<i>Arcyria cinerea</i>	2	18	3.70 ± 0.77	11.6 ± 8.5	64
<i>Arcyria denudata</i>	1	—	3.47	18.5	77
<i>Arcyria minuta</i>	1	—	3.84	16.5	33
<i>Badhamia</i> ? spec.	1	—	4.03	17	77
<i>Calomyxa metallica</i>	—	5	—	—	—
<i>Comatricha</i> ? laxa	—	1	—	—	—
<i>Didymium difforme</i>	1	—	5.84	4.5	52
<i>Didymium squamulosum</i>	12	1	4.52 ± 0.18	11.2 ± 2.5	36
<i>Echinostelium brooksii</i>	1	1	3.74	26	22
<i>Echinostelium fragile</i>	1	—	3.73	19	22
<i>Echinostelium minutum</i>	—	1	—	—	—
<i>Enteridium olivaceum</i>	1	—	4.12	38	52
<i>Enteridium</i> cf. <i>simulans</i>	—	1	—	—	—
<i>Leocarpus fragilis</i>	164	38	3.85 ± 0.61	16.6 ± 11.6	40
fructifications:	40	7	3.97 ± 0.10	14.6 ± 1.6	40
plasmodia assigned to <i>L. fragilis</i>	124	31	3.78 ± 0.09	17.2 ± 1.1	45
<i>Licea minima</i> Fr.	—	1	—	—	—
<i>Paradiacheopsis fimbriata</i>	3	3	3.68 ± 0.11	20.0 ± 1.3	18
<i>Paradiacheopsis solitaria</i>	72	6	3.64 ± 0.06	27.3 ± 12.5	32
<i>Perichaena corticalis</i>	—	9	—	—	—
<i>Perichaena vermicularis</i>	—	9	—	—	—
<i>Physarum bivalve</i>	4	2	4.10 ± 0.30	15.5 ± 6.3	47
<i>Physarum cinereum</i>	4	3	4.66 ± 0.41	8.0 ± 2.5	35
<i>Physarum</i> cf. <i>galbeum</i>	—	3	—	—	—
<i>Stemonitis fusca</i>	5	1	3.50 ± 0.25	26.1 ± 6.7	77
<i>Trichia</i> ? <i>persimilis</i>	—	1	—	—	—
Subtotal	273	104	—	—	—
<i>Mellitangium lichenicola</i> [Myxobacteria]	—	4	—	—	—
<i>Myxococcus</i> spec. [Myxobacteria]	—	11	—	—	—
<i>Pocheina rosea</i> [Acrasid amoebae]	1	—	5.26	8	6
Subtotal	274	119	—	—	—
Unidentified white to cream plasmodia	38	11	3.62 ± 0.09	20.3 ± 1.6	57
Unidentified deep brown plasmodium	1	—	3.39	14	52
Unidentified immature myxomycete fructification	—	1	—	—	—
Total	313	131	—	—	—

University of Arkansas (UARK).

2.4. Data analysis

Species accumulation curves were constructed according to the rarefaction formula using the program EstimateS (Version 9.1; Colwell, 2013; 100 randomizations), which computes also a number of estimators of species richness. In accordance with Unterseher et al. (2008), the Chao2 estimator (Chao et al., 2005) was chosen as the best estimator and calculated using the 'classical settings' of EstimateS.

Statistical calculations were performed using the programming language R (Core Team, 2013), and all records that could be identified to species were considered (Table 1). To compute correlations between environmental variables and myxomycete abundance we subdivided the respective observed range into ten parts, counted within each part the respective number of trees carrying the myxomycete species and compared it to the environmental variable's interval midpoint (Table 2). Correlation among environmental variables was checked and tested for significance based on Spearman's rank correlation (Table S1). To assess which environmental variables affect myxomycete occurrence, we tested pairwise for equality in the distributions of environmental variables between trees colonized and not colonized, utilizing the Kruskal–Wallis statistic (Table S2). Logistic regression models were employed to select those of the (often intercorrelated) environmental variables that yield some explanatory power in addition to one another (Table S3). As used here, the models estimated the likelihood of occurrence for the two most common species in the

Table 2

Spearman rank correlation between environmental variables and abundance of the two common myxomycetes *Paradiacheopsis solitaria* and *Leocarpus fragilis* (asterisk: significant correlations, $p = 0.05$).

	<i>P. solitaria</i>	p	<i>L. fragilis</i>	p
Vit	0.929	* 0.002	−0.286	0.501
pH	−0.238	0.582	0.886	* 0.003
Dbh	0.857	* 0.024	−0.929	* 0.007
Age	0.786	* 0.028	−0.810	* 0.022
Height	0.881	* 0.007	−0.857	* 0.011
Crown	0.857	* 0.024	−0.964	* 0.003
Comp	−0.183	0.644	0.393	0.295
Shrub	−0.767	* 0.021	0.435	0.242

study. Significance of model parameters was tested with the likelihood ratio test between the respective full and reduced model based on the χ^2 distribution.

For 16 species of myxomycetes and acrasid amoebae found in 191 cultures from *P. glauca* a canonical correspondence analysis was carried out, including the eight tree parameters described above as environmental variables. PC-ORD V6 for Windows (McCune and Mefford, 2011) was used with default settings (rescaling threshold = 0, number of segments = 26). Despite the large number of zeros (absence) in the species-samples matrix, the transformation of the data with the Beals smoothing function of PC-ORD (McCune, 1994) did not increase the explanation values of the ordination. Therefore this tool was not considered further.

To test for association of the two most common species on bark of *P. glauca*, the Cole index of association (Cole, 1949; corrected after

Ratcliff, 1982), was calculated and subjected to a χ^2 test. This index (range $-1 =$ complete avoidance to $1 =$ full association) indicates if two species do more or less often occur together in a culture than expected by chance.

For spatial analysis, mark connection functions (Illian et al., 2008) were computed from tree location and presence/absence of the two most common myxomycete species, to test if trees positive for myxomycetes were randomly distributed in space or were clustered. The mark connection function as used here represents an estimate of the probability that two trees at a given distance are both colonized by the respective myxomycete species. If one assumes complete spatial random distribution of myxomycetes among trees, this probability is expected to be independent from distance. To ensure statistical significance of results, pointwise 95% Monte-Carlo simulation envelopes were computed from the respective mark connection functions of 499 simulated point patterns, each obtained by a random permutation of the respective presence/absence mark (i.e., myxomycete occurrence) among trees (assigning the records of an investigated species with a random procedure to a tree). Spatial analysis was carried out with the R package spatstat (Baddeley and Turner, 2005).

3. Results

A total of 444 records of myxomycetes and myxomycete-like organisms were recovered from the 326 moist chambers prepared (260 moist chambers from bark of *P. glauca* and 66 moist chambers from additional bark samples; Schnittler et al., 2016). For *P. glauca*, 208 of 260 cultures (80%) were positive for myxomycetes and myxomycete-like organisms (including 17 cultures with indeterminable plasmodia which did not fruit); figures for additional samples were 58 of 66 cultures (88%). From the 428 myxomycete records, 39 plasmodia and one immature fructification could not be determined. Altogether, 27 taxa of myxomycete-like organisms (Schnittler et al., 2006), including 24 myxomycetes, two myxobacteria (determined after Reichenbach, 1993; recorded only on deciduous trees and shrubs), and one acrasid amoeba (Blanton, 1990; on spruce) were recorded. Table 1 shows the species list and the main ecological characteristics for all species recorded on *P. glauca*.

Compared to bark of spruce, bark of deciduous trees and shrubs was generally richer in both species and records. We found 16/20 taxa from 260/66 moist chambers with 274/119 records (1.05 vs. 1.80 records per culture) for the two types of substrata, respectively. For these counts, the yellow plasmodium (124/31 records) was assigned to *Leocarpus fragilis* (Table 1), whereas 39/11 records of other plasmodia and one immature fructification were neglected. Fig 1 shows the resulting species accumulation curves, resulting in 9.8 vs 18.7 species to be expected for 100 records for bark of spruce or bark of other trees.

Mean pH values for spruce bark were significantly lower than for the bark of deciduous trees (pairwise Mann–Whitney U-test, in all cases $p < 0.05$). Values for spruce bark were 3.69 ± 0.65 , range 2.46–5.84, $n = 260$; the outermost, dead twigs of spruce were similar (4.00 ± 0.30 , $n = 10$). Bark from deciduous trees and shrubs was clearly less acidic: *S. bebbiana* (5.19 ± 0.57 , $n = 10$), *Populus tremula* (6.08 ± 0.31 , $n = 10$), *B. neolaskana* (5.86 ± 0.45 , $n = 10$), *A. viridis* (5.17 ± 0.40 , $n = 10$) and *B. nana* (5.72 ± 0.38 , $n = 16$). For white spruce, bark pH declined with increasing tree size (Fig 2A).

If the 260 investigated spruce trees were divided into two cohorts, such <20 cm dbh (138 trees with 164 records) and such ≥ 20 cm dbh (122 trees with 110 records), the young trees had 14/1.19, the older trees 12/0.90 species/records per culture. A different delimitation of cohorts (setting the threshold at 15 or 25 cm dbh) led to similar results (data not shown). Already the smallest size

class (<5 cm dbh) was more productive than the average (1.43 vs 1.06 records per culture).

Of the 260 investigated spruce trees, only three species appeared with more than five records: *Didymium squamulosum* (12), *Leocarpus fragilis* (40 + 124 non-fruiting plasmodia), and *Paradiacheopsis solitaria* (72). For *L. fragilis*, the conspicuous, bright yellow plasmodia appearing in many cultures could clearly be assigned to this species, since they displayed a typical morphology (thick, wavy veins and constant color), and in most of the 40 fruiting specimens the plasmodium was observed in the process of fruiting; at least some parts of it converted to sporocarps.

The two most common species, *L. fragilis* and *P. solitaria*, seem to occupy different ecological niches (Fig 2): *L. fragilis* was generally found on smaller and younger, more vital trees (pH 3.85 ± 0.61 , dbh 16.6 ± 11.6 cm, age 112.8 ± 44.5 yr, vitality 2.94 ± 0.86) than *P. solitaria* (pH 3.64 ± 0.06 , dbh 27.3 ± 12.5 cm, age 151.5 ± 29.8 yr, vitality 3.42 ± 0.95). All these differences were highly significant (Mann–Whitney U-test, $p < 0.001$). The mean age for trees carrying the two species (Fig. 2D–E) was most likely overestimated, since for only 97 of 164 records of *L. fragilis*, and 42 of 72 records of *P. solitaria*, the host tree was sufficiently large to allow coring. Whereas 55% of all records of *L. fragilis* were made on trees <15 cm dbh, this applies for only 8% of all records from *P. solitaria* (Fig 3). This coincides with the figure found for the Cole index of association (0.047, non-significant, very weak association, $\chi^2 = 2.572$, test figure of the χ^2 distribution 3.841).

Which environmental variables explain the distribution of these two myxomycete species? Some variables were highly correlated (the following lines mention those significant at a level $p = 0.05$, Table S1). Obviously, dbh showed a strong positive correlation with age, tree height, and crown diameter (Spearman correlation >0.73). All of these four variables were positively correlated with each other, but negatively correlated with bark pH (< -0.43). Tree vitality showed a weak (>0.15) positive correlation with dbh, tree height and crown diameter. The competition index was negatively (<0.36) correlated with dbh, tree height and crown diameter (taller trees suffer less from competition). Shrub coverage showed a weak negative correlation with most other parameters. The triplot of a canonical correspondence analysis (CCA, Fig 4, eigenvalues for the first three axes 0.502, 0.298 and 0.143) visualizes these relationships. Species scores for the two species of *Paradiacheopsis* were associated with sample scores for larger trees; whereas species scores for typically litter-inhabiting Physarales (*Didymium difforme*, *D. squamulosum*, *L. fragilis*, *Physarum bivalve*, *Physarum cinereum*) were associated with sample scores for smaller trees.

If we look for correlations between environmental variables and occurrence of the two most common myxomycete species, the decisive variables for *L. fragilis* were pH, dbh, age, height and crown diameter; for *P. solitaria* these were vitality, dbh, age, height, crown diameter, but also shrub coverage (Table 2). A similar pattern was displayed when looking at significant differences in the distribution of the eight variables for colonized and non-colonized trees (Table S2). The logistic regression models (Table S3) show how changes in environmental conditions influence the likelihood of occurrence for the two myxomycete species. For *L. fragilis*, decisive factors were again dbh, but here occurrence decreased with dbh, and increased with pH (a higher value, i.e. more basic pH, increased the likelihood of occurrence). The likelihood of occurrence of *P. solitaria* increased with dbh (which can be seen as a proxy for the highly correlated parameters age, height and crown diameter) and declining tree vitality. The weak (but non-significant) association indicated by the Cole index coincides with the result of the logistic regression model, that the presence of one of the two species increased as well the probability of occurrence of the other.

The spatial distribution of both species within the plot (Fig S1)

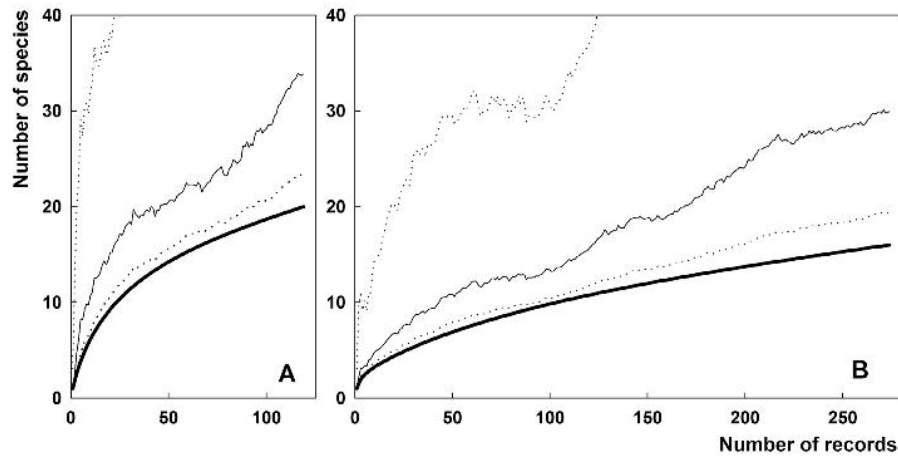


Fig. 1. Species accumulation curves of myxomycetes and myxomycete-like organisms (thick lines) for (A) various deciduous trees and shrubs (119 records, 66 cultures) and (B) bark of *Picea glauca* (274 records, 260 cultures). The means of the Chao2 estimator (thin line) and its lower and upper 95% confidence interval (dotted lines) are also shown.

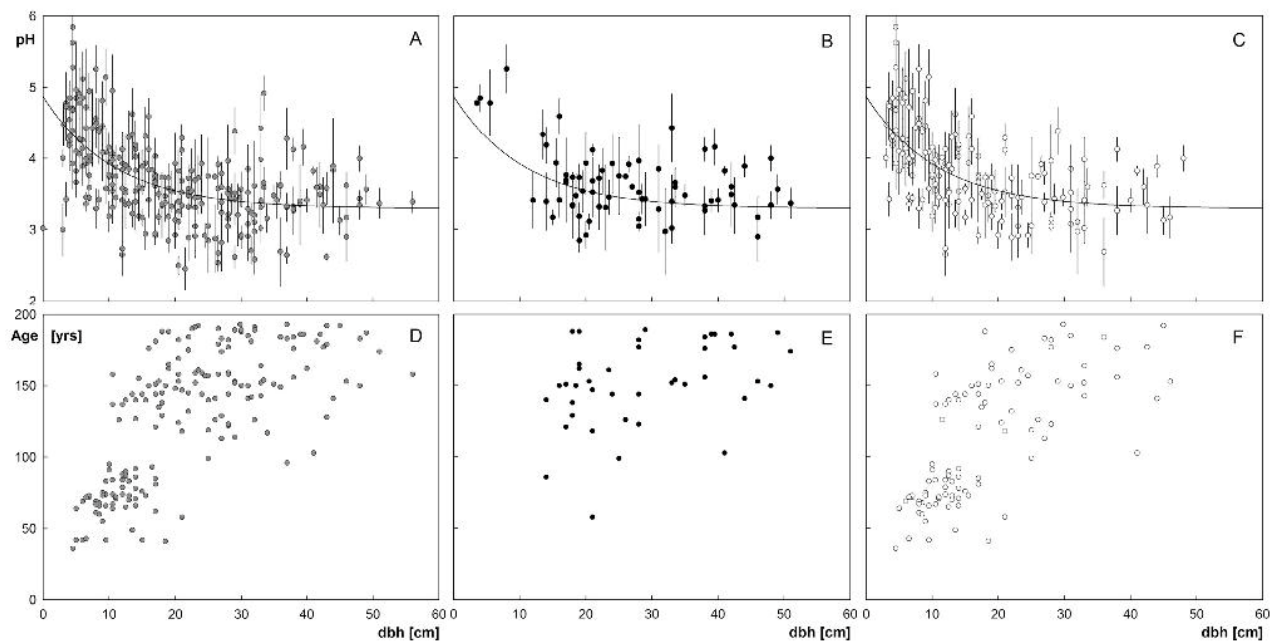


Fig. 2. A–C Dependence of bark pH (mean of three measurements per culture \pm SD) on tree dbh for 260 spruce trees (A); and for cohorts of trees inhabited by the myxomycetes *Paradiacheopsis solitaria* (B) and *Leocarpus fragilis* (C). Plot A was fitted by an exponential decay curve of the form $y = y_0 + a \times e^{-bx}$ with $y_0 = 3.30 \pm 0.07$, $a = 1.58 \pm 0.08$, $b = 0.09 \pm 0.01$, $R^2 = 0.326$. D–E Relationship between dbh and tree age for 175 spruce trees (D); and for cohorts of trees inhabited by the myxomycetes *P. solitaria* (E) and *L. fragilis* (F).

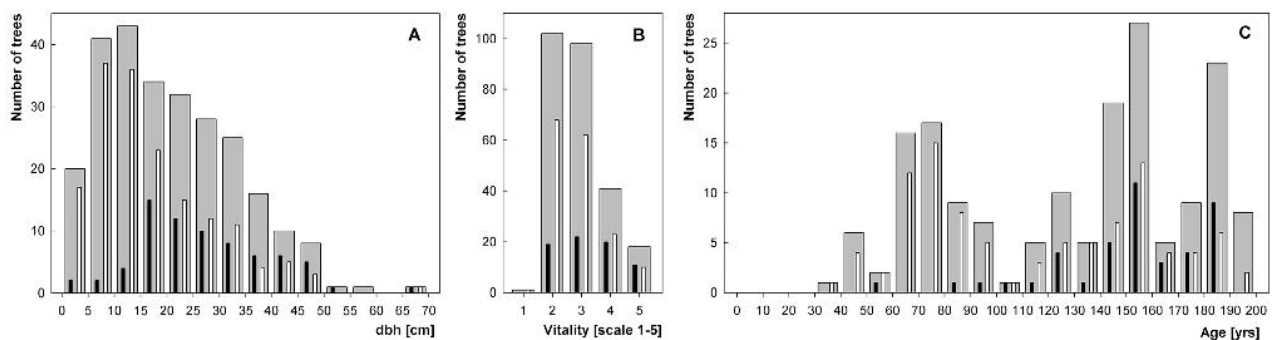


Fig. 3. Records of *Leocarpus fragilis* (narrow white bars) and *Paradiacheopsis solitaria* (narrow black bars) in relation to dbh (A), vitality (B) and age (C) of trees. Numbers of trees are indicated by broad grey bars; data on dbh and vitality were available for 260 trees, on age for 170 trees.

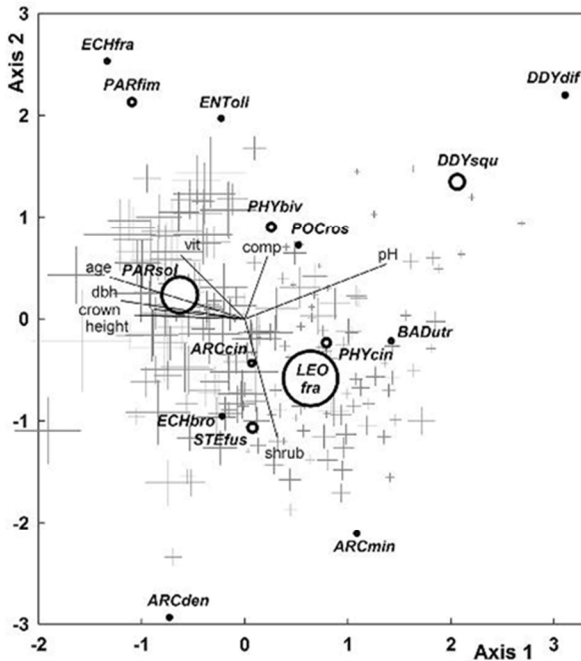


Fig. 4. Triplot of a canonical correspondence analysis including 15 taxa of myxomycetes and the acrasid amoebae *Pocheina rosea*, visualizing the direction of eight tree parameters (age, dbh, crown area, height, shrub cover around the tree, bark pH, competition index, and vitality) taken as environmental variables for 191 trees. Crosshairs mark the position of sample scores (trees positive for myxomycetes); circles mark the position of species scores. Sample scores are scaled according to tree dbh (smallest crosshair = 4 cm dbh); species scores according to abundance (smallest circle = 1 record).

does not significantly deviate from a random pattern. The mark connection function stays always within the 95% confidence envelope, although the deviations are largest within the first 5 m (positive for *P. solitaria*, i.e. two trees in this distance are more often inhabited by the myxomycete than expected by chance; negative for *L. fragilis*). In other words, trees that are positive for one of the two myxomycete species do not cluster; for any given distance between two trees the chance that both are positive for the species is not significantly higher than for assuming complete random distribution of the species.

4. Discussion

Corticolous myxomycetes form a distinct ecological guild comprising ca. 120 species considered to be more or less specialized on the bark of living trees and shrubs (Mitchell, 2004). Typical features are the small, often, but not always, stalked fructifications, the short development time (typically 5–10 d; compare Schnittler, 2001), and the regular occurrence in moist chamber cultures. Despite an extensive body of data on geographical distribution and diversity, much less is known about the ecology of corticolous myxomycetes. Most authors studying these organisms recorded only the plant providing the substratum.

In the few studies focusing on ecology, substratum pH has emerged as the most important ecological factor (Schnittler and Stephenson, 2000; Schnittler, 2001), influencing the occurrence of corticolous myxomycetes. Bark pH seems to be inversely correlated with species richness (Snell and Keller, 2003; Everhart et al., 2009), and our study confirms this. While desert shrubs and trees can show pH values exceeding 7, most deciduous trees in temperate zones display pH values between 6 and 7, whereas coniferous trees

have typical pH values between 3 and 4 (Snell and Keller, 2003). Many species of corticolous myxomycetes seem to be susceptible to low substratum pH, at least to the point that they fruit less abundantly on substrata with low pH (Wrigley de Basanta, 2004). Therefore, the low pH recorded for bark of *P. glauca* in our study region in Alaska produced only a limited species assemblage compared to that found on the bark of deciduous trees in the same area. The rarefaction analysis revealed nearly twice as many species expected on deciduous trees in comparison with *P. glauca*. As such, our survey is comparable with the results from boreal central Siberia, where bark of *Larix gmelinii* forms a comparable substratum (pH 2.6–4.7, mean from 46 trees: 3.7 ± 0.1 ; Novozhilov et al., 1999). In both cases, only members of the Stemonitales, especially *Comatracha* spp. and *Paradiacheopsis* spp., were abundant on the bark of spruce and larch and appeared to be specialized on this substratum. On spruce bark from Alaska, *P. solitaria* was most common; in Siberia it was *Paradiacheopsis fimbriata*. Both species are regarded as corticolous, whereas *L. fragilis*, the species found as most common in this survey, fruits typically on acidic (often needle) litter. Our analyses strongly suggest different pH optima for the two species, with *L. fragilis* being somewhat outside the optimum: only a small part (24%) of all the plasmodia assigned to this species fruited, and even for the fruiting records usually only the smaller part of a plasmodium formed fruiting bodies. As visualized by the CCA (Fig. 4), different ecological niches are the most likely explanation for the low Cole index of association (0.047) between the two species.

Another study investigating the ecology of corticolous myxomycetes on the deciduous North American tree *Quercus alba* (white oak) found a higher diversity on larger trees, when two cohorts were compared (Clayton et al., 2014). Small trees ($n = 17$, dbh < 16 cm, mean dbh 12 cm, mean pH 6.6) had on average 5.6 species per culture, large trees ($n = 17$, dbh > 32 cm, mean dbh 38 cm, mean pH 6.7) had 6.4 species per culture. For the larger and contiguous stand of *P. glauca*, we compared similar cohorts. Small trees ($n = 138$, dbh < 20 cm, mean dbh 11 cm, mean pH 4.0) had 1.2 species per culture, but large trees ($n = 122$, dbh ≥ 20 cm, mean dbh 32 cm, mean pH 3.4) had 0.9 species per culture. Clayton et al. (2014) discuss a possible ‘accumulation effect’ (higher diversity of corticolous myxomycetes in larger trees): the rough bark of older trees may (a) function as a more effective spore trap, (b) provides a more heterogeneous environment supporting a wider diversity of food organisms, and (c) allows a greater water holding capacity.

Not only pH (which was recorded in both studies), but also other bark features, like roughness and water retention, may change strongly and in a non-linear fashion with tree dbh. Together with the microclimate which is likely to be influenced by the density and extension of the tree crown, these factors determine the time the bark stays wet after rain. Since the development time differs between myxomycete species (Table 1), such differences are likely to be important for species’ occurrences. Although not specifically recorded by our study on *P. glauca*, this was mirrored by the coverage of lichens: trunks of trees above 5 cm dbh seemed to have been nearly free of lichens, whereas trunks of smaller trees had sometimes one third of the bark surface covered by lichens. A tree is a complex system of trunk and branches of different size, therefore overall microhabitat diversity is likely to increase over the life time of a tree (with the trunk having a stronger, more fissured bark which retains more water, local differences in epiphyte cover, soil accumulation on large branches). Consequently Clayton et al. (2014) reported higher myxomycete diversity in large oak trees. Our study, carried out with a coniferous tree that harbors less species than the white oak investigated by these authors did not show such an ‘accumulation’ effect. One reason is likely the low pH of the trunk of large trees, allowing only specialized myxomycete

species to survive on mature trees.

If existing, the accumulation effect may be caused by two different processes. First, if myxomycete occurrence is limited by dispersal capabilities, older (and on average larger) trees have more time to catch spores, and their rougher bark may be a more efficient spore trap than the smooth bark of younger trees. Second, increasing microhabitat diversity realizes local niches for more species. Unfortunately, these two processes are nearly impossible to disentangle.

Litter and soil represent continuous substrata: if sufficiently wet, there should hardly be barriers for migrating amoebae and/or plasmodia. In contrast, the bark of trees and shrubs dries out regularly, and if bark is wetted by rain, water moves downwards, washing amoebae down rather than allowing upward migration. As such, trees can be seen as habitat islands which may be easier to reach by airborne spores than by amoebae or plasmodia. If we assume this, numbers of spores produced, dispersal abilities, and the reproductive system of a species should play a crucial role, as shown in a simulation study (Schnittler and Tesmer, 2008).

Our study also considered tree age (Fig. 2 D–F). The most common species (*L. fragilis*) was most abundant on young trees. In the plot dbh vs tree age (Fig. 2D) three recruitment cohorts are recognizable: 25–100 years, 100–165, and 165–200 years. *L. fragilis* occurred on 47 of 59 trees (80%) of the first, 37 of 73 (51%) of the second, and 12 of 42 (29%) of the third and oldest cohort. This speaks against dispersal limitation. The different figures for *P. solitaria* (5, 34, and 31%) can be explained by the different pH values for the three age cohorts (mean figures are 3.9, 3.3 and 3.2, respectively). Both species are spatially randomly distributed (Fig. S1), but are not randomly distributed among trees with different features, as shown by the correlation between abundance and tree parameters (Table 2). However, the youngest trees that could be aged were older than 25 years. This does not rule out dispersal limitation for myxomycetes to reach a tree within the first years of its life. However, several lines of evidence indicate a high dispersal capacity of myxomycetes: short lived aerial litter in tropical forests regularly harbors myxomycetes (Black et al., 2004), and sterilized straw-sticks are colonized by protostelids (which belong to different groups of Amoebozoa and form fruit bodies developing one to a few spores) within several weeks (Moore and Spiegel, 1995, 2000). We thus assume that at least common species produce enough spores to colonize a major proportion of host trees already in young stands. Both species in the present study (*L. fragilis* and *P. solitaria*) have spores of ca. 10 µm diameter. In accordance, myxomycete spores have been successfully detected in air (Kamono et al., 2009), which is explained by the low sedimentation velocity of the spores (expected to range between 2 and 2.5 mm/s, Tesmer and Schnittler, 2007).

Increasing complexity of the bark microhabitat, or more simply a change in microhabitat features over the life of a tree, is more likely to occur: Clayton et al. (2014) monitored a different species composition of the myxomycete assemblages from small and large trees, and the first assemblage was not simply a depauperated version of the second. In the present study, we recorded only two species as very common, but changes in microhabitat parameters with tree size are also likely to cause their different distribution among trees. The first of the two species, *P. solitaria*, is a specialized myxomycete usually not found on other substrata than acidic tree bark. The second species, *L. fragilis*, occurred frequently also on bark of deciduous trees and shrubs (found in 56% of these cultures), whereas *P. solitaria* prefers coniferous trees (found in only 9% of bark cultures from deciduous trees and shrubs). The logistic regression models suggest that dbh and pH contribute in addition to each other to the likelihood of colonization with one of the two common myxomycete species, and dbh was found as decisive for

the colonization by both species, yet in a different manner (one species reacted positively, the other negatively to increasing dbh). Moreover, for *L. fragilis* within the subset of trees of known age, an alternative parametrization was found that allows exclusion of pH due to co-linearities between the environmental variables, and here occurrence is negatively correlated with tree age (Table S3).

A real colonization experiment, monitoring the colonization of sterilized bark by detecting first amoebae (perhaps by molecular methods), and later fructifications, may help to elucidate the contributions of the different steps of colonization: dispersal, establishment of amoebal populations, and finally the formation of fruit bodies. In addition, different structures of a tree, like the trunk, older and younger branches, should be sampled to elucidate the influence of microhabitat parameters on myxomycete occurrence.

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Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.funeco.2016.02.003>.

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Conclusions

CHAPTER 4

& Perspectives

CONTENTS

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4.1. Challenges and limitations

The accurate circumscription of species is an essential prerequisite for any diversity assessments and biogeographical studies. For myxomycetes, two species concepts were developed: the morphospecies concept bases on differences in morphological characters of the fructifications and is applied in virtually all diversity studies of the group. The biospecies concept, derived from compatibility studies of plasmodia raised in culture, remained largely theoretical since it is restricted to a few easily cultivable members of the Physarales. Therefore the traditional morphospecies concept is utilized for all diversity assessments conducted in Palearctic regions within this study. Consequently, the accuracy of determinations relies on well-developed fructifications collected in the field and from moist chamber cultures. For studies in tropical regions, there are many obstacles for this approach: fruiting bodies do not last long in the tropical climate, are produced sporadically due to erratic heavy rain falls, and (in contrast to studies in arid zones) moist chambers produce often few sporocarps which are not always well developed. Therefore, one cannot expect that a one-time assessment reflects properly the myxomycete assemblage of the region. This holds especially true for species usually seen in the field only, which are often apparently rare or absent. Often a full day of collecting results in less than 25 specimens, which is much less than at the peak season in temperate forests, where 100 or more specimens can easily be collected in the same time. In our studies in Ethiopia and the Philippines, many ecosystems suffered from intense land use, which severely changes forest structure and reduced both amount and quality of coarse woody debris. Furthermore, the time required for a solid survey and the personal experience in searching for fruiting bodies in the field are some of the major constraints in doing intensive field surveys. However, intense field work is the only way to compile a comprehensive species list, since not all species will fruit in moist chambers. Nevertheless, to compensate these difficulties and to achieve a fairly comprehensive survey, collecting substrates in a given locality and setting up numerous moist chamber cultures is a common alternative.

Determination of tropical myxomycete species is as well difficult, since a rather high proportion of the specimens in a given survey is usually in bad condition or represented by a few sporocarps only. As usually, it is difficult to decide if subtle differences in a given taxon may be attributed to phenotypic plasticity or represents true, genetically determined differences, or even cryptic speciation as evidenced for *Hemitrichia serpula* in this dissertation. This becomes especially important for the comparison of Neotropical and Palearctic myxomycete communities which is solely based on the morphological species concept. Here, we only can outweigh lacking quality (for instance, possible misidentifications) with quantity (using very large and carefully compiled data sets).

As acknowledged in this study, the few morphospecies so far investigated using independent genetic markers may be composed of several phylopecies (which may represent biospecies) that may (as in the case of the *Tubifera ferruginosa*-complex) or may be not (*Trichia varia*) show morphological differences. It is therefore highly recommended that intensive surveys in the tropics should be coupled with a molecular barcoding component to have a rough estimation of the relationship between morpho- and phylopecies, and to minimize determination errors.

4.2. Baseline knowledge in the myxomycete diversity of the Paleotropics

This study focused on two regions that are understudied in terms of myxomycete diversity. The survey in the Ethiopian highlands presented the first fairly comprehensive report on myxomycetes for the country and adds to the poorly known myxomycete diversity of the African Paleotropics. This descriptive study highlights evidence that similar to its unique flora, the east African mountain ranges harbor different and distinguishing myxomycete assemblage. Especially the hollow trunks of a giant lobelia (*Lobelia rhynchopetalum*), found abundantly in the high elevations of the Simien Mts. can serve as a natural moist chamber. For the last years, studies about Philippine myxomycetes are continuously expanding.

This study also updates the current knowledge of myxomycete diversity for the country. Now 150 total species coming from 26 provinces of the 81 provinces of the country are reported, covering ca. 20 large islands and small islets from the 7,107 islands the Philippines. One species, *Stemonaria fuscoidea*, is reported the first time for the Asian Paleotropics. A paper presented in this dissertation investigates the bioabsorption potential of some myxomycetes collected in ultramafic and volcanic soils. At this point, I would like to correct the statement from one paper (Rea-Maminta et al. 2015): species diversity of myxomycetes from ultramafic soils is not higher than that from volcanic soils, but as it can be derived from the rarefaction curves, it is better explored.

When we compare the results of this thesis with data from Neotropical studies, our assessments conducted in different, yet unexplored lowland areas in the Philippines confirm the following hypotheses: (a) in the Tropics, aerial microhabitats seem to be taxonomically more diverse than forest floor litter, (b) forest types with heterogenous plant communities will have higher diversity than monotypic agricultural plantations, (c) differences in the forest structure affects the occurrence of myxomycetes, (d) species richness follows the intermediate disturbance hypothesis and (e) anthropogenic activities tend to decrease myxomycete diversity. For the biogeographic study carried out with two molecular markers on the common tropical species *Hemitrichia serpula*, the Philippine population showed a higher molecular diversity (9 ribotypes per specimen) compared with other Southeast Asian countries like Thailand (1 ribotype) or Vietnam (4 ribotypes). These results suggest that as well for myxomycetes the Philippine archipelagic landscape, due to its past geographical history, offers an ideal living laboratory to test key concepts and theories, like the importance of long-distance dispersal by spores, gene flow in the context of island biogeography, to enlarge our understanding about the diversity and distribution of myxomycetes.

4.3. Major concluding points

The composition of myxomycete assemblages in the tropics is clearly not the same. Differences between Neotropic and Paleotropic assemblages are mainly attributable to geography and not the elevational gradient, i.e. lowland vs. highland forests. Moreover, there are some species that are found to be geographically restricted like, *Comatricha spinispora* and *Diderma pseudotestaceum* since they were recorded as occasionally occurring in only a certain region, in this case only in Vietnam. In theory, tropical ecosystems are stable for a long period of time and show similar macroecological conditions. Therefore, in difference to temperate region the apparent restriction of species (as noticed by recording its fructifications) cannot be explained by temperature, more likely seem vicariance events leading to isolation caused by distance. As such, the different distribution patterns for some morphospecies found in this study are likely to represent only the proverbial “tip of the iceberg” of the total assemblage of species actually present.

Such geographical differences are also visible in the case of a biogeographic study in a pantropical species, *Hemitrichia serpula*. The intraspecific variation in ribotypes of this species, found as well for other dark-spored (*Badhamia melanospora* and *Meriderma complex*) or bright-spored (*Trichia varia* and *Tubifera ferruginosa*-complex) myxomycetes can be explained by two mechanisms: (1) a given morphospecies shows allopatric speciation caused by geographic barriers, in addition, (2) still hypothetical mutations in mating type genes may cause sympatric speciation, leading to reproductively isolated biospecies. The unique combinations of the genotypes of the two investigated molecular markers provide evidence for cryptic speciation in *Hemitrichia serpula*, but these patterns are not completely congruent with variation in spore ornamentation. We interpret this as occasional hybridization events due to still ongoing speciation in this myxomycete species.

Therefore, we can conclude that myxomycetes are ubiquitous in the sense that long-distance dispersal by spores enables them to reach every suitable habitat here on earth, at least considering a geological time scale. Consequently, many morphospecies fill out their entire potential ranges and show a wide, most often cosmopolitan, distribution pattern, although they can be locally rare due to limited microhabitat availability. According to this line of thought, the “everything is everywhere” hypothesis for protistean biogeography is met, at least if the limitation “but the environment selects” is regarded. However, with a more differentiating species concept (biospecies) that looks deeper into the molecular diversity of widely distributed species, it seems apparent that gene flow along with long-distance dispersal of spores represent a joint mechanism that allows myxomycetes to occupy their whole potential environmental range. However, since the latter system is not strong enough to prevent differences in regional gene pools to develop, speciation within the myxomycetes seems inevitable.

Affidavits

Selbstständigkeitserklärung / Declaration

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Ernst-Moritz-Arndt-Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

I hereby declare that I have submitted this work so far neither at the Faculty of Science and Mathematics at the Ernst-Moritz-Arndt-Universität Greifswald nor at any other university with the purpose to earn a PhD degree.

Furthermore I declare that I have written this work as an independent effort and did not use any other sources and guides than those cited in the work. I did not copy any paragraphs of a third author without marking them as a citation.

Greifswald 18 January 2016

Nikki Heherson A. Dagamac

Erklärung bei Gemeinschaftsarbeiten Selbstständig

Hiermit erkläre ich, dass die in der folgenden Inhaltsübersicht mit meinem Namen gekennzeichneten Kapitel von mir selbständig verfasst worden sind:

1.	Abstract	Dagamac NHA
2.	Introduction	Dagamac NHA
3.	Publications	
3.1.	<i>Myxomycetes diversity of the Philippine archipelago</i>	
3.1.1.	Plasmodial slime molds of a tropical karst forest Quezon National Park, the Philippines	Dagamac NHA, Rea-Maminta MAD, dela Cruz TEE
3.1.2.	Rapid assessment of myxomycete diversity in the Bicol Peninsula	Dagamac NHA, dela Cruz TEE, Rea-Maminta MAD, Aril-dela Cruz JV, Schnittler M
3.1.3.	Diversity of plasmodial slime molds (myxomycetes) on coastal, mountain, and community forest of Puerto Galera, Oriental Mindoro, Philippines	Dagamac NHA, Rea-Maminta MAD, Batungbacal NS, Jung SH, Bulang CRT, Cayago AGR, dela Cruz TEE
3.1.4.	Myxomycete research in the Philippines: Updates & Opportunities	Dagamac NHA, & dela Cruz TEE
3.2.	<i>Filling the gaps of myxomycete diversity in the Paleotropics</i>	
3.2.1.	Myxomycetes diversity from the highlands of Ethiopia	Dagamac NHA, Hoffman M, Novozhilov YK, Schnittler M
3.2.2.	Biogeographical assessment of myxomycetes across the tropics	Dagamac NHA, Novozhilov YK, Stephenson SL, dela Cruz TEE, Rojas C, Schnittler M.
3.3.	<i>Molecular diversity of a tropical myxomycete population</i>	
3.3.1.	Speciation in progress? A phylogeographic study among <i>Hemitrichia serpula</i> (myxomycetes) population	Dagamac NHA, Rojas C, Moreno GH, Novozhilov YK, Schlueter R, Schnittler M
3.4.	<i>Other publications related to myxomycete diversity</i>	
3.4.1.	The occurrence of myxomycetes from a lowland montane forest and agricultural plantations of Negros Occidental, Western Visayas, Philippines	Alfaro JRA, Alcayde DLIM, Agbulos JB, Dagamac NHA, dela Cruz TEE
3.4.2.	Comparative diversity and heavy metal biosorption of myxomycetes from forest patches on ultramafic and volcanic soils	Rea-Maminta MAD, Dagamac NHA, Huyop FS, Wahab RAB, dela Cruz TEE
3.4.3.	Ecological factors limiting occurrence of corticolous myxomycetes – A case study from Alaska	Schnittler M, Dagamac NHA, Sauke M, Wilmking M, Buras A, Ahlgrimm S, Eusemann P
4	Conclusion & Perspectives	Dagamac NHA

Ich erkläre weiterhin, das ich die im folgenden beschriebenen Teile derjenigen Kapitel, bei denen ich nicht Alleinautor bin, selbstständig verfasst habe:

I hereby declare that I have independently written the following parts of chapters where I was not the sole author.

Chapter 3.1.1. Dagamac NHA, Rea-Maminta MAD, dela Cruz TEE. 2015. Plasmodial slime molds of a tropical karst forest Quezon National Park, the Philippines. Pacific Science 69(3):407-418.

I conceptualized the study design and methodologies that were employed in this paper. Sample collections in the field and the preparation of the moist chamber set-ups were conducted by me at the Microbiology lab of the Fungal Biodiversity and Systematics group, UST of TEE dela Cruz. With the assistance of MAD Rea-Maminta, we routinely checked the moist chamber and the identification of the specimens were carried out by the both of us. I did all the data analysis and interpretation included in the discussion of this manuscript. I have written the manuscript as the first and corresponding author with the approval of all my co-authors.

Chapter 3.1.2. Dagamac NHA, dela Cruz TEE, Rea-Maminta MAD, Aril-dela Cruz JV, Schnittler M. 2015. Rapid assessment of myxomycete diversity in the Bicol Peninsula, Philippines. Nova Hedwigia http://dx.doi.org/10.1127/nova_hedwigia/2015/0252.

I designed the idea and methodologies used for this paper. Fieldwork in the seven random lowland forest sites across Bicol Peninsula was done by me with the assistance of TEE dela Cruz, JV Aril-dela Cruz and M. Schnittler. Substrates that I collected were brought back to EMAU Greifswald and moist chambers were prepared by myself. Additional collected samples were also prepared as moist chambers in the Microbiology lab of the Fungal Biodiversity and Systematics group of TEE dela Cruz wherein JV Aril-dela Cruz and MAD Rea-Maminta routinely checked. Specimen determination was conducted by me, M. Schnittler and TEE dela Cruz. Data management, analysis, and interpretation were done by me with the assistance of M. Schnittler. I have written the manuscript as the first and corresponding author with the approval of all my co-authors.

Chapter 3.1.3. Dagamac NHA, Rea-Maminta MAD, Batungbacal NS, Jung SH, Bulang CRT, Cayago AGR, dela Cruz TEE. 2015. Diversity of plasmodial slime molds (myxomycetes) on coastal, mountain, and community forest of Puerto Galera, Oriental Mindoro, Philippines. J. Asia Pac. Biodivers. 8:322–329.

As part of the undergraduate research paper of the research group on Cryptogamic Diversity (TEE dela Cruz'), I participated in the field work and assisted in the preparation of the moist chamber cultures. Together with TEE dela Cruz, I helped the included undergraduate students to identify their specimens found both in the field collection and in their moist chambers at the Microbiology lab of the Research Center for Natural and Applied Sciences (RCNAS) in UST. Data management, analysis, and interpretation were done by me with the assistance of MAD Rea-Maminta. I have written the manuscript as the first and corresponding author with the approval of all my co-authors.

Chapter 3.1.4. Dagamac NHA, & dela Cruz TEE. Myxomycete research in the Philippines: Updates & Opportunities. Mycosphere 6(6):784–795.

This review paper was planned by me and TEE dela Cruz. I collated all the literatures that are associated for myxomycetes studies in the Philippines for the last 40 years. I have written the manuscript as the first and corresponding author with the approval of my co-author.

Chapter 3.2.1. Dagamac NHA, Hoffman M, Novozhilov YK, Schnittler M. Myxomycetes diversity from the highlands of Ethiopia. Nova Hedwigia (manuscript accepted).

I prepared the moist chamber (MC) for the various substrates and species list of the collected fruiting bodies in the field. For almost 12 weeks, I painstakingly checked the MCs and I helped in the preparation of the SEM for some of the rare specimens. I sequenced selected specimens for barcoding. Also, I contributed in the preparation of this manuscript.

Chapter 3.2.2. Dagamac NHA, Novozhilov YK, Stephenson SL, dela Cruz TEE, Rojas C, Schnittler M. Biogeographical assessment of myxomycetes across the tropics. (submitted in Journal of Biogeography)

Together with M. Schnittler, I assisted in the conceptualization of this research project. I initially contacted all possible putative coauthors that have done extensive surveys of myxomycetes in the tropics. With the dataset I have for the Philippine surveys, additional data was also used from the fieldwork I conducted in Thailand with M. Unterseher in 2013. I collated, meticulously rechecked and assembled a compilation of all of these different species databases for myxomycetes surveys done in the tropics. I did the data evaluation and interpretation. I have written the manuscript with considerations to the comments of all co-authors.

Chapter 3.3.1. Dagamac NHA, Rojas C, Moreno GH, Novozhilov YK, Schlueter R, Schnittler M. Speciation in progress: A phylogeographic study among *Hemitrichia serpula* (myxomycetes) population. (submitted in Fungal Diversity)

Together with M. Schnittler, I assisted in designing this research project. I did the molecular work with the assistance of A. Klahr at the EMAU Greifswald. Together with R. Schlueter, G. Moreno and Y. Novozhilov we conducted the morphological analysis via SEM of all the specimens used in this study. I did all the phylogenetic and population genetic analyses and together with C. Rojas, I assisted in construction of the species distribution modeling for the major clades in this paper. I also performed an event based historical biogeography reconstruction using the S-DIVA command employed in RASP. I have written the manuscript, considering the comments of all co-authors.

Chapter 3.4.1. Alfaro JRA, Alcayde DLIM, Agbulos JB, Dagamac NHA, dela Cruz TEE. 2015. The occurrence of myxomycetes from a lowland montane forest and agricultural plantations of Negros Occidental, Western Visayas, Philippines. *Fine Focus* 1: 7 – 20.

As part of the undergraduate research paper of TEE dela Cruz' group, I participated in the field work and assisted in the preparation of their moist chamber cultures. Together with TEE dela Cruz, I helped the undergraduate students to identify their specimens found both in the field collection and in their moist chambers at the Microbiology lab of the Research Center for Natural and Applied Sciences (RCNAS) in UST. Data management, analysis, and interpretation were done by me. I have written the manuscript as the corresponding author with the approval of all my co-authors.

Chapter 3.4.2. Rea-Maminta MAD, Dagamac NHA, Huyop FS, Wahab RAB, dela Cruz TEE. 2015. Comparative diversity and heavy metal biosorption of myxomycetes from forest patches on ultramafic and volcanic soils. *Chemistry & Ecology* 31(8):741 – 753.

This manuscript is part of the Master's thesis of MAD Rea-Maminta under the supervision of TEE dela Cruz. I joined in the field work where we collected substrates for moist chambers and soil samples for the chemical soil analysis. I also participated in the species determination and ecological analysis of her dataset. I contributed in the preparation of this manuscript by reviewing the final version of the text that was submitted for publication.

Chapter 3.4.3. Schnittler M, Dagamac NHA, Sauke M, Wilmking M, Buras A, Ahlgrimm S, Eusemann P. Ecological factors limiting occurrence of corticolous myxomycetes – A case study from Alaska. *Fungal Ecology* 21: 16-23.

I set up the moist chamber for all of the bark samples collected in Alaska. Furthermore, I routinely checked the moist chambers and generated the important dataset that was used for the evaluation of the environmental parameters accounted for this paper. I also took part in the species determination and ecological analysis of the dataset. I contributed in the preparation of this manuscript by writing some parts of the introduction, materials and methods and reviewing the final version of the text that was submitted for publication.

Greifswald 18 January 2016

Nikki Heherson A. Dagamac

Die Unterschrift weiterer Autoren kann aus technischen Gründen nicht eingeholt werden. Da die betreffenden Kapitel jedoch in Zeitschriften veröffentlicht sind, haben alle Mitautoren in die Publikation eingewilligt

Prof. Dr. Martin Schnittler
(Wissenschaftlicher Betreuer)

Erklärung zur Abgabe einer elektronischen Kopie der Dissertation

Declaration on the submission of an electronic copy of the PhD thesis

Mathematisch-Naturwissenschaftliche Fakultät

Einverständniserklärung nach § 4 Abs. 1 Nr. c Promotionsordnung

Faculty of Science and Mathematics

Declaration of consent according to § 4 sect. 1 Nr. c Doctoral Degree Regulations

Hiermit erkläre ich, dass von der Arbeit eine elektronische Kopie gefertigt und gespeichert werden darf, um unter Beachtung der datenschutzrechtlichen Vorschriften eine elektronische Überprüfung der Einhaltung der wissenschaftlichen Standards zu ermöglichen.

I hereby declare my consent to produce and store an electronic copy of this thesis for the purpose to enable an electronic examination of the thesis with the goal to exclude plagiarism and to obey good scientific practices.

Datum:

Unterschrift: Nikki Heherson A. Dagamac

The man who makes a success of an important venture never wails for the crowd. He strikes out for himself. It takes nerve, it takes a great lot of grit; but the man that succeeds has both. Anyone can fail. The public admires the man who has enough confidence in himself to take a chance. These chances are the main things after all. The man who tries to succeed must expect to be criticized. Nothing important was ever done but the greater number consulted previously doubted the possibility. Success is the accomplishment of that which most people think can't be done.

- C.V. White

NIKKI HEHERSON ALDEA DAGAMAC

Mobile Phone Nos.: (+63)9279157766 / (+49)17687869057 Email Address: nhadagamac@gmail.com / nhad-05@yahoo.com

Current Address: Zi 3.31, Max Kade Haus, Hans Beimler Str. 9, 17491, Greifswald, Germany

EDUCATIONAL BACKGROUND

- DOCTORAL DEGREE** : **Doctor of Natural Sciences (Dr. rer. nat)**
(2012 – Present) **Deutscher Akademischer Austauschdienst (DAAD) Scholarship Awardee**
Institut für Botanik und Landschaftsökologie
Ernst Moritz Arndt Universität Greifswald, Greifswald, Germany
- MASTERAL DEGREE** : **Master of Science major in Biological Sciences (M.Sc.)**
(2008 – 2010) **DOST-PCHRD ASTHRD Graduate Scholarship Grantee**
Graduate School, University of Santo Tomas, España, Manila, Philippines
- TERTIARY EDUCATION** : **Bachelor of Science in Biology (B.Sc.)**
(2004 – 2008) **QC Student Youth Development Program Scholarship Grantee**
College of Science, University of Santo Tomas, España, Manila, Philippines
- SECONDARY EDUCATION** : **High School Diploma (Valedictorian)**
(2000 – 2004) Diliman Preparatory School, Diliman, Quezon City, Philippines

PROFESSIONAL EXPERIENCE

- November 2010 – March 2012** **Instructor 3**
Department of Natural Sciences, College of Nursing,
University of Santo Tomas, España, Manila, Philippines
- June 2010 – March 2012** **Assistant Professor 3 /**
November 2009 – March 2010 **Lecturer**
Department of Biological Sciences, Centro Escolar University
Mendiola, Manila, Philippines
- July 2008 – December 2008** **Research Assistant**
UST – Collection of Microbial Strains (USTCMS)
Research Center for the Natural Sciences
University of Santo Tomas España, Manila, Philippines

SKILLS & COMPETENCIES

- Languages:** Filipino (Mother Tongue), English (excellent writing, reading and speaking skills)
Deutsch (good writing, reading and speaking skills)
- Technicals:** Proficient with Windows 10, MS Office, Adobe Photoshop, various phylogenetic, ecological and population genetic software, basic knowledge of R

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Nikki Heherson A. Dagamac

LIST OF PUBLICATIONS

Dagamac NHA, Rea-Maminta MAD, Batungbacal NS, Jung SH, Bulang CRT, Cayago AGR, dela Cruz TEE. **2015**. Diversity of plasmodial slime molds (myxomycetes) on coastal, mountain, and community forest of Puerto Galera, Oriental Mindoro, Philippines. **Journal of Asia Pacific Biodiversity** <http://dx.doi.org/10.1016/j.japb.2015.10.004>.

Rea-Maminta MAD, **Dagamac NHA**, Huyop FS, Wahab RAB, dela Cruz TEE. **2015**. Comparative diversity and heavy metal biosorption of myxomycetes from forest patches on ultramafic and volcanic soils. **Chemistry & Ecology** **31(8):741 – 753**.

Dagamac NHA, dela Cruz TEE, Rea-Maminta MAD, Aril-dela Cruz JV, Schnittler M. **2015**. Rapid assessment of myxomycete diversity in the Bicol Peninsula, Philippines. **Nova Hedwigia** **98** http://dx.doi.org/10.1127/nova_hedwigia/2015/0252

Dagamac NHA, , Rea-Maminta MAD, dela Cruz TEE. **2015**. Plasmodial slime molds of a tropical karst forest, Quezon National Park, the Philippines. **Pacific Science** **69(3):411 – 422**.

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Bennett RM, **Dagamac NHA**, Fernandez EVM, Uba MO, Ching MW, **2012**. *In vitro* degradation of anthracene by *Mycobacterium* sp. GIPAH – 01 isolated from Guimaras Island, Philippines. **Asian Journal Experimental Biological Science** **3(4): 682 – 687**.

Dagamac NHA, dela Cruz TEE, Pangilinan MVB, Stephenson SL, **2011**. List of species collected and interactive database of myxomycetes (plasmodial slime molds) for Mt. Arayat National Park, Pampanga, Philippines. **Mycosphere** **2(4): 449 – 455**

dela Cruz TEE, Santiago KAA, Ramirez CSP, Torres JMO, **Dagamac NHA**, Yap J, Ching M, Yulo PRJ, **2011**. Occurrence of cellular slime molds (dictyostelids) in Subic Bay Natural Forest Reserve, Zambales, Philippines. **Philippine Journal of Systematic Biology** **5: 99 – 104**

Macabago SAB, **Dagamac NHA**, dela Cruz TEE. **2010**. Diversity and distribution of plasmodial myxomycetes (slime molds) from La Mesa Ecopark, Quezon City, Philippines. **Biotropia** **17(2) 51-61**

dela Cruz TEE, Pangilinan MVB, Cruz RJ, de Jesus EE, Puylong RG, **Dagamac NHA**, **2010**. A checklist of plasmodial myxomycetes (slime molds) from Subic Watershed Forest Reserve, Zambales, Philippines. **Acta Manilana** **58: 41-45**

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Dagamac NHA, Sogono PG, Cabalfin RCB, Adducul ACY, dela Cruz TEE, **2008**. Fungal Root Endophytes from Musa spp. as biological control agents against the plant pathogen *Fusarium oxysporum*. **Acta Manilana** **56: 27 – 35**

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Even though this dissertation is only published solely under my name, many important people have made their contribution in its creation. I am thankful to all of those people and organizations who have made this dissertation work possible. Because of them, my whole graduate life as a PhD student in a foreign land is what I would forever treasure my entire lifetime.

- To my Doktorvater, **Prof. Dr. Martin Schnittler**, for being more than just a research and academic mentor who have welcomed me in his working group. You became an adviser to me by giving me all those scientific inputs that guided me throughout my research undertakings, for training me to be an independent worker and to start thinking meticulously and critically. But you become like my second father when you had shared your amazing, funny and crazy stories in the fieldwork from different countries, when you gave me wisdom about life and career and when you kindly gave your support to all of my professional and personal plans. Your enthusiasm and deep passion in your field of interest is something I hope I can emulate someday.
- **PD Dr. Martin Unterseher and his wonderful family** who were the first German family that welcomed me in their home. Beyond all the help in all the ecological knowledge and in analyzing enormous genetic sequences, I am inspired on how you as a person especially that humility and generosity you always showed me. Your character as a well-balanced man as a scientist, a family man and as a very dear friend is something I will always remember.
- **Dr. Thomas Edison E. dela Cruz** for introducing me to the world of myxomycetes and trusting my abilities. For the guidance and wisdom that tested my character and for your magnificent family that I have become part of, I will forever be grateful.
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- Financial support of my studies here in Germany is supported by the **Deutscher Akademischer Austausch Dienst (DAAD)**.
- To the staff of other institutes in the EMAU Greifswald namely **Dr. Rabea Schlüter** and the rest of the staff of Institute of Microbiology for letting me used any time the SEM in her laboratory and sharing to me the perks of being a diligent scientist. **Imme Burkart-Juergens** of the Welcome Center of the International Office for all the good times that you organized those cultural trips and monthly "Stammtisch".
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- To all the **USTIGers**: Reuel, Paul, Mam Cess, Kuya Mike, Mam Pia, Kuya Axel, Kuya Dan, Kuya Ronald, & Jeremy for all the memorable get aways and lakwatsa in Europe.
- To **PD Dr. Barbara Schulz and Fritz** for welcoming me in their home everytime I go in Braunshweig. Best memories I have in Germany are found in your place.
- To all the teachers and mentors and advisers for the last 28 years in my life starting from my younger grade school years until my graduate school life. So many of you had made an impact in me and your names may not be listed in this page but your names will forever be written in my heart.
- To my ever dearest friends back in the Philippines and all of the other international friends and German families I have met here in Europe. Without naming every single one of you in this acknowledgement page, I can say that I had the best moments of my life learning your culture and perspectives about life. Each person has a special place in my treasured collection of good memories.
- To my **FAMILY** back home in the Philippines, your continued encouragement and support was the reason why I remained strong amidst being far from my comfort zone. I am always thankful that I have the best family in the world!
- For the strength and guidance in many unforeseen ways, **Lord Almighty** you were the source of this wisdom and never ending blessings. To God be the Glory!

NHAD

Two words that pay a multitude of dividends are uncommon to some; the most simple of expressions serves to make each recipient valued as a person; the two words are thank you which when extended and expressed often say more about the giver than any other two words in the English language."

- Byron Pulsifer